MINISTRY OF HEALTH OF UKRAINE

LVIV NATIONAL MEDICAL UNIVERSITY NAMED AFTER DANYLO HALYTSKY

Department of Clinical Immunology and Allergology

WORKING PROGRAM OF DISCIPLINE

«CLINICAL IMMUNOLOGY AND ALLERGOLOGY»

for training of specialists of the second (master's) level of higher education field of knowledge 22 "Healthcare"

specialty “222 “Medicine”

V year

LVIV - 2021

MINISTRY OF HEALTH OF UKRAINE

LVIV NATIONAL MEDICAL UNIVERSITY NAMED AFTER DANYLO HALYTSKY

"APPROVED"
The first vice-rector for
scientific and pedagogical work
corresponding member of AMS of Ukraine
professor M.R. Gzegotsky
_____ 2021

"CLINICAL IMMUNOLOGY AND ALLERGOLOGY"

Methodological Recommendations

for training of specialists of the second (master's) level of higher education field of knowledge 22 "Healthcare"

specialty "222 "Medicine"

V year

Approved
at the faculty meeting at the department of clinical immunology and allergology

« 30 » august in 2021
Protocol №1

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Head of the department prof. V.V. Chopyak

Approved
by the field-specific methodological commission for therapeutic discipline

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Head of the field-specific methodological commission prof. O.M. Radchenko

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The program was discussed and approved:

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1. EXPLANATORY NOTE

Program of Clinical Immunology and Allergology for students of higher medical educational establishments of III-IV accreditation levels has been established for specialties "General Medicine" 7.110101 in accordance with existing regulations, and the curriculum of training future doctors for educational qualification level "Specialist". Undergraduate students study the subject "Clinical Immunology and Allergology" in the fifth year (during 9^{th} -10^{th} semesters).

The program is based on the following regulations:

- Education and qualification characteristics (EQC) and educational and vocational program (EVP) training approved by the Ministry of Health of Ukraine on 16.04.03 № 239 "On approval of the components of education standards for the specialty 1101 - Medicine";

- Recommendations for curriculum development training courses approved by the MOH of Ukraine № 152 of 24.03 2004 " On approval of recommendations for curriculum development training courses" as amended by the order of Ministry of Health of Ukraine № 492 dated 12.10.2004 "On Amendments and Additions with recommendations on curriculum development training courses ";

- Order of the Ministry of Health of Ukraine № 148 from 31.01.03 "On measures for implementation of the Bologna Declaration on higher medical and pharmaceutical education ";

- Pilot curriculum developed on the principles of the European Credit Transfer System (ECTS) and approved by the Order of the Ministry of Health of Ukraine of 31.01.2005 № 52.

- Order of the Ministry of Health of Ukraine № 52 dated 31.01.2005 "On the approval and introduction of new curriculum training educational qualification of "specialist" training "doctor" of higher educational establishments of III-IV accreditation levels of Ukraine in the field of "medicine", "pediatrics".

- Order of the Ministry of Health of Ukraine № 414 of 2007 "On amendments to the Order of Ministry of Health of Ukraine № 52 of 31.01.2005"

Clinical Immunology and Allergology as an academic discipline:

a) is based on other basic disciplines (medical biology, medical and biological physics, bio-organic and biological chemistry, histology, cytology and embryology, human anatomy, pathological morphology, physiology and pathophysiology, microbiology, virology and immunology, as well as the basics of internal medicine, pediatrics, general surgery, infectious diseases, obstetrics and gynecology, pharmacology) and integrates with these disciplines;

b) helps students to explore the features of the clinical profile of vocational and practical disciplines.

c) provides the ability to apply knowledge of clinical immunology and allergology at further education and training activities in accordance with the principles of evidence-based medicine.

Organization of educational process is carried out by the credit transfer system according to the Bologna process.

According to the curriculum training (order number from the Ministry of Health of Ukraine, 2007), students learn discipline "Clinical Immunology and Allergology" (3 credits for the specialty "General Medicine", "Pediatrics" and "Medical and preventive care") in the fifth year at higher medical educational establishments.

The program is structured for the 1st module of the discipline, which consists of 3 blocks of content modules:

Module 1 (5th year).

Basics of clinical immunology and allergology

(specialties "General Medicine")

Contents:

• Immunological status. Measurement principles and directions of immune correction.

• Immunodeficiency diseases and immunodependent pathology

• Allergic diseases

Program of Clinical Immunology and Allergology in the fifth year for the specialties: "General Medicine" involves learning the basics of clinical immunology and allergology at its main sections: general immunology, immunopathology: primary and secondary immunodeficiency, autoimmune diseases, transplant immunology, immunology of reproduction, oncoimmunology, allergology, to focus on a study of immunopathogenesis, clinics of immune and allergic diagnosis, treatment and prevention of immunodependent diseases.

The main objective is to familiarize students with various aspects of clinical immunology. The emphasis is on getting skills of providing immunological allergic history, physical examination and differential diagnosis of frequent clinical manifestations and diseases. Students are participating in the diagnostic and treatment processes of outpatients (mostly) and in-patients under the supervision of assistants, associate professors and professors, who also provide familiarization with the procedures that are frequent and usual in the practice of clinical immunologist and allergist. Physicians, clinical assistants and associate professors of the department are the most important participants of this program. Each student records and shows clinical data of the patients three times during the cycle.

The types of learning activities of students according to the curriculum are: a) lectures b) practical classes, c) independent students work (ISW). Thematic plan of lectures, practical classes, ISW provide the implementation of all the topics in the educational process that make up the content modules. Thematic lectures reveal problem areas, relevant sections of clinical immunology and allergology. Teaching tools like multimedia presentation slides, training films, patients' case demonstrations are most used in the lecture course. Lectures and practical training components are relevant to the practical work. Practical classes are conducted at clinical sites of the department. Expected duration of practical training in the fifth year is 5 academic hours. The objectives of clinical practical classes in clinical immunology and allergology are following:

- make the student become the participant in the process of providing medical care to patients from the time of their examination, diagnosis, treatment till discharge from the hospital or in -patient admission;
- acquire professional and practical skills; teamwork skills, physicians and other members of health care provision;
- form a responsible student as a future specialist in accordance with the level of training, his/her improvement during training and profession. The students are

provided with a detailed work plan of the department and the tools for its realization. This plan should include:

- Methods of investigation that a student should know;
- Algorithms (protocols) of examinations, diagnosis, treatment and prevention according to the standards of evidence-based medicine;

Supervision of the patients includes:

• clarification of patients' complaints, history of the disease, medical history and examination of the systems of the body;
• physical examination of the patient and determination of the main symptoms of the disease ;
• analysis of immunological laboratory data and data of allergy examination of the patient;
• statement of a diagnosis of the patient;
• purpose of treatment;
• identification measures of primary and secondary immunization ;
• report about the results of team work of the students in the study group, the analysis of the correctness of the diagnosis under the supervision, differential diagnosis, the amount of the assigned inspection, treatment tactics, assess of prognosis and disability.

In practical classes students are encouraged to keep a diary in which they should make a summary of the patients examined during the practical classes, fix the statement of the diagnosis, the patient's plan of examination and treatment plan. ISW makes up 30 % in the curriculum. It includes:

• study of topics not included in the plan of classes;
• students work in the offices of the departments of clinical sites, including immunological laboratories, allergic diagnosis rooms, interpretation of laboratory data and allergy research methods in extracurricular time;
• mastering practical skills using phantoms and work with patients (according to the list);
• work in the computer lab to prepare for the Step 2.

Teachers and supporting staff of the department help students to fulfill their individual work. During the practical training and the final module control teachers monitor and evaluate the individual work. Topics submitted for independent study are evaluated only during the final testing.

Departments and Courses of Clinical Immunology and Allergology have the right to redistribute training hours within the structural modules within the program depending on the organizational and technical capabilities, areas of scientific research, environmental characteristics of the region, but must complete the whole subject of the claims in accordance with the ultimate goals of EQC and EVP for field of study and curriculum.

A tentative curriculum subjects
"Clinical Immunology and Allergology"
for medical students on specialties:
7.110101 " General medicine"

The structure of the discipline	Number of hours, including				Year of study	Type of control
	Total hours / credits	Audience		ISW		
		Lectures	Practical lessons			
Total hours / credits ECTS	**75/2,5**	**10**	**40**	**25**	**5**	
Module 1: Fundamentals of Clinical Immunology and Allergology semantic modules– 3	**75 hours / 2,5 credits ECTS**	**10**	**38**	**25**	**5**	**The final module control**
including - the final control of module 1			**2**			**The final module control**

Note: 1 ECTS - 30 hours.; Classroom activity - 70%, IWS - 30%;

2. Purpose of study of educational discipline

Purpose of the Clinical Immunology and Allergy is based on EQC and EVP training of the doctor by profession and is the basis for building the structure of the discipline. Description of tasks formulated in the form of skills through targeted actions is based on the outcomes for each module or semantic module specific objectives formulated in the form of specific skills targets that achieve the final result of the study subjects.

The final task of discipline:

№139 To determine the nature and principles of treatment of immunological disorders in patients with different pathologies, form dispensary groups and risk groups, conduct immunization.

№154 To identify clinical, haematological and immunological features of immune disorders in patients with acute, chronic and relapse pathology, to put previous clinical diagnosis.

№ 212 To classify symptoms and syndromes of immunological disorders.

№ 234 To conduct differential diagnostics of hereditary and acquired immune disorders in various pathologies on the basis of immunological anamnesis, family tree analysis, data, clinical and laboratory examination of the patient.

№ 247 To draft examination of the patient, analyze the findings of research based on immunological processes, patient's age, health status, season.

Table of contents of the program
Module 1
Basis of clinical immunology and allergology
Total hours: 90/3 credits
(lectures - 10, practical classes -40, IWS -40)

Ultimate goals of the module

Students should be able to:

• Demonstrate the ability to conduct a survey and physical examination of patients with congenital to acquired immunodeficiency states according to a leading patient complaints and history of the disease.

• Demonstrate the ability to conduct a survey and physical examination of patients with Allergic disorders under the leading patient complaints and history of the disease.

• Demonstrate the ability to use various diagnostic methods that help in making decisions on the management of patients with various immunodeficiency states and immunodependent diseases.

• Apply the principles of evidence-based medicine in making diagnostic and therapeutic decisions in immunodependent and allergic diseases.

• Show the ability to determine the etiologic and pathogenetic factors in the development of various immunodeficiencies, immunodependent and allergic diseases.

• Show the ability to identify the typical clinical picture of immunodeficiencies, immunodependent and allergic diseases.

• Demonstrate ability to diagnose and treatment plan represent immunological abnormalities in patients with different pathologies immunodependent to form risk.

• find the main course options and complications immunodependent and allergic diseases.

• Demonstrate the capability to assess the health of patients and the use of appropriate recommendations for immuno - and allergoprevention.

• To demonstrate the ease of use of health information technology and critical peer reviews of the medical literature on the diagnosis and treatment immunodependent pathology.

• Demonstrate the ability to justify and apply methods for understanding clinical manifestations of the disease.

• Show a basic understanding of ethical principles and their application in the treatment of patients

Students must provide treatment of the patients (new or those already being treated) with the following diseases:

- Congenital immunodeficiency
- Acquired immunodeficiency
- Immunodependent disease
- Allergic disease.

Organization of educational process should ensure the participation of students in the conduct of not less than 1 hospitalized or ambulatory patients. If it is not likely

to get access to patients in any category, students fill out the medical history of educational diagnoses / problems relevant category. The need for writing such a history is defined by an assistant / associate professor (department head teacher) based on viewing data on the availability of appropriate patients in the office or in the outpatient reception.

Daily reports of inspection of patients and students remain available. Assistant / Associate Professor for the required number of control patients and patients often display common immunodependent and allergic diseases without unnecessary repetition.

Didactic classes are held during morning checks, lectures and classes. An assistant controls each student to receive the necessary competence in the following areas: physical examination and questioning of the patient, oral report, filling in the documents, diagnostic decision making (critical thinking). In addition, assistants supervise activities of students in order to be sure that they have mastered the practical skills.

Topical Module 2: **Immune status, main principles and directions of immunological correction**

Specific objectives:

Students should be able to:

• To explain the concept of "immunity" and the factors of congenital and acquired immunity.
• Implement the conclusions of the immune system on the basis of laboratory research.
• Analyze changes in immune status based on the patient's age, health status and season.
• Explain the basic concepts of immunological studies to determine the quantitative and qualitative indicators of immunity.
• Conduct a survey and physical examination of patients with immune disorders.
• Justify the use of the basic diagnostic methods used in clinical immunology, define the indications and contraindications for their conduct.
• To determine the etiologic and pathogenetic factors of acquired immunodeficiency.
• Identify features of antiviral immune defense.
• Identify the features of antibacterial host defense.
• Highlight the features of antifungal host defense.
• Interpret data of the leukogram and of the immunogram based on clinical data, the stage of the immune response , immunological anamnesis.
• To demonstrate the moral and ethical principles of medical specialist and principles of professional subordination.

TOPIC 1. **Structure and principles of the immune system. Immune inflammation. Developmental Immunology.**

Definition and types of immunity. Central and peripheral organs of the immune system. Factors of innate immunity: cellular (monocyte - macrophage system, and granulocyte killer cells), humoral (complement system, cytokines, etc.). Antigens and their characteristics. Specific immunity, its features, stages of formation and cooperation of immune cells that are involved in the formation of the immune response. Populations (T -and B- lymphocytes) and subsets (T- helper (Th) 1, Th2, Th3, Th9,Th17, Th22; T-regulatory cells, T- CTL) lymphocytes stages of maturation and differentiation of function. Immunoglobulins, structure and function, thymus-dependent and thymus-independent mechanism of synthesis of antibodies. Structure and properties of circulating immune complexes. Major histocompatibility complex: structure, properties and function. Regulation of immunity.

TOPIC 2 Analysis of the immune system functioning.

Features of immunological history. Clinical methods for the analysis of the immune system. Instrumental methods for analysis of the immune system. Laboratory methods for the analysis of the immune system: humoral innate protective factors ; assessment of cellular immunity; comprehensive assessment of local immunity.

Comprehensive analysis of the immune status by causing the person. The main complaint of patients with immune disorders. Features of immunological diagnosis. Determination of the main symptoms and syndromes of immune disorders . Physical symptoms of immune pathology. Methods of physical examination of the patient with disorders of the immune system (ultrasound, radiology, immunohistochemistry, etc.

Immunogram, the interpretation of results. Possibilities and limitations of immunological methods in the practical work. Features of statement of immunological diagnosis.

Age features of bone marrow, thymus and peripheral organs of the immune system. Age features functioning of immune cells. Age features of inflammatory reactions. The part of the mother's body in the formation of the immunity of the child. Immune system of the fetus, the newborn child and at different ages.

Thymus and aging. Immunoregulatory processes at old age. Immune theory of aging. Immune pathology in the elderly. The peculiarities of therapy based on age.

TOPIC 3. Immunotropic therapy immunorehabilitation, immunization.

Classification of immunotropic drugs, mechanism of action, side effects. The principles of clinical application of immunotropic drugs, indications and contraindications for the prescription, selection of doses; immunological control of therapeutic effectiveness: immunosuppressive drugs; immunocorrective drugs; blockers of mediators of immune response; anti-inflammatory drugs; replacement therapy; therapy with cytokines, anti- receptor drugs and others.

Basic principles of immunological prevention of bacterial and viral infections. The main types of immunorehabilitation, its strategy, tactics and fundamentals.

Topical Module 2: Immunodeficiency diseases and immunodependent pathology.

Specific objectives:

Students should be able to:

• Conduct a survey and physical examination of patients with congenital and acquired immunodeficiency states.
• Identify factors in the development imunopathogenic immunodependent disease (a disease caused by the human immunodeficiency virus (HIV), herpes virus disease, Chlamydia infection, infectious mononucleosis, etc.):
• justify the use of fixed immunodiagnostic methods used in clinical immunology, define the indications and contraindications for their conduct in patients with various immunodependent diseases.
• Interpret data phenotyping pair donor - recipient (index histocompatibility) in preparation for the transplantation of organs and cells.
• justify the use of immunosuppressive therapy in post transplant period.
• To determine the clinical and laboratory signs of pre-acute , acute and chronic rejection crises .
• To conduct differential diagnostics between the crisis of rejection and infectious complications in patients after organ transplantation.
• Identify laboratory signs of systemic and local immunosuppressive mechanisms during normal pregnancy.
• Identify mechanisms of immunodependent forms of infertility.
• Interpret data of phenotyping pair male - female (index histocompatibility) in the diagnosis of infertility immunodependent form .
• To analyze the results to identify indicators that characterize factors antitumor protection in patients with suspected tumor.
• Interpret data definition of tumor associated antigen for early detection of tumors in evaluating the effectiveness of treatment and to determine the presence or absence of metastases ;
• justify the use of immunotropic therapy in patients with tumors;
• Be able to use clinical - immunological criteria in the diagnosis of autoimmune disease;
• Identify the basic immunological mechanisms in the development of autoimmune diseases;
• Justify the use of immunosuppressive therapy in patients with autoimmune disorders;
• To demonstrate the moral and ethical principles of medical specialist and professional subordination principles

TOPIC № 4. Immunodiagnosis and immunotherapy in cancer patients. Immunodiagnosis and immunotherapy in transplantology. Immunodependent infertility.

Anti-tumor and pro-tumor interaction mechanisms of the immune system body "host" and "tumor". Factors of immunological resistance of the tumor. The concept of tumor-

associated antigens. Immunosuppressive effect of tumors. Immune changes in cancer patients. Immunodiagnosis, including differential diagnosis according to the CD-phenotype of tumor cells. Current approaches to the immunotherapy of patients with cancer.
Basic concepts, terminology (auto -, alo -, Xena - transplant). Pre-transplantation monitoring. Mechanisms of the rejection allografts: above acute, acute and chronic. Postoperative infectious complications, diagnostic criteria. Immunosuppressive therapy: mechanisms of action, principles of destination, complications. New immunological methods of diagnosis and therapy in transplantology.
Immunological relations between systems "father - mother", "mother - fetus".
Immune status of pregnant women**.** Immunodependent form of infertility in marriage. Causes and immunological mechanisms of antisperm auto-antibidies in man and in woman immunodiagnosis. Immunooncological methods of treatment. Immune conflicts in the system "mother - fetus": diagnosis, treatment, prophylaxis.
Immunological aspects of contraceptive study.

TOPIC № 5. **Primary and secondary immunodeficiency disease. HIV / AIDS**

Congenital/primary and acquired/secondary immunodeficiency disease. HIV / AIDS
Congenital immunodeficiency disease: definition, classification, mechanisms of development. Clinical signs of immunological tactics of a doctor, treatment approaches: combined , T - i B - dependent immunodeficiencies caused by disturbance of the immunity and phagocytic deficiency of complement proteins.
Acquired immunodeficiency disease: definition, causes , mechanisms of development , classification, diagnostics. The role of acquired immunodeficiency diseases in pathogenesis of the different diseases. Early detection in the organism of secondary immunological insufficiency. Basic approaches to the treatment.
Rapid fatigue syndrome; chronic fatigue syndrome.
The etiology, immunopathogenesis, diagnostics and immunotherapy of AIDS. Immunological methods in the diagnosis of AIDS. Dynamics of the immunogram of HIV -infected patients and AIDS. Immunization of HIV.

TOPIC № 6. Autoimmune disease (immune-diagnostics, immunotherapy).
The concept of autoimmune reactions, autoimmune syndrome, an autoimmune disease. Mechanisms breakdown of immunological tolerance, the role of genetic factors. Immunopathogenetic mechanisms of biostructures damage of the human body in autoimmune diseases. The role of immunological methods for the study of early verification of the diagnosis of autoimmune diseases. Autoimmune dependence in immune pathogenesis of various human diseases. Modern methods of immune organ and systemic autoimmune diseases. Current approaches to application of immunotropic new generation drugs in the treatment of patients with autoimmune diseases.

Topical Module 3
Allergic diseases. Specific objectives:

Students should be able to:

• Conduct a survey and physical examination of patients with allergic diseases.
• To determine the etiologic (groups of allergens) and pathogenic (types of immune responses) factors of allergic diseases.
• Explain the basic concepts of allergy research (laboratory tests, skin tests, provocation tests, etc.)
• Develop a plan for examination of patients with Allergic diseases, justify the application of the basic diagnostic methods used in allergy, determine the indications and contraindications for their conduct, possible complications;
• Identify different versions of the course and complications of allergic diseases;
• Conduct differential diagnosis, justify and formulate diagnosis of major allergic syndromes based on data analysis of laboratory and instrumental examination.
• Identify the prediction conduct primary and secondary prevention of allergic diseases;
• Conduct a differential diagnosis of allergic diseases and pseudo allergies.
• Appoint against allergic therapy, to evaluate its effectiveness.
• Demonstrate the moral and ethical principles of medical specialist and professional subordination principles.

TOPIC № 7. Atopic diseases.

The role of genetic factors and the environment in the immunopathogenesis of allergy. Modern understanding of allergies and atopy. Atopy as a systemic disease.
Types and main stage(s) of immunological reaction. Modern aspects of allergic diagnosis. Screening methods in assessing allergies. Elimination and provocative tests in allergy. Types of skin tests.
Principles of treatment of allergic diseases. Specific immunotherapy, indications and contraindications.
Features of immunopathogenesis of asthma, hay fever, allergic rhinitis, urticaria, and others. Drug allergies: causes, immunopathogenesis, clinical allergic diagnosis and prevention.

TOPIC № 8. Acute allergic conditions

Cell - mediated allergic disease (serum sickness, Arthus phenomenon , allergic alveolitis, etc.).: Immunopathogenesis, clinical immunodiagnosis, immunotherapy.
Differential diagnostics of diseases caused acute allergic reactions. Anaphylaxis. Pathomorphology, pathogenesis , clinic and emergency care. Anaphylactic reaction to the introduction of allergenic extracts in skin testing and conducting specific immunotherapy. Anaphylactic reactions to food.
Pathogenesis and classification of urticaria and angioedema. Acute urticaria and angioedema, their diagnosis and treatment.

THEMATIC PLAN OF LECTURES

for the 5-year students of medical faculties on clinical immunology and allergology

№	Lecture theme	Hours
1.	Tasks of clinical immunology and allergology. Assessment of the state of immune system.	2
2.	Immunodeficiency diseases. HIV-infection: diagnostics, treatment, prevention.	2
3.	Autoimmune diseases: immune diagnostics and immunotherapy. Allergic diseases: clinics, diagnostics, treatment.	2
	Total	6

Thematic plan

practical training for of 5th year medical students

of Clinical Immunology and Allergology

Topical module 1. Immune status, principles of analysis and direction of immune correction

№	Topic	Total hours
1	Structure and principles of functioning of the immune system. Immune inflammation. Age Immunology.	3
2	Analysis of the immune system	3
3	Immunotropic therapy immunorehabilitation, immunization	3
Total		9

Topical Module 2: : Immunodeficiency diseases and immunodependent pathology

№	Topic	Total hours
4	Immunodiagnosis and immunotherapy in cancer patients. Immunodiagnosis and immunotherapy of transplantology. Immunodependent infertility.	3
5	Congenital and acquired immunodeficiency disease. HIV / AIDS	3
6	Autoimmune disease (immune-diagnostics, immunotherapy).	3
Total		9

Topical Module 3 Allergic diseases

№	Topic	Total hours
7	Atopic disease: immunopathogenesis, clinical manifestations, diagnosis, treatment	3
8	Acute allergic conditions	2
	The final modular control	1
Total		24

TOPICS OF SELF-GUIDED WORK

for the 5-year students of medical faculties on clinical immunology and allergology

№ з/п	Theme	Hours
1.	Mucosal immunity: laboratory diagnostics and approaches to correction	5
2.	Assessment of the state of antibacterial, anti-fungal and anti-parasite immunities in patients	5
3.	Herpetic immunotropic infections	10
4.	Hey fever: diagnostics and immune therapy	5
5.	Drug allergy	5
Total		30

9. Analysis of success

Evaluation - is one of the final stages of training activities and determines the success of training. Assessment of the discipline is set as the average of the scores of all modules that structurize discipline.

Assessment for the module is defined as a sum of current educational activities (in points) and evaluation of the final module control (in points), which is suggested in the assessment of theoretical knowledge and practical skills according to the lists in specified discipline program.

The maximum number of points that a student can gain by studying each module is - 80, including the current educational activities - 120 points (60%), the results of the final module control - 80 points (40%).

The maximum number of points that the student can get for the current activity in a module is calculated by multiplying the number of points that corresponds with "5" (15 points), the number of topics in the module, and is 120 points (15x8) points.

The minimum number of points that a student must score at a module for admission to the final module control is calculated by multiplying the number of points that corresponds with "3", the number of topics in the module, and makes up 72 points.

The program will apply this system to convert the traditional system of points :

The traditional system	**Converted into points**
«5»	**15 points**
«4»	**12 points**
«3»	**9 points**
«2»	**0 points**

The maximum number of points that the student can get after completing the module is 96 points. It is calculated by multiplying the number of points that corresponds with "5" on a number of topics in the module (15 * 8 = 120 points).

The minimum number of points that the student can get when a module is calculated by multiplying the number of points corresponds with "3", the number of topics in the module (9 X 8 = 72 points).

Independent work of students, which is provided in the subject line along with the audience work is measured during continuous control on the relevant topics session. Assimilation of topics is considered only for private study, evaluated at the final module control as "zarakh"/accounted.

The final module control is carried out after completion of all topics of the module on the last control class module.

The students who visited all classes according to the curriculum, and scored not less than the minimum number of points for the module, are allowed to take the final module control. Students, who have not passed the module, should have the individual curriculum and permission to make up the academic debt to a certain fixed period. The maximum number of points that a student can get while preparing to the final module control is 72 points. The final control is considered to be passed if the student scored at least 50 points.

METHODICAL INSTRUCTION

Practical class №1

1. **THEME. STRUCTURE AND PRINCIPLES OF FUNCTIONING OF IMMUNE SYSTEM. IMMUNE INFLAMMATION. AGE IMMUNOLOGY** (5 academic hours).

2. **Background:** to familiarize students with the subject of **c**linical immunology and allergology; the current understanding of the antigen recognition (structure of antigen receptors of T and B cells, MHC molecules and MHC processing); regulation of the immune response (development of the immune system, the anatomy of the organs involved in the immune system, mechanisms of B and T cells regulation) and the processes in the formation of immune response.

3 Aim:

- ***Study****:* Students need to learn the basic modern data on the structure and function of all parts of the immune system;

- ***Professionally oriented****:* Students should know the basic stages of the immune response and the types of regulation;

- ***Educational****:* to form students' sense of responsibility for the timeliness and appropriateness of professional activities.

4. Materials: Equipment to run powerpoint presentation.

Main books. Short information on the topic.

5. Interdiscipline integration

5.1. *Interdiscipline integration*: The theme of this practical lesson is related to the themes set out in the course of general immunology: the organs and cells of the immune system, the structure and function of immunoglobulins, types of immune response, antigens, innate and acquired immunity.

Subject	Know	Can
Anatomy	Haemopoietic organs of immune system	Name primary and secondary lymphoid organs, lymphatic ducts
Biology, histology, embryology.	Immunity as mechanism of functional and structure homeostasis. Essentials of immuno-embryogenesis.	Distinguish basic phylogenetic levels of immunity evolution, primitive cell-mediated immunity and integral cell-mediated and humoral immunity
Biochemistry	Chemical structure and biological action of mediators of cell-mediated immunity	Immunophoretic determination of immunoglobulins.

Pathophysiology	Phagocytosis in the focus of inflammation, immunological tolerance, reactivity, allergy.	Immunocompetent cells, participating in defense reactions of cell-mediated and humoral immunity
Normal physiology	Interaction of nervous, endocrine and immune system for functional and structure homeostasis maintaining	On practical classes determine interaction of nervous, endocrine and immune system.
Pathological anatomy	Role of immune disorders in mesenchymal and protein dystrophies, mucous and fibrinoid degeneration of connective tissue	Determine mesenchymal and protein dystrophies, mucous and fibrinoid degeneration of connective tissue. Types of tissue reactions in collagenoses.
Microbiology, virusology	Specific and non-specific mechanisms of defense. Types of antigenes, immunity, allergies.	Perform agglutination test, Coombs' test, precipitation. Titration of complement, bacteriolysis and haemolysis tests, immunofluorescence test
Propedeutics of internal diseases	Role of immune system in pathogenesis of different diseases.	Interpretation of rosette test, Manchini immunoglobulins, phagocytic activity of blood.

6. Student has to know:

1. Define “immunity”, factors of innate and adaptive immunity.
2. Main data about immune system structure and function.
4. Characteristics of phagocytosis, complement system, T- and B-lymphocyte systems, scheme of main stages of immune response.

Study questions.

1. Characterize antiviral immunity development.
2. Characterize antibacterial immunity development.
3. Characterize immunity development in case of autoimmune disorders.
4. Characterize antifungal immunity development.
5. Point indications for immunological investigation.
6. Characterize immune state of patient.
7. Estimate results immunological tests of I and II level in different conditions.

Main part

An immune system is a system of biological structures and processes within an organism that protects against disease by identifying and killing pathogens and tumor cells. It detects a wide variety of agents, from viruses to parasitic worms, and needs to distinguish them from the organism's own healthy cells and tissues in order to function properly.

There are two groups of immune system organs.

- Primary (central)--organs where *immature* lymphocytes develop
  - ✓ Thymus
  - ✓ Bone marrow
- Secondary (peripheral)--tissues where antigen is localized so that it can be effectively exposed to *mature* lymphocytes
  - ✓ Lymph nodes
  - ✓ Appendix
  - ✓ Peyer's Patches (of GI tract)
  - ✓ Tonsils
  - ✓ Adenoids
  - ✓ Spleen
  - ✓ MALT (Mucosal-Associated Lymphoid Tissue)
  - ✓ GALT (Gut-Associated Lymphoid Tissue)
  - ✓ BALT (Bronchial/Tracheal-Associated Lymphoid Tissue)
  - ✓ NALT (Nose-Associated Lymphoid Tissue)
  - ✓ VALT (Vulvovaginal-Associated Lymphoid Tissue)

The Cells of the Immune System

T-Cells -- T lymphocytes are usually divided into two major subsets that are functionally and phenotypically (identifiably) different. The T helper subset, also called the CD4+ T cell, is a pertinent coordinator of immune regulation. The main function of the T helper cell is to augment or potentiate immune responses by the secretion of specialized factors that activate other white blood cells to fight off infection.

Another important type of T cell is called the T killer/suppressor subset or CD8+ T cell. These cells are important in directly killing certain tumor cells, viral-infected cells and sometimes parasites. The CD8+ T cells are also important in down-regulation of immune responses. Both types of T cells can be found throughout the body. They often depend on the secondary lymphoid organs (the lymph nodes and spleen) as sites where activation occurs, but they are also found in other tissues of the body, most conspicuously the liver, lung, blood, and intestinal and reproductive tracts. **Natural Killer Cells** -- Natural killer cells, often referred to as NK cells, are similar to

the killer T cell subset (CD8+ T cells). They function as effect or cells that directly kill certain tumors such as melanomas, lymphomas and viral-infected cells, most notably herpes and cytomegalovirus-infected cells. NK cells, unlike the CD8+ (killer) T cells, kill their targets without a prior "conference" in the lymphoid organs. However, NK cells that have been activated by secretions from CD4+ T cells will kill their tumor or viral-infected targets more effectively.

B Cells -- The major function of B lymphocytes is the production of antibodies in response to foreign proteins of bacteria, viruses, and tumor cells. Antibodies are specialized proteins that specifically recognize and bind to one particular protein that specifically recognize and bind to one particular protein. Antibody production and binding to a foreign substance or antigen, often is critical as a means of signaling other cells to engulf, kill or remove that substance from the body.

Granulocytes or Polymorphonuclear (PMN) Leukocytes -- Another group of white blood cells is collectively referred to as granulocytes or polymorphonuclear leukocytes (PMNs). Granulocytes are composed of three cell types identified as neutrophils, eosinophils and basophils, based on their staining characteristics with certain dyes. These cells are predominantly important in the removal of bacteria and parasites from the body. They engulf these foreign bodies and degrade them using their powerful enzymes.

Macrophages -- Macrophages are important in the regulation of immune responses. They are often referred to as scavengers or antigen-presenting cells (APC) because they pick up and ingest foreign materials and present these antigens to other cells of the immune system such as T cells and B cells. This is one of the important first steps in the initiation of an immune response. Stimulated macrophages exhibit increased levels of phagocytosis and are also secretory.

Macrophages -- Another cell type, addressed only recently, is the dendritic cell. Dendritic cells, which also originate in the bone marrow, function as antigen presenting cells (APC). In fact, the dendritic cells are more efficient apcs than macrophages. These cells are usually found in the structural compartment of the lymphoid organs such as the thymus, lymph nodes and spleen. However, they are also found in the bloodstream and other tissues of the body. It is believed that they capture antigen or bring it to the lymphoid organs where an immune response is initiated. Unfortunately, one reason we know so little about dendritic cells is that they are extremely hard to isolate, which is often a prerequisite for the study of the functional qualities of specific cell types. Of particular issue here is the recent finding that dendritic cells bind high amount of HIV, and may be a reservoir of virus that is transmitted to CD4+ T cells during an activation event.

The Immune Response

An immune response to foreign antigen requires the presence of an antigen-presenting cell (APC), (usually either a macrophage or dendritic cell) in combination with a B cell or T cell. When an APC presents an antigen on its cell surface to a B cell, the B cell is signaled to proliferate and produce antibodies that specifically bind to that antigen. If the antibodies bind to antigens on bacteria or parasites it acts as a signal for PMNs or macrophages to engulf (phagocytose) and kill them. Another important

function of antibodies is to initiate the "complement destruction cascade." When antibodies bind to cells or bacteria, serum proteins called complement bind to the immobilized antibodies and destroy the bacteria by creating holes in them. Antibodies can also signal natural killer cells and macrophages to kill viral or bacterial-infected cells.

If the APC presents the antigen to T cells, the T cells become activated. Activated T cells proliferate and become secretory in the case of CD4+ T cells, or, if they are CD8+ T cells, they become activated to kill target cells that specifically express the antigen presented by the APC. The production of antibodies and the activity of CD8+ killer T cells are highly regulated by the CD4+ helper T cell subset. The CD4+ T cells provide growth factors or signals to these cells that signal them to proliferate and function more efficiently. This multitude of interleukins or cytokines that are produced and secreted by CD4+ T cells are often crucial to ensure the activation of natural killer cells, macrophages, CD8+ T cells, and PMNs is listed in the chart below.

Layered defense

The immune system protects organisms from infection with layered defenses of increasing specificity. Most simply, physical barriers prevent pathogens such as bacteria and viruses from entering the organism. If a pathogen breaches these barriers, the innate immune system provides an immediate, but non-specific response. Innate immune systems are found in all plants and animals. If pathogens successfully evade the innate response, vertebrates possess a third layer of protection, the adaptive immune system, which is activated by the innate response. Here the immune system adapts its response during an infection to improve its recognition of the pathogen. This improved response is then retained after the pathogen has been eliminated, in the form of an immunological memory, and allows the adaptive immune system to mount faster and stronger attacks each time this pathogen is encountered.

Components of the immune system

Innate immune system	**Adaptive immune system**
Response is non-specific	Pathogen and antigen specific response
Exposure leads to immediate maximal response	Lag time between exposure and maximal response
Cell-mediated and humoral components	Cell-mediated and humoral components

No immunological memory	Exposure leads to immunological memory
Found in nearly all forms of life	Found only in jawed vertebrates

Both innate and adaptive immunity depend on the ability of the immune system to distinguish between self and non-self molecules. In immunology, self molecules are those components of an organism's body that can be distinguished from foreign substances by the immune system. Conversely, non-self molecules are those recognized as foreign molecules. One class of non-self molecules are called antigens (short for antibody generators) and are defined as substances that bind to specific immune receptors and elicit an immune response.

Adaptive immunity

The adaptive immune system evolved in early vertebrates and allows for a stronger immune response as well as immunological memory, where each pathogen is "remembered" by a signature antigen. The adaptive immune response is antigen-specific and requires the recognition of specific "non-self" antigens during a process called antigen presentation. Antigen specificity allows for the generation of responses that are tailored to specific pathogens or pathogen-infected cells. The ability to mount these tailored responses is maintained in the body by "memory cells". Should a pathogen infect the body more than once, these specific memory cells are used to quickly eliminate it.

Lymphocytes

The cells of the adaptive immune system are special types of leukocytes, called lymphocytes. **B cells and T cells** are the major types of lymphocytes and are derived from hematopoietic stem cells in the bone marrow. B cells are involved in the humoral immune response, whereas T cells are involved in cell-mediated immune response.

Both B cells and T cells carry receptor molecules that recognize specific targets. T cells recognize a "non-self" target, such as a pathogen, only after antigens (small fragments of the pathogen) have been processed and presented in combination with a "self" receptor called a major histocompatibility complex (MHC) molecule. There are two major subtypes of T cells: the killer T cell and the helper T cell. Killer T cells only recognize antigens coupled to Class I MHC molecules, while helper T cells only recognize antigens coupled to Class II MHC molecules. These two mechanisms of antigen presentation reflect the different roles of the two types of T cell. A third, minor subtype are the γδ T cells that recognize intact antigens that are not bound to MHC receptors.

In contrast, the B cell antigen-specific receptor is an antibody molecule on the B cell surface, and recognizes whole pathogens without any need for antigen processing. Each lineage of B cell expresses a different antibody, so the complete set of B cell antigen receptors represent all the antibodies that the body can manufacture.

Killer T cells directly attack other cells carrying foreign or abnormal antigens on their surfaces.

Killer T cell are a sub-group of T cells that kill cells that are infected with viruses (and other pathogens), or are otherwise damaged or dysfunctional. As with B cells, each type of T cell recognises a different antigen. Killer T cells are activated when their T cell receptor (TCR) binds to this specific antigen in a complex with the MHC Class I receptor of another cell. Recognition of this MHC:antigen complex is aided by a co-receptor on the T cell, called CD8. The T cell then travels throughout the body in search of cells where the MHC I receptors bear this antigen. When an activated T cell contacts such cells, it releases cytotoxins, such as perforin, which form pores in the target cell's plasma membrane, allowing ions, water and toxins to enter. The entry of another toxin called granulysin (a protease) induces the target cell to undergo apoptosis. T cell killing of host cells is particularly important in preventing the replication of viruses. T cell activation is tightly controlled and generally requires a very strong MHC/antigen activation signal, or additional activation signals provided by "helper" T cells (see below).

Helper T cells

Function of T helper cells: Antigen presenting cells (APCs) present antigen on their Class II MHC molecules (MHC2). Helper T cells recognize these, with the help of their expression of CD4 co-receptor (CD4+). The activation of a resting helper T cell causes it to release cytokines and other stimulatory signals (green arrows) that stimulate the activity of macrophages, killer T cells and B cells, the latter producing antibodies. The stimulation of B cells and macrophages succeeds a proliferation of T helper cells.

Helper T cells regulate both the innate and adaptive immune responses and help determine which types of immune responses the body will make to a particular pathogen. These cells have no cytotoxic activity and do not kill infected cells or clear pathogens directly. They instead control the immune response by directing other cells to perform these tasks.

Helper T cells express T cell receptors (TCR) that recognize antigen bound to Class II MHC molecules. The MHC: antigen complex is also recognized by the helper cell's CD4 co-receptor, which recruits molecules inside the T cell (e.g. Lck) that are responsible for the T cell's activation. Helper T cells have a weaker association with the MHC: antigen complex than observed for killer T cells, meaning many receptors (around 200–300) on the helper T cell must be bound by an MHC:antigen in order to activate the helper cell, while killer T cells can be activated by engagement of a single MHC:antigen molecule. Helper T cell activation also requires longer duration of

engagement with an antigen-presenting cell. The activation of a resting helper T cell causes it to release cytokines that influence the activity of many cell types. Cytokine signals produced by helper T cells enhance the microbicidal function of macrophages and the activity of killer T cells. In addition, helper T cell activation causes an upregulation of molecules expressed on the T cell's surface, such as CD40 ligand (also called CD154), which provide extra stimulatory signals typically required to activate antibody-producing B cells.

γδ T cells

γδ T cells possess an alternative T cell receptor (TCR) as opposed to CD4+ and CD8+ (αβ) T cells and share the characteristics of helper T cells, cytotoxic T cells and NK cells. The conditions that produce responses from γδ T cells are not fully understood. Like other 'unconventional' T cell subsets bearing invariant TCRs, such as CD1d-restricted Natural Killer T cells, γδ T cells straddle the border between innate and adaptive immunity. On one hand, γδ T cells are a component of adaptive immunity as they rearrange TCR genes to produce receptor diversity and can also develop a memory phenotype. On the other hand, the various subsets are also part of the innate immune system, as restricted TCR or NK receptors may be used as pattern recognition receptors. For example, large numbers of human Vγ9/Vδ2 T cells respond within hours to common molecules produced by microbes, and highly restricted Vδ1+ T cells in epithelia will respond to stressed epithelial cells.

An antibody is made up of two heavy chains and two light chains. The unique variable region allows an antibody to recognize its matching antigen.

B lymphocytes and antibodies

A B cell identifies pathogens when antibodies on its surface bind to a specific foreign antigen. This antigen/antibody complex is taken up by the B cell and processed by proteolysis into peptides. The B cell then displays these antigenic peptides on its surface MHC class II molecules. This combination of MHC and antigen attracts a matching helper T cell, which releases lymphokines and activates the B cell. As the activated B cell then begins to divide, its offspring (plasma cells) secrete millions of copies of the antibody that recognizes this antigen. These antibodies circulate in blood plasma and lymph, bind to pathogens expressing the antigen and mark them for destruction by complement activation or for uptake and destruction by phagocytes. Antibodies can also neutralize challenges directly, by binding to bacterial toxins or by interfering with the receptors that viruses and bacteria use to infect cells.

ANTIBODIES OR IMMUNOGLOBULINS
Definition:

Glycoprotein in serum and tissue fluid

*** Produced by:**

B-lymphocytes in response to exposure to antigen

*** React specifically with antigen**

*** Five classes of Antibodies:**

IgM Properties

- ✓ First Ig made by fetus and B cells
- ✓ Present in colostrum and mother milk protect newly born.
- ✓ Fixes complement

IgG Properties

- ✓ Major serum Ig
- ✓ Major Ig in extravascular spaces
- ✓ The only Placental transfer Ig
- ✓ Fixes complement
- ✓ Phagocytes – opsonization

IgA Properties

- ✓ Found in serum and body secretion:
- ✓ Tears, saliva, gastric and pulmonary
- ✓ secretions
- ✓ Major secretory Ig on Mucous surfaces give Local Immunity by coating m.o, bacteria or viruses preventing their adherence to mucosal cells•Does not fix complement (unless aggregated)
- ✓ Present in colostrum and mother milk protect newly born.
- ✓ Least common serum Ig
- ✓ Binds to basophils and mast cells (Does not require Ag binding)
- ✓ Allergic and hypersensitivity reactions
- ✓ Parasitic infections (Helminths)
- ✓ Binds to Fc receptor on eosinophils
- ✓ Does not fix complement

IgD Properties

- ✓ Present in very small amount in serum
- ✓ B cell surface Ig
- ✓ Does not bind complement

IgE Properties

- ✓ Least common serum Ig
- ✓ Binds to basophils and mast cells (Does not require Ag binding)
- ✓ Allergic and hypersensitivity reactions
- ✓ Parasitic infections (Helminths)
- ✓ Binds to Fc receptor on eosinophils
- ✓ Does not fix complement

Activation of B-cells

Two mechanisms:

1) T-dependent antigen:

- Most antigen require T-helper cells to activate B-cells
- Antigen is phagocytosed by macrophages or B-cells
- Macrophages or B process present Ag to T-cells
- These activate T-cells to produce lymphokines .lymphokines (IL-2,IL-4,IL,5) stimulate B-cells to divide and differentiate into plasma cells specific antibody
- Plasma cells form or differentiate into memory cells
- All classes of antibody (IgG,IgM,IgA,IgD,IgE) are T-cell dependant

2) T-independent antigens:

- Activation of B-cells directly without help of T-cells (e.g. bacterial capsular polysaccharides)
- IgM antibody is primarily produced

Primary and Secondary antibody responses

Primary antibody response	**Secondary antibody response**
* First exposure to antigen	* Subsequent exposure
* Lag period: days or weeks (slow onset)	* Lag period: hours (rapid onset)
* Small amount immunogl. low Ab level with gradual increase	* Large amount immunogl. high Ab with rapid increas
* Ab Persist for short duration decline rapidly	* Persist for long periods Weeks then (monthes or years)
* Antibody is IgM	* Antibody is IgG

Antigens

- Epitope - The distinct molecular surface features of an antigen capable of being bound by an antibody (a.k.a. *antigenic determinant*). Antigenic molecules, normally being "large" biological polymers, usually present several surface features that can act as points of interaction for specific antibodies. Any such distinct molecular feature constitutes an epitope. Most antigens therefore have the potential to be bound by several distinct antibodies, each of which is specific to a particular epitope. Using the "lock and key" metaphor, the antigen itself can be seen as a string of keys - any epitope being a "key" - each of which can match a different lock. Different antibody idiotypes, each having distinctly formed complementarity determining regions, correspond to the various "locks" that can match "the keys" (epitopes) presented on the antigen molecule.
- Allergen - A substance capable of causing an allergic reaction. The (detrimental) reaction may result after exposure via ingestion, inhalation, injection, or contact with skin.

- Superantigen - A class of antigens which cause non-specific activation of T-cells resulting in polyclonal T cell activation and massive cytokine release.
- Tolerogen - A substance that invokes a specific immune non-responsiveness due to its molecular form. If its molecular form is changed, a tolerogen can become an immunogen.
- Immunoglobulin binding protein - These proteins are capable of binding to antibodies at positions outside of the antigen-binding site. That is, whereas antigens are the "target" of antibodies, immunoglobulin binding proteins "attack" antibodies. Protein A, protein G and protein L are examples of proteins that strongly bind to various antibody isotypes.

Origin of antigens

Antigens can be classified in order of their class.

Exogenous antigens

Exogenous antigens are antigens that have entered the body from the outside, for example by inhalation, ingestion, or injection. The immune system's response to exogenous antigens is often subclinical. By endocytosis or phagocytosis, exogenous antigens are taken into the antigen-presenting cells (APCs) and processed into fragments. APCs then present the fragments to T helper cells ($CD4^+$) by the use of class II histocompatibility molecules on their surface. Some T cells are specific for the peptide:MHC complex. They become activated and start to secrete cytokines. Cytokines are substances that can activate cytotoxic T lymphocytes (CTL), antibody-secreting B cells, macrophages, and other particles.

Some antigens start out as exogenous antigens, and later become endogenous (for example, intracellular viruses). Intracellular antigens can be released back into circulation upon the destruction of the infected cell, and become exogenous antigens once again.

Endogenous antigens

Endogenous antigens are antigens that have been generated within previously normal cells as a result of normal cell metabolism, or because of viral or intracellular bacterial infection. The fragments are then presented on the cell surface in the complex with MHC class I molecules. If activated cytotoxic $CD8^+$ T cells recognize them, the T cells begin to secrete various toxins that cause the lysis or apoptosis of the infected cell. In order to keep the cytotoxic cells from killing cells just for presenting self-proteins, self-reactive T cells are deleted from the repertoire as a result of tolerance (also known as negative selection). Endogenous antigens include xenogenic (heterologous), autologous and idiotypic or allogenic (homologous) antigens.

Autoantigens

An autoantigen is usually a normal protein or complex of proteins (and sometimes DNA or RNA) that is recognized by the immune system of patients suffering from a specific autoimmune disease. These antigens should, under normal conditions, not be the target of the immune system, but, due to mainly genetic and environmental factors, the normal immunological tolerance for such an antigen has been lost in these patients.

Tumor antigens

Tumor antigens or Neoantigens are those antigens that are presented by MHC I or MHC II molecules on the surface of tumor cells. These antigens can sometimes be presented by tumor cells and never by the normal ones. In this case, they are called tumor-specific antigens (TSAs) and, in general, result from a tumor-specific mutation. More common are antigens that are presented by tumor cells and normal cells, and they are called tumor-associated antigens (TAAs). Cytotoxic T lymphocytes that recognize these antigens may be able to destroy the tumor cells before they proliferate or metastasize.

Tumor antigens can also be on the surface of the tumor in the form of, for example, a mutated receptor, in which case they will be recognized by B cells.

Nativity

A *native antigen* is an antigen that is not yet processed by an APC to smaller parts. T cells cannot bind native antigens, but require that they be processed by APCs, whereas B cells can be activated by native ones.

CYTOKINES

✓ Low molecular weight soluble proteins (polypeptides) produced in response to microbes and other antigens

✓ They act via cell surface receptors to mediate and regulate the amplitude and duration of the immune-inflammatory responses, through activation of macrophages, controlling growth and differentiation of T and B cells

Functional Categories of Cytokines

Cytokines classified according to their biologic actions into three groups:

1) Mediators and regulators of innate immunity

- Produced by activated microphages and NK cells in response to microbial infection - they act mainly on endothelial cells and leukocytes to stimulate the early inflammatory response to microbes

2) Mediators and regulators of acquired immunity

- Produced mainly by T lymphocytes in response to specific recognition of foreign antigens

- They include IL-2, IL-4, IL-5,, IL-13, IFN, Transforming growth factor-β (TGF-β) and lymphotoxin (TNF- β)

3) Stimulators of haematopoiesis

- Produced by bone marrow, stromal cells, leukocytes
- Stimulate growth and differentiation of leukocytes
- Stem cell factor, IL-3, IL-7, GM-CSF

Interferons (IFNs)

Interferons (IFNs): are proteins secreted in response to viral infections or other stimuli

** They include:*

- INF-α produced by leucocytes induced by virus infected cells
- INF-β produced by fibroblasts - INF-γ produced by NK cells,TH1 cells, CD8 T-cells**Action of INF-α and IFN-β :**
- Prevent viral replication
- Increase MHC-I expression on viral infected cells helping their recognition by CD8 T-cells
- Increase cytotoxic action of Nk cells
- - Inhibit cell proliferation and tumor growth

-**Action of IFN-γ :**

- Activate Macrophages
- Increase expression of MHC-I and II on APCs
- Enhance cytotoxic actions of Nk cells

Promotes production of TH1 and inhibits proliferation of TH2

MAJOR HISTOCOMPATIBILITY COMPLEX (MHC)

The MHC is a closely linked complex of genes that govern production of the major histocompatibility

* In humans, MHC resides on the short arm of chromosome 6

* Three genes (HLA-A, HLA-B, HLA-C) code for the class I MHC proteins

* Several HLA-D loci determine the class II MHC proteins i.e. DP, DQ and DR

* HLA genes are very diverse (polymorphic) i.e. there are many alleles of the class I and II genes

* Between the class I and class II gene loci, there is a third locus (Class III)

* This locus contains genes encoding tumor necrosis factor, lymphotoxin and two complement components (C2 and C4)

- Class III antigens do not participate in MHC restriction or graft rejection

MHC Class I Antigens

Class I MHC antigens are: HLA-A, HLA-B and HLA-C

* These antigens are glycoproteins found on surfaces of all nucleotide human cells and on platelets

* HLA-A contains 24 different antigenic specificities,
HLA-B contains 52 and HLA-C contains 11

* Class I MHC antigens are involved of MHC restriction of cell mediated cytotoxicity

MHC Class II Antigens

Class II antigens are: HLA-DP, HLA-DQ, HLA-DR antigens

These antigens are glycoproteins found on the surface of macrophages, B-cells, Dentritic cells, Langerhans cells of skin and activated T cells

HLA-DP contain 6 different antigenic specificities, HLA-DQ contains 9 and HLA-DR contains 20

* Helper T-cells recognize antigens on antigen-presenting cells only when the antigens are presented on the surface of cells in association with class II MHC

* Class II antigens react with the CD4 molecule on the helper T-cells which secrete cytokines

3. Final part Test-control

Initial knowledge level

1. Which of the following is a major interleukin produced by CD4+ T helper 1 (TH1) lymphocytes?

A. IL-1
B. IL-2
C. IL-4
D. IL-6
E. IL-8

2. Which of following are presented in association with antigens processed by the exogenous antigen presentation pathway?

A. Fc receptors
B. IgG heavy chains
C. MHC class I molecules
D. MHC class II molecules
E. T cell receptor (TCR)

3. Which of the following events occurs first in the differentiation sequence of human B cells in the bone marrow?

A. Cytoplasmic mu chains present in the B cell
B. Immunoglobulin heavy chain rearrangement
C. Immunoglobulin light chain rearrangement
D. Surface IgD and IgM present on the B cell
E. Surface IgM present on the B cell

4. Which of the following is the most important costimulatory signal provided to a T cell from an antigen-presenting cell?

A. B7 molecules interacting with CD 28
B. B7 molecules interacting with LFA- 1
C. ICAM-I interacting with LFA-1
D. LFA-3 interacting with CD 28
E. MHC class II interacting with T cell receptor

5. Cytotoxic T cells induced by infection with virus A will kill target cells

A. from the same host infected with any virus
B. infected by virus A and identical at class I MHC loci to the cytotoxic T cells
C. infected by virus A and identical at class II MHC loci to the cytotoxic T cells
D. infected with any virus and identical at class I MHC loci to the cytotoxic cells
E. infected with any virus and identical at class II MHC loci to the cytotoxic cells

6. Which of the following genes involved in the synthesis of immunoglobulins are linked on a single chromosome?

A. C gene for gamma chain and C gene for alpha chain
B. C gene for gamma chain and C gene for kappa chain
C. V gene for kappa chain and C gene for the epsilon chain
D. V gene for lambda chain and C gene for kappa chain
E. V gene for lambda chain and V gene for heavy chain

7. What is the role of the macrophage during antibody formation?

A. Activation of cytotoxic CD8 T cells

B. Delayed hypersensitivity reaction
C. Lysis of virus-infected cells
D. Processing antigen and presenting it to T helper CD4 cells
E. Synthesis of immunoglobulin
8. which of the following complement activities would a deficiency of the complement protein C4 inhibit?
A. Completion of the classic pathway to the splitting of C3
B. Formation of C3b for opsonization
C. Formation of C5 convertase via the alternative pathway
D. Formation of C5a for chemotactic attractant for neutrophils
E. Formation of the membrane attack complex
9. A superantigen is a bacterial product that
A. binds to B7 and CD28 costimulatory molecules
B. binds to the β chain of TCR and MHC class II molecules of APC stimulating T cell activation
C. binds to the CD4 + molecule causing T cell activation
D. is presented by macrophages to a larger-than-normal number of T helper CD4 + lymphocytes
E. stimulates massive amounts of IgG synthesis because of its large size
10. The blood from an 8-year-old boy was analyzed by flow cytometry. The exact number of B cells was counted. Which of the following cell surface markers was likely used to identify the B cells in this blood sample?
A. CD3
B. CD4
C. CD8
D. CD19
E. CD56
11. Which type of cells will T cells that have a low affinity for MHC class I molecules differentiated in the thymus become?
A. CD 8 + cytotoxic lymphocyte
B. Gamma-delta T cell
C. Natural killer cell
D. T helper 1 cell
E. T helper 2 cell
12. Which marker or markers are present on B cells and could be used to specifically identify such cells in a flow cytometric analyzer?
A. CD 3
B. CD 8
C. CD 14
D. CD 16 and CD 56
E. CD 19 and CD 20
13. Which of the following is the primary opsonin in the complement system?
A. C1q
B. C3b
C. C5

D. C5a
E. Factor B
14. Which of the following is an IgG2 molecule composed of?
A. One alpha, one gamma2, and two kappa chains
B. One gamma1 chain and two kappa chains
C. Two gamma1 chains and one kappa and one lambda chain
D. Two gamma1 chains and two kappa chains
E. Two gamma2 chains and two kappa chains
15. Avidity is important because:
A. it amplifies the binding strength of low affinity Fab's.
B. Fc receptor binding depends on it.
C. G-protein-mediated signal transduction will not occur without it.
D. it result in the activation of high affinity antibody-producing clones.
E. none of the above.

Final knowledge level

1.A researcher develops a specific antibody to the complement component C3b. Assume that intravenous administration of the antibody prevents the biological effects of C3b. Which of the following biological functions would the administration of the antibody be expected to interfere with?
A. Decreased appetite
B. Fever
C. Increased collagen synthesis by fibroblasts
D. Increased leukocyte adherence to endothelium
E. Opsonization to facilitate phagocytosis
2. A 7-year-old girl is walking across a vacant lot and steps on a nail. The next day, her foot is sore and the wound appears inflamed. During these early stages of infection, which of the following compounds exert the most powerful chemotactic effect on neutrophils, causing them to migrate into the inflamed area?
A. C5a and IL-8
B. IL-1 and tumor necrosis factor
C. LTC4 and LTD4
D. PGI2 and PGD2
E. Thromboxane and platelet activating factor
3. Which of the following enzymes does the neutrophil use to initiate the production of toxic oxygen compounds that kill bacteria?
A. Hydrogen peroxide
B. Myeloperoxidase
C. NADPH oxidase
D. Superoxide
E. Superoxide dismutase
4. C3 is cleaved to form C3a and C3b by C3 convertase. C3b is involved in all of the following EXCEPT
A. altering vascular permeability.

B. promoting phagocytosis.
C. forming alternative-pathway C3 convertase.
D. forming C5 convertase.
5.Natural killer cells are
A. B cells that can kill without complement.
B. cytotoxic T cells.
C. increased by immunization.
D. able to kill virus-infected cells without prior sensitization.

6."Isotype switching" of immunoglobulin classes by B cells involves
A. simultaneous insertion of VH genes adjacent to each CH gene.
B. successive insertion of a single VH gene adjacent to different CH genes.
C. activation of homologous genes on chromosome 6.
D. switching of light-chain types (kappa and lambda).
7. Idiotypic determinants are located within
A. hypervariable regions of heavy and light chains.
B. constant regions of light chains.
C. constant regions of heavy chains.
D. the hinge region.
8. The membrane IgM and IgD on the surface of an individual B cell
A. have identical heavy chains but different light chains
B. are identical except for their CH regions
C. are identical except for their VH regions
D. have different VH and VL regions
9. During the maturation of a B lymphocyte, the first immunoglobulin heavy chain synthesized is the
A. Mu chain.
B. gamma chain.
C. epsilon chain.
D. alpha chain.
10. Which one of the following substances is NOT released by activated helper T cells?
A. interleukin-1
B. gamma interferon
C. interleukin-2
D. interleukin-4

11. The antibody-binding site is formed primarily by
A. the constant regions of H and L chains.
B. the hypervariable regions of H and L chains.
C. the hypervariable regions of H chains.
D. the variable regions of H chains.
E. the variable regions of L chains.
12. The class of immunoglobulin present in highest concentration in the blood of a human newborn is

A. IgG.
B. IgM.
C. IgA.
D. IgD.
E. IgE.

13. Which one of the following must antigen-presenting cells that activate helper T cells express on their surfaces?
A. IgE
B. gamma interferon
C. class I MHC antigens
D. class II MHC antigens

14. The role of the macrophage during an antibody response is to
A. make antibody.
B. lyse virus-infected target cells.
C. activate cytotoxic T cells.
D. process antigen and present it.

15. Complement can enhance phagocytosis because of the presence on macrophages and neutrophils of receptors for
A. factor D.
B. C3b.
C. C6.
D. properdin.

CORRECT ANSWERS:

Initial knowledge level:

1.B; 2.D; 3.B; 4.A; 5.B; 6.A; 7.D; 8. A; 9.B; 10.D; 11.A; 12.E; 13.B; 14. E; 15. A

Final knowledge level:

1.E; 2.A; 3. C; 4.A; 5.D; 6.B; 7.A; 8.B; 9.A; 10.A; 11.B; 12.A; 13.D; 14.D; 15.B

Control questions.

1.Subject and aims of clinical immunology and allergology. History of immunology development. Main branches of further research.

2. Contemporary views on immune system structure, function and ontogenesis. Primary and secondary organs of immune system.

3. Principles of functioning of immune system in children and senior**.**

4. Innate cells factors of defense, their interaction in immune response.

5. Monocyte-macrophage system: functional characteristic, role in immune response realization. Contemporary views on phagocytosis.

6. Killing effect as part of immunobiological supervision. Types of killer cells. Their function, properties. Role of granulocytes in immune response.

7. Humoral factors of innate immunity.

8. Complement system. Biological consequences of its activation.

9. Antigenes, their structure, functions. Haptens.

10. Stages of T- abd B-lymphocytes maturation and differentiation.

11. T-lymphocytes. Structure of T-cell receptor. T-cell populations. Main markers and clusters of differentiation.

12. Th 1 and Th2. Importance of balance (Th1\Th2).

13. T-regulatory cells, main function.

14. Apoptosis as special form of cell death. Its role it physiological and pathological processes.

15. B-lymphocytes, markers and functions. Structure of receptor.

16. T-dependent and T-independent immune responses.

17. Immunoglobulins: structure, functions, classes. Role pf immune complexes in pathology.

18. Cytokines – mediators of immune system, Interleukins, classification, function and role in immune processes.

19. Growth factors. Tumor necrotic factors, interferons and adhesive molecules. Characteristic and role in immune response.

20. Immune system of mucosae. Gut-associated lymphoid tissue (GALT).

21. Contemporary views on structure and function of MHC

22. Structure of HLA antigens. Predisposition to diseases according to HLA phenotype.

List of practical skills.

Perform examination of immune response

Structure of class.

Plan and organizational structure of the lesson:

Preparatory stage: organization of lesson and test control of incoming level of knowledge (5 academic hours or 225 minutes)

№	Content	Level of learning	Methods of the learning control	Materials of the methodological support	Time, min.
1.	<u>The preparatory phase</u> Control input level of knowledge, skills. Organs and cells of immune system innate and adaptive immunity	 I I	1. Front rapid survey	Tests. Scheme.	25
2.	<u>The main stage</u> Description of immune system Immune response and immunocompetent cells Regulation of immune response Complement system Cytokines	 II II II II-III	2. Individual oral examination. 1. Test control.	1.Tables. 2. Folio-grams. 3. Tests I-III 1. 2. Slideshow.	180
3.	<u>The final stage</u> Monitoring and adjustment of professional knowledge, skills and abilities: immune system, immune response, innate and adaptive immunity, HLA-system, age immunology	 III III III III III	1. Testing. 2. Solving custom applications. 3. Oral examination.	1. Tests III-IV levels. 2. Situational problems.	15
4.	To sum up the lessons. Homework for the next topic.				2 3

Issues were considered during the class

Control questions:

1. Definition of immunity and its types.
2. Primary and secondary organs of immune system.
3. Factors of innate immunity: cells-mediated (monocyte-macrophage system, killers, granulocytes) , humoral (complement system, cytokines ect.).
4. Antigens and their characteristic.
5. Adaptive immunity, its properties, stages of formation and cooperation of immune cells participating in immune response.
6. Populations (T- and B-lymphocytes) and subpopulations (T-helpers 1 and 2, T-regulatory, CTL) of lymphocytes.
7. Stages of maturation and differentiation of lymphocytes function.
8. Immunoglobulins, structure, function.
9. Thymus-dependent and non-thymus-dependent mechanism of antibodies synthesis.
10. Structure and properties of circulating immune complexes.
11. Major histocompatibility complex (MHC): structure, properties, function.
12. Regulation of immunity.
13. Age immunology – basic principles.

Educating tasks and control (added)

Self student's work:

1. Make a list of age-specific central and peripheral organs of the immune system.
2. Develop a table (scheme) of the main differences in the functioning of organs and cells of the immune system, depending on age.
3. To form the main features of the immune system in old age.

Main theoretical aspects of theme.

1. Immune, neurological and endocrine regulation of functions.
2. Apoptosis as regulator of immune response.
3. Immunology of mucosae.

References:

1. Advances in Immunology. Edited by Frederick Alt. Hardbound (2008). – 240 pages

2. Abul K. Abbas, Andrew H. H. Lichtman, Shiv Pillai Cellular and Molecular Immunology. - Saunders; 7 edition (2011). – 560 p.

3. Roitt's Essential Immunology, Includes Desktop Edition. Peter J. Delves, Seamus J. Martin, Dennis R. Burton, Ivan M. Roitt. Wiley-Blackwell; 12 edition (2011). – 560 p.

4. How the Immune System Works, Includes Desktop Edition. Lauren M. Sompayrac. Wiley-Blackwell; 4 edition (2012). – 152 p.

5. Lecture Notes: Immunology, 6th Edition. Ian Todd, Gavin Spickett. Wiley-Blackwell (2011). – 480 p.

6. Essentials of Clinical Immunology, 6th Edition. by Helen Chapel, Mansel Haeney, Siraj Misbah, Neil Snowden. Wiley-Blackwell (2014). – 376 p.

7. Immunology: A Short Course, 6th Edition. Richard Coico, Geoffrey Sunshine. Wiley-Blackwell (2009) . – 416 p.

METHODICAL INSTRUCTION

Practical class №2

1. **THEME. CLINICAL AND LABORATORY EVALUATION OF HUMAN IMMUNE STATUS** (5 academic hours).

2. Background: Due to large number of factors that cause a decrease in immune reactivity and a fundamental role of immunopathological mechanisms in the development of many diseases, it is very important to study modern clinical and laboratory methods for the diagnosis of the state of the human immune system.

3. Aims:

- *Academic:* level based on theoretical training material. Students should familiarize themselves with the history of laboratory immunology, know physical and chemical basics of immunological laboratory techniques and equipment that is used for their implementation.
- *Professionally-oriented:* Students should understand the need to assess the patient's immune system in prognosis of the underlying disease, be able to base clinical and laboratory assessment to make statement on the immune system of the patient.
- *Educational:* make the students realize the sense of responsibility for the timeliness and appropriateness of professional activities and the need to continually improve their skills.

4. Equipment: schemes, immunograms, set of equipment for the laboratory (microscope, thermostat spectrophotometer, automatic dispensers, reagents).

5. Integrative ties of topic:

5.1. *Interdiscipline integration*: The theme of this practical lesson is related to the topics of the same series of practical lessons, "Structure and function of the immune system", "Immunology of reproduction", "The immune system and the tumor".

5.2. *Out-disciplinary integration*:

Subjects	To know	To be able to
1. Physics	The laws of refraction of light and fluorescence	Work with a light microscope to measure the extinction of samples on a spectrophotometer
2. Inorganic Chemistry	Ionic strength, the strength of van der Waals forces, laws of chemical dissociation valence	Prepare a buffer with a specific pH, using a pH meter
3. Histology	cell structure	Recognize under the microscope nucleus cytoplasm, the cell membrane
4. Biochemistry	The structure of the protein, fat, carbohydrates, enzymes.	Perform simple color biochemical reactions

	Main routes of metabolism of substances in the body.	
5. Pathoanatomy	Structural changes of the immune system	Microscopically evaluate morphologic features imunopathology
6. Pathophisiology	Immunological reactivity. Fundamentals of immunopathological processes. Membranopatiyi	Interpret changes in hemogram and proteinogram
7. Propaedeutic therapy	Pathology of membranes	Carry out inspection, palpation and percussion, to evaluate the results of instrumental methods.

6 . Summary theme session.

6.1. Study Questions.

6.1.1. History of Laboratory Immunology.

In cases of abnormalities in the test results, "first-level" or the presence of specific views (e.g. clinical severity of the immunological defect) it is recommended to conduct a more thorough analysis by "second level" tests.

“Second level" tests:

1. Bactericidal neutrophils in spontaneous and activated nitroblue tetrazolium test with (NBT-test)
2. Determination of cytokines in serum (e.g. IL -1 , IL- 2 , IL- 4 , IL- 10, granulocyte-macrophage colony-stimulating growth factors; tumor necrotizing factor alpha)
3. Determination of specific antigens and antibodies in serum and body fluids
4. Determination of specific tumor markers in serum
5. Determination of immunoregulatory substances in serum (beta-2-microglobulin, prostaglandin hormones, cyclic nucleotides).

6.1.6 Basic laboratory methods that are used in clinical immunology and allergy

1. Antigen-antibody reaction in vitro (serum) used in clinical diagnostics to identify and determine the number of antigens and antibodies (titration)
2. Methods that are performed to directly detect antigen-antibody reaction and the presence of immune complexes (precipitation, immunoelectrophoresis, double diffusion in agar gel, linking complement radial immunodiffusion, bacterial agglutination, hemolysis, passive hemagglutination, hemagglutination inhibition) (Figure)
3. Combined serological methods, which require additional factors for the detection of antigen-antibody complexes, as well as special equipment (e.g. fluorescent dyes, enzyme peroxidase, isotope iodine 125) (Figure)
4. Tests identify immune cells in order to ascertain the number of phagocytic cells and lymphocyte populations and subpopulations (Figure).

5. Methods for the identification of specific allergens and antibodies to them using tests in vivo (scarification method) and in vitro - test RIST (radioimmunosorbent test) and RAST (radioallergosorbent)
6. Methods for detection of immune complexes in serum and biological fluids using the complement component C1q and Raji cells
7. Methods of preparation and use of monoclonal antibodies: obtaining monoclonal antibodies based on the use of mouse hybridoma use relates to laboratory practice, as well as the treatment of tumors and the development of new vaccines.
8. Immunogenetic methods: typing system for HLA (detection leukocyte antigens encoded by genes 1 and 2 major histocompatibility complex class, index histocompatibility between hypothetical donor-recipient pairs).
6.1.7 . Interpreting laboratory immunological assay.
Use of laboratory tests in clinical immunology: critical considerations of eight widely used diagnostic procedures
The recent expansion of clinical immunology has been accompanied by the introduction of a variety of immunological diagnostic tests in clinical laboratories. Due to increasing demands from clinicians for such procedures, their use has often been exaggerated and there is a general feeling that a better definition of the indications for such tests, made in relation to patients' needs, would be beneficial.
Obviously, immunological tests, like any other diagnostic test, can be graded according to their usefulness in the care of patients. Some tests are essential for diagnosis, prognosis or monitoring of disease; many tests are useful but optional for routine investigations; other tests are of interest only for research purposes. In addition, a number of immunological tests are useless in some circumstances. There is a consensus amongst immunologists that an effort should be made to reduce their share in the continuously increasing cost of medical laboratory investigations. This requires self-limitation in the routine application of some immunological techniques, without detriment to patient care.
It has been restricted to the analysis of eight widely used diagnostic procedures.
For each procedure, two aspects have been considered. First, the main methods which can at present be recommended are outlined and their pitfalls discussed. Technical details are not included since they are readily available. Second, particular attention has been given to the definition of the clinical conditions for which the test would be essential for diagnosis, those for which the test would be helpful in assessing or monitoring the disease activity and those conditions for which the test should only be used in clinical research.
The conclusions of this committee reflect the present status of the art and do not preclude future improvements. It was the feeling that the primary goal of clinical immunology should be to help the patient in the most cost-effective manner.

QUANTITATION OF IMMUNOGLOBULINS
The assessment of the three major immunoglobulin classes in body fluids involves three laboratory techniques: serum electrophoresis, quantitation of major immunoglobulin classes and immunoelectrophoresis.

The measurement of IgE requires more sensitive techniques (Measurement of Total and Specific IgE). There is no clinical indication for the measurement of serum IgD. Quantitation of the immunoglobulin classes by immunological methods is important in a limited number of clinical conditions. This test is too often performed without good indication. *Methodological considerations*

Many methods for the quantitative assessment of immunoglobulins have been described. Two of them are currently of the most value and of comparable accuracy: (1) radial immunodiffusion (RID) and (2) nephelometry. When patient load is relatively low, RID will probably remain the method of choice. However, with a high patient load and if a nephelometer is already available, nephelometry is useful.

Radial immunodiffusion This has a constant coefficient of variation which, under optimal conditions, may be less than 10% except at extremely low concentrations. The limit of accurate protein measurements, using low concentrations of antisera, is about 10 mg/l (10 pg/ml).

Techniques using limited diffusion are more accurate than those with timed diffusion. With normal sera, results can be obtained after 24 hr of diffusion but more time may be required for the assessment of very high or very low levels.

Pitfalls. RID is sensitive to differences in diffusion constants; special precautions should be taken to ensure that immunoglobulins in the standard and test sera are not split or aggregated and are in the same form. For instance, reliable measurements of such proteins as low-molecular-weight IgM and secretory IgA cannot be made unless a standard preparation of these kinds of immunoglobulin is used.

Nephelometric techniques

These are increasingly used for quantitating serum immunoglobulin levels. Both turbidimetric procedures and the detection of antigen-antibody complexes by light scattering can be applied. Discrepancies in results have arisen from the use of different standards by different laboratories. The WHO makes available reference preparations for the five classes of human serum immunoglobulins and it is recommended that working standards should be related to these preparations. All antisera, including those from commercial sources, must be shown to be specific in the test for which they are being used. Hybridoma-derived monoclonal antibodies may be useful in the future; however, many monoclonal antibodies do not precipitate antigen when used alone so that mixtures of such antibodies may be required. With these antibodies it may also become easier to quantitate subtypes and subclasses of the immunoglobulins. Normal values Concentrations of immunoglobulins in sera vary with age, geographical environment and sex. Each laboratory should measure serum immunoglobulin concentrations on a matched control group.

Clinical indications

Quantitation of serum immunoglobulins is essential in suspected primary or secondary immunodeficiency (ID), even when no abnormality is seen in electrophoresis. Concentrations of immunoglobulins cannot be used, however, as the sole criterion for diagnosis of primary ID. Reports have been made of persons with selective IgA deficiency without any evidence of associated disease, and IgA is undetectable in approximately 0 03-02% of the normal population. On the other hand, failure to respond to one or more antigens can sometimes be observed in patients with normal or

high levels of all immunoglobulins. Thus normal immunoglobulin concentrations do not exclude antibody deficiency. Monitoring of serum immunoglobulin levels is essential in patients with severe forms of hypogammaglobulinaemia who receive gammaglobulin substitution therapy.

Quantitation of serum Ig is helpful in distinguishing 'benign' idiopathic monoclonal gammopathies from paraproteinaemieas caused by myeloma. In the latter case, the levels of normal immunoglobulins are usually decreased while they usually are unaltered in the 'benign' form. In this context it should be stressed that monoclonal immunoglobulins tend to give falsely high values in immunodiffusion assays. When large enough amounts of the monoclonal protein are present, it is more accurate to measure the protein by the area under the spike on serum protein electrophoresis. The value of quantifying serum immunoglobulins for other clinical purposes has been established in only a few additional instances, such as the determination of IgM levels in the cord blood of infants suspected of congenital infections, and as an aid in the diagnosis of trypanosomiasis and of tropical splenomegaly. Optionally, for research purposes, immunoglobulins may be quantitated in diffuse hypergammaglobulinaemia in conditions such as some lymphoproliferative diseases. liver cirrhosis and systemic lupus erythematosus. More promising might be immunoglobulin studies in the families of patients with immunodeficiency or homogeneous immunoglobulins which may clarify the role of genetic factors.

Other body fluids

Urine. Quantitation of immunoglobulins in the urine is possible but fraught with problems. For instance, Ig molecules may be split, urinary light chains exist as monomers, making standardization difficult. For the demonstration of Bence-Jones proteins, the combination of protein electrophoresis and immunoelectrophoresis is more useful.

Cerebrospinalfluid (CSF). Quantitation of immunoglobulin should be performed on unconcentrated CSF since concentration procedures will lead to the aggregation of immunoglobulins, especially IgG, and a falsely low value by RID.

Quantitation of immunoglobulins in CSF is of interest in diseases such as multiple sclerosis and subacute sclerosing panencephalitis where the concentration of IgG relative to the total protein or albumin is often, but not always, increased. In African trypanosomiasis the increase of CSF Ig is an indication of invasion of the central nervous system by the parasites.

IMMUNOELECTROPHORETIC ANALYSIS OF IMMUNOGLOBULINS IN BIOLOGICAL FLUIDS

Immunoelectrophoresis (IEL) permits ready identification of the major immunoglobulin classes. It is the method of choice for the identification of monoclonal immunoglobulins since it detects simultaneously their electrophoretic and antigenic homogeneity. It is not a good quantitative technique. It should not be used for the systematic screening of serum proteins.

Methodological considerations

IEL is a useful method to study immunoglobulins in other fluids in addition to serum, e.g. urine, cerebrospinal fluid, saliva and intestinal juice. In the latter instances, it is usually necessary to concentrate the proteins before performing IEL and to run a serum sample simultaneously from the same patient.

The medium of choice for IEL is either agar or agarose, using where possible the same type of gel for the serum protein electrophoresis.

IEL requires the use of potent and specific antisera. It is recommended to use, in the first step, polyvalent antisera containing precipitating antibodies to the various Ig classes and light-chain types. In order to identify monoclonal immunoglobulins, monospecific antisera to the various IG heavy and light chains are often required. These antisera are commercially available and should always be checked for their content of precipitating antibodies and for their specificity. The determination of the heavy-chain class of a monoclonal immunoglobulin requires sometimes, but not always, the use of class-specific antisera. Such antisera are necessary for the diagnosis of IgD or IgE myeloma. The identification of the heavy-chain subclass of monoclonal IgG or IgA components is mainly of value in research. The identification of the K- or A-light-chain type is necessary for the diagnosis of Bence-Jones proteins and optional for myeloma proteins. The light-chain type may, however, have prognostic significance in myeloma. IEL with anti-K and anti-A antisera allows the detection of small monoclonal components in the presence of diffuse hyperimmunoglobulinaemia, and sometimes the detection of multiple monoclonal components. Pitfalls. Because of poor availability of antigenic determinants for cross-linking, many anti-light-chain antisera are unable to precipitate some whole monoclonal immunoglobulin molecules, especially IgAA, and/or some free light chains (Bence-Jones proteins). Thus for the diagnosis of heavy-chain diseases (in particular a-chain disease), the use of additional procedures is necessary; e.g. IEL with antisera containing precipitating antibodies to conformational determinants of the Fab region or immunoselection combined with IEL, using potent antisera to light chains or to Fab which are incorporated into the gel. As in all immunoprecipitation procedures, antigen excess may preclude the visualization of a precipitin line especially when horse antisera are used. This is particularly the case when analysing Bence-Jones proteins. When a cryoglobulin is present in the serum, the immunoelectrophoretic analysis of the whole serum should be performed after heating at 370C and re-solution.

In order to permit ready identification of some IgM proteins and to ascertain their monoclonal nature by light-chain typing, additional procedures may be necessary, such as the addition of a reducing agent to the fluid under study (in order to convert 19S IgM into 8S subunits) or preliminary separation of IgM from IgG by physico-chemical techniques. Immunofixation, a technique more recently developed for the identification of monoclonal immunoglobulins, may be particularly useful in such instances.

In interpreting immunoelectrophoretic patterns one should be aware of possible associations of monoclonal immunoglobulins with other proteins, such as serum albumin, ai-anti-trypsin and lipoproteins.

Clinical indications

Serum. IEL is essential under the following conditions:

(a) When the clinical haematological and/or pathological findings lead to the diagnosis or
suspicion of the following diseases: myeloma, Waldenstrdm's macroglobulinaemia, heavy-chain diseases, amyloidosis and immunoglobulin deposition disease.
(b) In the presence of the following biological abnormalities:
(i) An abnormal narrow band on serum protein electrophoresis; however, it should be stressed that IEL allows the detection ofmonoclonal components in situations without a distinctive electrophoretic pattern.
(ii) Presence of a cryoglobulin; IEL is necessary to identify the proteins of the cryoprecipitate and to distinguish single-class homogeneous cryoprecipitating immunoglobulins from mixed cryoglobulins with or without a monoclonal component. IEL should also be done on whole serum.
(iii) Presence of a Bence-Jones type of proteinuria.
(iv) Pyroglobulin; serum hyperviscosity; discrepancy between immunoglobulin level as appreciated by electrophoretic and immunochemical procedures.
IEL may be useful in some immunoproliferative disorders such as chronic lymphocytic leukaemias (detection of p-chain disease and of monoclonal immunoglobulins) and cold agglutinin disease and in diseases such as Gaucher's disease or mucinar papulosis (monoclonal components) and trypanosomiasis (elevated polyclonal IgM).
IEL may be useful for research purposes in several instances such as primary immunodeficiencies (in addition to measurement of Ig levels which is necessary); bone marrow grafts in patients with leukaemias, marrow aplasia, or severe combined immunodeficiency; some autoimmune diseases; some haematological conditions such as myelomonocytic leukaemias; various infections such as cytomegalovirus or congenital toxoplasmosis; systematic survey of family members of patients with monoclonal gammopathies.
Urine. IEL is essential in myeloma (with or without whole homogeneous serum Ig); amyloidosis, immunoglobulin deposition disease; in all cases in which a monoclonal Ig has been found in serum, whatever the clinical conditions; in cases in which an abnormal narrow band has been found on the urinary protein electrophoresis pattern.
IEL is optional in malignant lymphoproliferative diseases other than myeloma (macroglobulinaemia, chronic lymphatic leukaemia, lymphoma, heavy-chain diseases) and in primary immunodeficiencies. Other fluids. IEL of cerebrospinal fluid proteins is also useful in the search for oligoclonal components in patients with subacute sclerosing panencephalitis, or in myeloma or macroglobulinaemia with neurological involvement. In multiple sclerosis, the technique of isoelectrofocusing is more productive.
IEL of the intestinal juice is essential in 'immunoproliferative small-intestinal disease' with suspicion of a-chain disease when the abnormal protein is not detected in the serum of the patient.

MEASUREMENT OF TOTAL AND SPECIFIC IgE

Measurement of total immunoglobulin E

IgE is the most important mediator in atopic disease. Moreover, it is highly increased in some parasitic diseases. The clinical usefulness of IgE-level determination, however, is of limited value.

Methodological considerations

The recommended methods to measure serum IgE (usually present in jug/l quantities) are ELISA techniques and solid-phase radioimmunoassay. The common principle of the two methods is to use insolubilized anti-IgE antibody. This reagent can be used either in a competitive binding assay using radiolabelled IgE and IgE standard or in a non-competitive assay using radiolabelled anti-IgE. Like other competitive assays, the first one is subject to non-specific inhibition by other serum factors and of limited sensitivity and is not recommended. The advantages of non-competitive assays are increased sensitivity and precision and the fact that they are usually free from interference by non-specific serum factors.

Although radioimmunoassays were used initially, the advantages and potential of ELISA enzymoimmunological assays, especially in developing countries, should foster their increasing use. The main advantages of ELISA techniques are the avoidance of isotope markers, the long shelf-life of the reagents and the evaluation by means of a photometer instead of the y-counter. The only limitation is that the ELISA techniques developed to date are not sufficiently sensitive to measure very low IgE levels. Radioimmunoassay is therefore the method of choice in paediatric patients, in immunodeficiencies and for analysis of cord blood, supernatants of cell cultures, etc. For the higher sensitivity and reproducibility required for research purposes, suitable double-antibody assays have been described. The values obtained must be compared with those of a control group matched according to age and geographical location. A WHO international reference preparation is available.

Indications

Determination of total IgE is not essential except in the diagnosis of the rare hyper-IgE syndrome associated with eosinophilia and recurrent infections described by Buckley. Determination of total IgE may sometimes be useful in differentiating IgE-mediated from non-IgE-mediated disorders when this cannot be done by clinical means. Such disorders include perennial rhinitis, bronchial asthma, dermatitis, chronic urticaria and food intolerance. However, IgE levels are of limited value since total IgE level can be in the normal range in IgE-mediated diseases (e.g. hay fever) and it can be increased by non-atopic mechanisms such as infestation with Laboratory tests in clinical immunology parasites. The results should therefore be interpreted with caution taking into account all other pertinent clinical information. Serial determinations (e.g. during immunotherapy) are usually of limited value, with the possible exception of allergic bronchopulmonary aspergillosis.

In prospective investigations, IgE elevation in early childhood may be a useful indicator of high risk for atopic diseases. Determination of total IgE can be considered as a tool for research in certain immunodeficiencies and in atopic families.

Measurement of specific IgE

Methodological considerations

Two main techniques are available. One using radiolabelled anti-IgE (radioallergosorbent test:
RAST) and the other one, enzyme-labelled anti-IgE (ELISA). Modifications of established standard RAST procedures should be used cautiously. Frequently, increased sensitivity is achieved at the cost of decreased specificity. The potential advantages of immunoenzyme assays for specific IgE are the same as those for total IgE.
The interpretation of results is hampered by a number of pitfalls:
(1) The commonly used and commercially available kits yield results related to a single reference serum. For this reason, comparison with other results is almost impossible.
(2) RAST classes for different allergens are not comparable.
(3) Impurity of most allergen preparations.
(4) Interference by antibodies of other immunoglobulin classes present in the same serum sample.
One main obstacle is the difficulty of transforming RAST results into levels of clinical sensitivity which are meaningful for the practicing physician.
Indications
The measurement of specific IgE is not essential in any clinical situation. It is no alternative to careful history taking and skin tests. Usually skin tests are more specific than specific IgE assay and closer to the clinical manifestation. However, in vivo tests can be subject to non-specific (irritant) influences.
Measurement of specific IgE is useful in the following situations: dermographism or severe dermatitis which preclude skin testing; situations in which symptomatic treatment influencing skin reactions cannot be stopped (e.g. antihistamines); extremely high level of sensitization in which skin testing would be dangerous for the patient; allergens which cannot be used for skin testing (toxic, water-insoluble or highly sensitizing substances); food allergies in which skin tests are less reliable; interpretation of doubtful skin tests. In this connection it should be stressed that the antigenic composition of skin-test solutions is not necessarily the same as that used as a substrate in the in vitro test. Properly used, measurement of specific IgE can reduce the frequency of provocation tests. Measurements of specific IgE are used for research purposes in various IgE-mediated diseases and in some parasitic infestations. Measurement of specific IgE should not be regarded as a 'screen' for allergic diseases or requested for the evaluation of allergic conditions in which IgE-mediated mechanisms are not involved (e.g. contact dermatitis).

COMPLEMENT MEASUREMENTS

Complement consists of a series of proteins that undergo sequential activation as a consequence of interaction with a variety of agents. Measurement of complement can be achieved either by functional measurement of the whole system, by functional measurement of individual components or by immunochemical measurement of individual components, using specific antisera. These measurements are static, representing the balance between synthesis and consumption. Elevated complement levels occur due to increased synthesis, especially following acute inflammation and

trauma, and low levels are found due to increased consumption and/or decreased synthesis. The latter may be genetically determined.

Methodological considerations

The total complement haemolytic assay (CH50) assesses the ability of serum to lyse a standard suspension of sheep erythrocytes optimally sensitized with anti-sheep red cell rabbit antibody. The test, as usually performed, principally assesses the functional activity of the components which generate the classical pathway C3 convertase, and of C3 itself. It is also a test of the presence of functionally active terminal components C5-C9, although it is not sensitive to variations in the level of these components. There are many ways of performing this test, but the technique which is most reproducible and clinically applicable is that described by Mayer. Variations in this procedure use different concentrations of cells and/or different volumes of reactants and incubation times. It is also possible to measure CH50 by automated methods.

Pitfalls. The value is dependent on the conditions of the test, and variations in level may occur if the red cells are aged or not standardized properly, are low in potassium, or are not adequately sensitized. For this reason, a standard serum with a known value should be included in all batches of estimations. Also, inadequate collection and storage of test specimens may give falsely low values.

Sera should be separated within 1 hr of collection of blood and stored at -70'C before testing. Where this is not possible, the use of EDTA plasma has been recommended. In sera containing cryoglobulin, falsely low functional and immunochemical complement levels may be obtained. A reference standard preparation for CH50 is available through the WHO.

Functional measurement of individual components is seldom necessary in clinical practice unless a genetic complement defect is suspected. Antisera are available to most complement proteins, in particular C3, C4, Clq, C1 esterase inhibitor and factor B. Estimation of individual components by immunochemical techniques is adequate for the vast majority of clinical purposes, and is particularly useful in poorly stored specimens. Although genetic defects rarely occur which result in the synthesis of abnormal molecules without functional activity, in general, immunochemically estimated component levels reflect in vivo functional levels.

The immunochemical estimation of C3 and C4 and other complement proteins can be carried out either by the single radial diffusion test or by some form of nephelometry. Rocket electroimmunodiffusion is not recommended because of the changes in electrophoretic mobility of the molecules on storage. International reference standard preparations for C3, C4, Clq and factor B are available through the WHO. The specificity of the antiserum used in the analysis is important and, for C3, antisera specific for C3c only should be used.

Estimation of C3 and C4 together form the most useful routine measurements of complement components. In some conditions the C4 may be abnormally low although the CH50 may be normal. Sometimes a low CH50 is due principally to a low C2, but antisera to C2 are not widely available, and functional tests of this protein are difficult to carry out in routine laboratories. Cl esterase inhibitor levels are principally of value in the differential diagnosis of angio-oedema.

Clinical indications CH50 complement estimations are essential only in those conditions in which a genetic defect in complement is suspected, e.g. in patients presenting with recurrent infections, especially recurrent meningitis, with hereditary angio-oedema or with established immune complex diseases occurring in families. For the confirmation of angio-oedema, estimation of the C1 esterase inhibitor level is essential, and if a normal immunochemical level is obtained then a functional assay should be performed since 10-15% of kindreds are associated with the production of non-functional molecules. If the CH50 is normal, functional assays of individual components are unnecessary except to detect heterozygous states.

Complement estimations (CH50, C3 and C4) are helpful in the assessment and monitoring of patients with glomerulonephritis, in established immune complex diseases such as systemic lupus Laboratory tests in clinical immunology erythematosus and certain forms of vasculitis, and in conditions such as dengue haemorrhagic fever. In conditions in which low levels are found, these frequently return to normal in remission, and complement levels can be used to monitor treatment.

Routine complement tests are of little value in most other acute and chronic inflammatory or infectious diseases.

DETECTION OF IMMUNE COMPLEXES IN HUMAN BIOLOGICAL FLUIDS

There is good evidence that immune complexes (IC) are involved in the pathogenesis of tissue lesions in a variety of human diseases.

Since 1972, more than 30 methods for the detection of circulating IC have been devised and used extensively. It was expected that this type of technology would provide ideal tools for the diagnosis of diseases due to immune complexes. However, these expectations have not been fully realized.

Methodological considerations

Most methods have been designed for the detection of immunologically aggregated immunoglobulins without considering the nature of the antigen(s) involved in the IC. These methods are the most widely used for clinical purposes. Some methods are based on physico-chemical differences between monomeric Ig and aggregated Ig. Precipitation in polyethylene glycol (PEG) has been widely used as a routine method. Although it may be useful to concentrate complexes, it is not specific for immune complexes since, even at low concentrations, a variety of large serum proteins are also precipitated. The quantitation of total protein or even individual proteins in PEG precipitate is not recommended as a measurement of IC levels.

Biological methods are based on the recognition of IC in humoral or cell-receptor systems. Although all of these methods detect IC they do not allow for a direct quantitation of IC proteins. Tests using Fc receptors on macrophages, K cells or platelets have been largely abandoned for two reasons: (a) high sensitivity to interfering factors, and (b) difficulty in achieving reproducibility. Although interfering factors can lead to false-positive results, it appears now that, in most cases, a positive result is likely to indicate the presence of IC when the following methods are used: Clq solid-phase or fluid-phase binding tests; conglutinin assays; monoclonal RF inhibition; Raji-cell assay. These tests have been found the most acceptable in recent WHO/IUIS collaborative studies.

Some of them (e.g. solid-phase C lq or conglutinin) can be used to detect the class of antibody present in the complex by the use of appropriate specific antisera at the final stage.

The main pitfalls of these four methods are the following:

(a) These methods will detect non-specifically aggregated Ig as well as immunologically aggregated Ig. Some of the methods require a pretreatment of the same (heating at 560C) which may induce Ig aggregation.

(b) The collection and the storage of samples for IC detection should be done with care, avoiding bacterial contamination and repeated freezing-thawing. Blood should be allowed to clot for 2 hr at 370C before separation of serum. The temperature of storage should be - 700C.

(c) Tests using Clq may be influenced by the presence of heparin, endotoxins or free DNA in the test sample.

(d) Methods using rheumatoid factors (RF) are unsuitable for IgM-containing IC and cannot be used with sera containing RF or in the presence of elevated IgG levels. It has also proved difficult to standardize RF preparations.

(e) In the case of the Raji-cell assay, false-positive results may be obtained in the presence of anti-lymphocyte antibodies. The cells require particular care in culture conditions to avoid variations in sensitivity.

(f) For the above reasons, the results of the different tests for IC may not always be directly comparable.

(g) The quantitation of IC has been done until now without comparable reference preparations.

Therefore, published results expressed in pg of complexes or as pg equivalents of heat-aggregated IgG are not comparable from one laboratory to the next. Reference preparations of aggregated IgG and of preformed IC (tetanus toxoid antigen-antibody complexes) are now available on request.

Although antigen-specific detection ofimmune complexes should be the main goal in this type of investigation, information regarding the nature of the antigen(s) involved in the in-vivo-formed IC has only been obtained in restricted clinical conditions, using methods developed for that particular purpose (e.g. microbial antigens, DNA, etc.). Information obtained through the analysis of IC purified from serum indicates that IC may often result from specific interactions between immunoglobulin molecules (RF, anti-idiotypes). Thus the presence of IC in serum samples does not imply the presence of a particular antigen of exogenous, microbial or autologous origin.

Clinical indications

The detection of IC is not essential in any clinical condition. The presence of IC in serum is not specific for an immune complex disease. IC-induced lesions (e.g. glomerulonephritis) can exist without detectable circulating IC while IC are often present in serum without evidence of typical immune-complex-associated lesions.

The detection of IC may be helpful for assessment and monitoring of disease activity in conditions such as rheumatoid arthritis and systemic lupus erythematosus. It is also of value in monitoring the effects of plasma exchange therapy. It may also have a prognostic value in some malignancies such as acute leukaemia.

In all conditions where an IC disease is suspected, a direct analysis of tissue samples (e.g. kidney, skin) should be done when possible. Such examinations cannot be replaced by the detection of circulating IC.

AUTOANTIBODIES BY INDIRECT IMMUNOFLUORESCENCE

The most widely used method for detection of autoantibodies directed against tissue antigens is indirect immunofluorescence (IIF). However, many other methods in common use provide diagnostic information by employing defined antigens. In the future, more procedures using purified antigens can be expected.

Methodological considerations and pitfalls The IF procedure involves the application of a patient's serum to a section of appropriate human or animal tissue, removal of unbound globulin by repeated washing and subsequent addition of antiserum to human immunoglobulin (prepared by immunization of an experimental animal) which has been conjugated with a fluorescent tag. The site of antibody fixation can be visualized with fluorescence microscopy. Rather than a fluorescent dye, antibody can be labelled with an enzyme such as peroxidase and appropriate cytochemical methods used to trace antibody localization. The most important variables involved in a reproducible technique are: (1) the type of substrate employed including source, method of fixation, storage and preparation, (2) duration of incubation and washing of the patient's serum, and (3) specificity and sensitivity of the antiglobulin conjugate. An essential part of each test is the incorporation of known positive and negative sera as controls.

The four groups of autoantibodies that are most requested are antibodies to nuclei, to thyroid, to mitochondria and to smooth muscle. It is possible to prepare composite blocks of several tissues processed at one time.

To test for anti-nuclear antibodies (ANA), appropriate substrates are cryostat sections of rodent liver or kidney, but human leucocytes are used in special cases. Fixed tissue culture cells are available commercially, but they are visually less satisfactory than tissue sections because they give more non-specific fluorescence. Different patients' sera may produce different patterns of nuclear staining. Antibodies producing the homogeneous pattern are mainly directed against nucleohistones. The peripheral pattern is probably due to antibodies against native DNA. The antibodies associated with speckled staining are directed against soluble nuclear antigens such as the Sm or ribonucleoprotein antigens. The nucleolar patterns are due to reaction with RNA. The substrate for demonstrating thyroid autoantibodies consists of frozen human or monkey thyroid tissue and the procedure is the same as described for ANA. At least two distinct autoantibodies can be differentiated by IIF. They are directed against the thyroid epithelial cells or colloid respectively. A positive test of patient's serum on unfixed slides appears as bright fluorescence of the epithelial cells. The autoantibody responsible for this reaction is directed to a microsomal lipoprotein of the epithelial cell. Autoantibodies reacting to colloid can be seen only when using methanol-fixed slides. These autoantibodies can also be demonstrated effectively using haemagglutination tests with red blood cells coated with respective antigens.

For the demonstration of mitochondrial antibodies, rat kidney is usually employed as substrate and immunofluorescence is seen in the cytoplasm of epithelial cells lining the ducts. Smooth muscle antibodies are generally tested with rat stomach sections as substrate.

Clinical indications

Requests for unspecified screening for autoantibodies should be discouraged. Clinicians should rather ask for precise autoantibody tests appropriate to the clinical context.

Tests for ANA are essential for the diagnosis of systemic lupus erythematosus (SLE). The occurrence of ANA in low titres is relatively common and is associated with a variety of disorders. Even sera from normal individuals show a low incidence of ANA, especially in aged populations. Therefore, the greatest use of the ANA is to exclude the diagnosis of SLE, since the vast majority of all active SLE cases are positive. Further confirmation of the diagnosis of active SLE requires the demonstration of antibodies to native (double-stranded) DNA which can be demonstrated by IIF (with Crithidia lucilleae kinetoplast) or by other techniques; the demonstration of antibodies to Sm antigen is also of great diagnostic value in this condition.

Tests for ANA are useful in the diagnosis of 'mixed connective tissue disease' (speckled pattern associated with antibodies to RNP) and the autoimmune form of chronic active hepatitis. They are also helpful in many cases of drug-induced SLE and a characteristic pattern of nucleolar stain occurs in progressive systemic sclerosis. ANA is sometimes of value in the study of family members of patients with SLE as it may lead to earlier detection of this disease.

Tests for thyroid autoantibodies are essential for the diagnosis of chronic thyroiditis and spontaneous adult myxoedema. Over 90% of thyroiditis patients have autoantibodies directed against either cell microsomal antigen, thyroglobulin, or both. A positive test, however, does not eliminate the diagnosis of such conditions as adenocarcinoma or Graves' disease, since 20% of these patients have antibodies to thyroid antigen, although titres are generally lower than in those patients with thyroiditis.

Antibodies to mitochondria are characteristic but not specific for primary biliary cirrhosis and antibodies to smooth muscle are frequently found in high titre in the sera of patients with chronic active hepatitis. Both of these groups of autoantibody are found in many other conditions, but the tests may become more useful when purified antigens are available.

Other autoantibodies of clinical interest are found in certain uncommon diseases. For instance, antibodies to the intercellular substance of stratified squamous epithelium are present in pemphigus, while a different fluorescent pattern involving the basement membrane of stratified epithelium is characteristic of pemphigoid. Antibodies to muscle striation are often detected in the sera of patients with myasthenia gravis. However, in this condition, a more useful test is the detection of antibodies to the acetylcholine receptor, which can be detected by radioimmunoassay. Autoantibodies to adrenal cortex found in chronic cases of idiopathic adrenal insufficiency or to pancreatic islets in some cases of insulin-dependent diabetes mellitus are not frequent enough to be of diagnostic value, but are useful for clinical research.

B AND T CELL DETERMINATION

A major advance in the study of the lymphoid populations was made when it was shown that they could be characterized by certain cell surface markers. This has since generated a considerable number of studies of the enumeration of T and B cells in health and disease. Although these studies have been disappointing for most clinical

purposes, they have helped in the characterization of cellular markers and in our understanding of human lymphocyte physiology. Methodological considerations and pitfalls Lymphoid cell separation. Most of the studies of human T and B cells are performed on human peripheral blood and use the Ficoll-Isopaque method for mononuclear cell separation. Such preparations contain a variable number of monocytes which it is important to distinguish from lymphocytes. This can be most easily achieved by either latex particle ingestion or peroxidase staining. It is advisable to carry out study of cell markers on freshly drawn samples of blood and to check the viability of the cells since this may influence cell surface characteristics.

T-cell markers. At the present time, two types of methods are recommended to detect all peripheral T cells: they are the formation of sheep red cell rosettes (E rosettes) and the use of T-cell-specific monoclonal antibodies.

E resetting is the most commonly employed and recommended assay for enumerating T cells. Different laboratories have reported great variability in the percentage of E rosettes in the normal population; these variations are still frequent although they have been mostly overcome as a result of the better standardization of techniques. The source of sheep red blood cells, their conservation, the presence of small amounts of serum (fetal calf serum or human AB serum) as well as the careful handling of the rosette preparations are important factors. Serum factors (like antibodies to cell surface components or lipoproteins) may interfere with rosette formation in certain conditions by coating the cells and competing with the sheep red blood cells for their binding sites. In such situations, short-term culture (1-18 hr) of the cells is usually very helpful in removing or shedding these substances.

Specific anti-T-cell antisera are now used increasingly for the detection of all T cells in peripheral blood lymphocytes and in the lymphoid organs. Such reagents are directed against the E receptor or against other common T-cell membrane determinants. The most promising and reliable reagents are monoclonal antibodies. The preferred method for the use of such antibodies is by indirect fluorescent labelling rather than by cytotoxicity, which is less accurate.

T-cell subsets were defined initially by the presence of receptors for the Fc of IgM or IgG. However, recently defined monoclonal antibodies are more reliable and accurate reagents for defining T-cell subsets.

B-cell markers. Surface membrane Ig is the most reliable B-cell marker if properly carried out. Membrane immunoglobulin (SmIg) is most commonly identified by fluorochrome-labelled anti-immunoglobulin antisera. The recommended reagents for the enumeration of B cells are antisera raised against the Fab portion and/or mixture of anti-K and -) light chain. They are commercially available but should be checked very carefully for specificity. Monospecific reagents to the various Ig chains are used for characterization of the heavy and light chains on the cell membrane and in the cytoplasm.

The following pitfalls should be emphasized: (1) involvement of Fc receptors that may bind autologous as well as the reagent's Ig. This is largely overcome through incubation and the use of labelled F(ab)2 reagents for immunofluorescence; (2) specificity and potency of reagents; (3) inadequacy of monocyte identification; (4) potential

interference by autologously reacting anti-lymphocyte antibodies, conferring positive surface staining to an otherwise SmIg-negative cell.

Anti-immunoglobulins have also been labelled by enzymes, isotopes or red cells for the determination of SmIg. Using a similar approach to that employed for T cells, antisera and/or monoclonal antibodies reacting specifically with all B cells have been described recently. Antibodies to B-cell subsets have also been reported and await further characterization.

A group of other markers present on B-cell membranes have been described. Some, such as the complement receptor and the receptor for Fc, are not specific for B cells. Therefore, procedures such as the EAC rosettes are not recommended at present for routine enumeration of B cells. However, studies of these receptors as well as that of the Epstein-Barr virus and mouse red blood cell receptors to differentiate B cells and B-cell subsets could be used for research purposes. In summary, the recommended basic methods for T and B cell determination are at present the SmIg, E rosettes and, where available, suitable monoclonal antibodies.

Clinical indications

Enumeration of T and B cells is essential in the assessment and monitoring of primary immunodeficiencies and useful in the diagnosis of secondary immunodeficiency and for the classification of lymphoproliferative disorders. It should include as large a number of reagents as possible including monospecific anti-Ig and monoclonal antibodies to lymphoid populations. In addition, study of T- and B-cell subsets may be useful in selected patients and mainly for research purposes since enumeration of B and T cells has so far not proved to be of clinical value in infectious, autoimmune, or non-lymphoid malignant diseases.

LYMPHOCYTE RESPONSE TO MITOGENS IN THE EVALUATION OF CELL-MEDIATED IMMUNITY

The investigation of cell-mediated immunity (CMI) is important in the evaluation of the host immunological competence. For this purpose, a group of in vivo and in vitro procedures are commonly used.

It is essential that these assays should be employed in an orderly fashion so as to obtain pertinent information, minimize abuse and overcome pitfalls. Delayed hypersensitivity skin testing using two or more common recall antigens (streptokinasestreptodornase, PPD, Candida, trycophyton, mumps) should be the first assay to perform. It is only following this initial stage, if the results obtained suggest possible alterations of CMI, that cell function should be explored in vitro. In addition to mitogen responses, lymphocyte response to foreign antigens and alloantigens should also be investigated.

The following remarks will deal exclusively with proliferative response to mitogens. Methodological considerations and pitfalls Proliferative response of lymphocytes to several mitogens is best measured by radioactive thymidine uptake. Mononuclear cells separated by Ficoll-Hypaque from peripheral blood in the micromethod should be used. Results are commonly expressed as total radioactivity uptake.

In order to optimize the assay, it is essential to define culture conditions, standardize the biological and commercial reagents and control the number of cells in the culture

(including concentrations of monocytes). In addition, due to the great variability inherent in the systems, the
regular use of normal controls is critical. These should consist of both controls matched for the patients as well as controls for computing the daily variation of the laboratory. Results could then be expressed as relative proliferative response index which takes into consideration the above-mentioned factors. Dose-response curves are also of importance to select for the optimal response while suboptimal concentrations of mitogens may be of advantage in the study of certain disease states such as some immunodeficiencies. In the evaluation of the proliferative response, the level of background should be taken into account since it may clearly affect the final results. The use of overnight culture prior to the addition of the mitogen may help to explain depressed proliferative responses secondary to inhibitory factors. The most commonly used mitogens are phytohaemagglutinin, concanavalin A and pokeweed extract; the first two are mainly T-cell mitogens whereas the latter is a T- and B-stimulator. It is, Sensitization with 1-chloro-2,4-dinitrobenzene (DNCB) is at present the only way to explore the primary response in vivo but should be performed only in selected patients. However, likely that these as well as some other mitogens stimulate poorly defined subpopulations of T and B cells.

Clinical indications

The assessment of the lymphocyte response to mitogens is not indicated for routine use and should be used rather selectively. Abnormal results from single isolated CMI assays are clinically meaningless and will not necessarily indicate abnormalities of CMI in the patients.

Evaluation of cell-mediated immunity is essential in assessing a suspected or proven primary immunodeficiency. Evaluation of CMI is useful in (a) assessment of secondary immunodeficiencies, including those associated with chronic infections and (b) monitoring and evaluating the application of immunostimulatory therapy. It may be helpful for research purposes in diseases with possible impairment of immune function, such as autoimmune processes, cancer and in evaluation of the effect of immunosuppressive drugs.

Issues were considered during the class

The approach of using a universal method of evaluation:

- Immunological analysis in conjunction with the assessment of the clinical picture and the details of physical and instrumental examinations
- A comprehensive analysis of more informative than the evaluation of each separate
- Analysis of immunological dynamics more informative as to the diagnostic and prognostic respect
- The absence of abnormalities in Immunogram the presence of clinical disease should be considered atypical response of the immune system
- Laboratory evaluation of the immune system - not the only, albeit a very important stage of disease diagnosis, monitoring its progress and effectiveness of treatment.

The approach of using different sets of methods and tests:

- Evaluation stage antigen recognition (the study of the expression of the T cell receptor on lymphocytes, antigen presentation process, the number of adhesion molecules

(integrins, adhesins, etc.). Cells in a mixed lymphocyte culture, genetic analysis of alotypes HLA)
- Evaluation stage activated lymphocytes (lymphocyte phenotyping activation markers CD23, CD25, CD69, HLA-DR) by PHA stimulation, identification of secondary messengers (cAMP, cGMP), the study of immune cells in response cytokine signals)
- Evaluation stage of proliferation of lymphocytes (the study of lymphocyte response to mitogens, specific antigens, growth factors)
- Evaluation stage of differentiation of lymphocytes (effector function) - the study of production of antibodies, cytotoxic function of T lymphocytes, natural killer cells, cytokine production
- Evaluation of the regulation of the immune response (grade helper and suppressor / regulatory functions of lymphoid cells, the analysis of functional properties of T-helper 1 and 2 Gogo types, their production of cytokines.

Th1 and Th2 Cytokine Blood Test Panel

For those with a confirmed autoimmune condition, the Th1 Th2 test is possibly the most important test. The test points out imbalances in the immune system by looking at cytokines, proteins that the immune system relies on to communicate.

Bad communication results in complications for those with autoimmune conditions. The information this test provides can help your doctor develop a strong and effective treatment plan for you, especially when seeking alternative medicine support.

How Can the Th1 Th2 patient?

The immune system works like a seesaw. On one side you have Th1 cytokines that initiate the first line of defense. On the other side you have Th2 cytokines which help product antibodies to protect you from future invasions. However, when one side goes up, the other side goes down. This can contribute to a weak immune system and potentially promote autoimmune conditions.

Running this test will help figure out where the imbalance is. Because certain botanicals used in natural medicine can boost Th1 cytokines and Th2 cytokines, this test can help you and your doctor develop an effective plan to help balance a weak immune system and turn the volume down on autoimmune attacks.

For those with autoimmune concerns, it is suggested to run the CD4 CD8 ratio test along with this Th1/Th2 test.

Final part: Test-control
Initial knowledge level

1. Which of the following statements about smallpox is FALSE?
A) Powdered scabs from smallpox patients were used to induce immunity as early as 1,000 A.D.
B) Smallpox killed or left scars on most of its victims.
C) Edward Jenner used material from cowpox lesions to vaccinate against smallpox.
D) Smallpox only infects humans.
E) All statements are true.
2. Which of the following statements about vaccine in a virus is FALSE?
A) Vaccinia virus has been genetically engineered to make experimental vaccines.
B) Vaccinia virus causes smallpox.
C) The origin of the vaccinia virus that we now have is uncertain.
D) Vaccinia virus may be a hybrid of cowpox and smallpox viruses.
E) All statements are true.
3. An effective vaccine against smallpox has been available since before 1796, yet smallpox was not officially declared eradicated until
A) 960 A.D.
B) 1977.
C) 1979.
D) 2000.
E) possibly sometime in the future.
4. The change from negative serum without specific antibodies to serum positive for specific antibodies is called
A) Immunoassay.
B) Serology.
C) Immunochange.
D) Seroconversion.
E) Immunoconversion.
5. Which of the following statements is FALSE?
A) It takes at least 7-10 days to produce detectable antibodies against an antigen.
B) Blood can be tested for either antibodies or antigens.
C) Thin sections of tissue are most commonly used to test for the presence of antigens.
D) Serial dilutions are used to determine the amount of antibodies in a serum sample.
E) All of the above are true.
6. Which of the following statements about monoclonal antibodies is FALSE?
A) They are specific for one epitope.
B) They are produced by cells that replicate for a limited time.
C) They are produced by hybridomas.
D) They are of the same immunoglobulin class and have the same variable regions.
E) All of the above are true.

7. Precipitation reactions require
A) excess antigen.
B) excess antibody.
C) an optimal proportion of antibodies and antigen.
D) complement.
E) double diffusion gel.
8. Which of the following accurately describes possible results of immunoelectrophoresis?
A) A line of precipitate forms at the location of antigen that is recognized by antibodies.
B) A line of precipitate forms where each antigen meets an antibody in the area of optimal proportions.
C) A person that lacks immunoglobulins will have not lines of precipitation with anti-human antibodies.
D) A myeloma patient will have a heavy thick line for a single class of immunoglobulin.
E) All are correct.
9. Which of the following statements about agglutination is FALSE?
A) Agglutination reactions are easier to see than precipitation reactions.
B) Hemagglutination inhibition assays can be used to detect antibodies against viruses that cause hemagglutination.
C) Agglutination reactions involve large particles.
D) In indirect agglutination tests, antibodies are first attached to latex beads or other particles.
E) All of the above are correct.
10. All of the following tests involve antigens fixed to a solid support except
A) ELISA.
B) Western blot.
C) Indirect fluorescent antibody test.
D) Complement fixation test.
E) All of the above.

11. Receptors associated with innate immunity recognize microbes by detecting:
A) insulin.
B) pathogen associated molecular patterns (PAMPs).
C) Fc's.
D) complement.
E) none of the above.

12. Immunoglobulin classes must distinguished by the type of:
A) light chains they possess.
B) carbohydrate on their light chains.
C) constant regions in their light chains.
D) heavy chains they possess.

E) none of the above.

13. The variable regions in the light chains participate in:
A) Fc receptor binding.
B) epitope binding.
C) affinity of the complement receptors.
D) interaction of the Fab with cytokines.
E) none of the above.

14. IgD participates in antigen recognition by:
A) immature T cells.
B) NK cells.
C) macrophages.
D) B cells.
E) none of the above.

15. Antibody affinity is not determined by the amino acid sequence in:
A) the constant regions of the immunoglobulin molecule.
B) the variable regions of the immunoglobulin molecule.
C) the Fc of the immunoglobulin molecule.
D) the J-chain.
E) a, c and d.

Final knowledge level

1. Which of the following assays is the principle clinical use for the identification of antibodies against HIV?
A) Radioallergosorbent test.
B) Indirect fluorescent antibody test.
C) Western blot.
D) Complement fixation test.
E) Direct agglutination test.
2. Which of the following techniques uses a laser beam?
A) ELISA.
B) Fluorescence-activated cell sorter.
C) RIA.
D) Western blot.
E) All of the above.
3. What type of immunity is induced by the hepatitis B vaccine?
A) naturally acquired active immunity.
B) naturally acquired passive immunity.
C) artificially acquired active immunity.
D) artificially acquired passive immunity.

E) none of the above.
4. Which of the following statements about attenuated vaccines is FALSE?
A) They are used to protect against chickenpox, mumps, measles, and rubella.
B) They are not safe for pregnant women.
C) They may revert to virulent strains.
D) If given orally they may induce mucosal immunity.
E) All of the above are correct.
5. Inactivated whole agent vaccines are used to protect against all of the following diseases except
A) cholera.
B) plague.
C) tetanus.
D) anthrax.
E) hepatitis A.
6. Which of the following types of vaccines does not usually require repeated booster doses?
A) inactivated whole agent.
B) subunit.
C) attenuated.
D) recombinant.
E) toxoid.
7. Vaccines are currently under development for all of the following except:
A) HIV.
B) cancer.
C) hepatitis B.
D) genital herpes.
E) All of the above.
8. All of the following are promising new types of vaccines currently under development except:
A) edible vaccines.
B) DNA vaccines.
C) peptide vaccines.
D) antibiotic vaccines.
E) All of the above
9. All of the following are tests for humoral immunity except:
A) mitogen test.
B) precipitation test.
C) agglutination test.
D) immunofluorescence test.
E) Western blot.
10. Which of the following statements about monoclonal antibodies is FALSE?
A) They have the same constant region.
B) They are used in pregnancy tests.
C) They have the same variable regions.

D) They are naturally produced by an animal in response to immunization with a single antigen.
E) Monoclonal antibodies from a mouse are recognized as foreign proteins by humans.

11. Which of these is not associated with adjuvants?:
A) forms an antigen depot.
B) provides non-specific T cell stimulation.
C) activates antigen-presenting cells.
D) activates the complement cascade.
E) none of the above.

12. IgG binding to neutrophils cells is mediated by:
A) Fc-dependent cellular homing mechanisms.
B) sensitization of Mast cells and basophils.
C) Fc receptors specific for IgG.
D) ICAM's.
E) none of the above.

13. Complement damage is generally limited to the immediate area in which complement is
activated because of the:
A) short half-lives of the activated complement components and their rapid inactiva- tion.
B) very low concentrations of the inactivated complement components in serum.
C) the inability to activate the system in the presence of IgG antibodies.
D) once activated, the destructive activities of complement are non-specific.
E) none of the above

14. Sensitization to foods is minimized by secretory IgA antibodies by:
A) The inflammatory response that occurs in the presence of food, these antibodies and
complement.
B) Destroying the antigen presenting cells that would normally present the food antigens to T cells in the gut.
C) Blocking the penetration of intact food products into the gut.
D) All of the above
E) None of the

15. The location of complement activation is determined by:
A) the location of Fc receptors.
B) the location of dendritic cells.

C) the location of specific antibody/antigen complexes.
D) b and c.
E) none of the above.

CORRECT ANSWERS
Initial knowledge level:
1.E; 2.B; 3C; 4.D; 5.C; 6.B; 7.C; 8.E; 9.E; 10.D; 11.B; 12.D; 13.B; 14.D; 15.E
Final knowledge level:
1.C; 2.B; 3.C; 4.E; 5.C; 6.C; 7.C; 8.E; 9.A; 10.E; 11.D; 12.C; 13.A; 14.C; 15.C

Structure of class.
Plan and organizational structure of the lesson:
Preparatory stage: organization of lesson and test control of incoming level of knowledge (5 academic hours or 225 minutes)

№	Content	Level of learning	Methods of the learning control	Materials of the methodological support	Time, min.
1.	The preparatory phase Control input level of knowledge, skills.	I	1. Front rapid survey	Tests. Scheme.	25
2.	The main stage - Collection of immunological and allergic history - Clinical and instrumental examination of the patient - Laboratory methods (general clinical and immunological) survey - Assessment of survey results Resolution of situational problems.	II II II II	2. Individual oral examination. 2. Test control.	1.Tables. 2. Folio-grams. 3. Tests I-III 1. 2. Slideshow.	180
3.	The final stage Monitoring and adjustment of professional knowledge, skills and abilities: basic principles-purpose of routine and specific immunological diagnostic	III III	1. Testing. 2. Solving custom applications. 4. Oral examination.	1. Tests III-IV levels. 2. Situational problems.	15
4.	To sum up the lessons. Homework for the next topic.				2 3

Educating tasks and control (added)
Self student's work:

1. Describe an immunogram of the patients with acute infectious diseases.
2. Describe an immunogram of the patients with chronic infectious diseases.
3. Describe an immunogram of the patients with acute bacterial diseases.
4. Describe an immunogram of the patients with chronic bacterial diseases.
5. Describe an immunogram of the patients with acute fungal diseases.
6. Describe an immunogram of the patients with chronic fungal diseases.

Main theoretical aspects of theme.

1. Specific immunological tests in patients with different types of immune insufficiency.
2. Specific immunological tests in patients with allergic diseases.
3. Specific immunological tests in patients with autoimmune diseases.
4. Specific immunological tests in patients with cancer.
5. Specific immunological tests in patients with chronic inflammation.

6.1. Questions:
1. Milestones laboratory immunology.
2. Immunological and allergy history.
3. Basic approaches to clinical examination of organs and systems in patients with immune disorders and allergies.
4. Methods for assessing cellular factors of nonspecific (innate) immunity.
5. Methods for evaluation of humoral factors of nonspecific (innate) immunity.
6. Methods for assessing cellular factors specific (adaptive) immunity.
7. Methods for assessing humoral factors specific (adaptive) immunity.
8. Methods for in-depth evaluation of the immune system.
9. Modern laboratory technology.
10. Approaches to the interpretation of laboratory immunological assays.

6.1. Practical skills.
- To be able to collect immunological and allergy history
- To be able to objectively examine the patient's organs and systems under conditions of allergy and immunopathology
- To be able to justify the feasibility of implementation of patient immunological laboratory investigations and give an overall evaluation.

References:

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3. Roitt's Essential Immunology, Includes Desktop Edition. Peter J. Delves, Seamus J. Martin, Dennis R. Burton, Ivan M. Roitt. Wiley-Blackwell; 12 edition (2011). – 560 p.
4. How the Immune System Works, Includes Desktop Edition. Lauren M. Sompayrac. Wiley-Blackwell; 4 edition (2012). – 152 p.
5. Lecture Notes: Immunology, 6th Edition. Ian Todd, Gavin Spickett. Wiley-Blackwell (2011). – 480 p.
6. Essentials of Clinical Immunology, 6th Edition. by Helen Chapel, Mansel Haeney, Siraj Misbah, Neil Snowden. Wiley-Blackwell (2014). – 376 p.
7. Immunology: A Short Course, 6th Edition. Richard Coico, Geoffrey Sunshine. Wiley-Blackwell (2009) . – 416 p.

METHODICAL INSTRUCTION

Practical class №3

1. **THEME. IMMUNOTROPIC THERAPY. VACCINATION AND ITS COMPLICATIONS** (5 academic hours).

Background: Actuality: active immunization is the main method of preventing many infectious diseases both in childhood and in adulthood. It is used for active immunization routine and emergency prevention of a number of infectious diseases. The rate of dangerous infections such as diphtheria, mumps, measles has increased dramatically due to lower collective immunity. Since 1995 the onset of the TB epidemic in Ukraine has been officially announced. When the therapy is not provided on time or provided on the late stage - severe, sometimes life-threatening human complications can develop.

The importance of Immunotropic therapy is the ability of correct implementation of immunotropic drugs in case of already working infection and the presence of the lowered immune reactivity, which can be a basis for the development of various pathological processes.

2 Aim. - *Academic:* level based on theoretical training material. Students should familiarize themselves with the history of immunology, know physico-chemical basis of immunological laboratory techniques and equipment that is used for their implementation.

- *Professionally-oriented:* Students should understand the need to assess the patient's immune system in prognosis of the underlying disease, be able to provide clinical and laboratory assessment, make diagnosis of the immune system of the patient.

- *Educational:* form students' sense of responsibility for the timeliness and appropriateness of professional activities and the need to continually improve their skills.

3. The purpose of the lesson:

2. See the historical aspects of vaccination.
3. Know the types of vaccines and their purpose.
4. Assess the state of health of the person vaktsynuyetsya and determine the need for and the possibility of vaccination based on history, clinical picture and laboratory immunological studies
5. Assess immunization of patients with immune deficiencies.
6. Be able to diagnose post-vaccination complications and therapeutic measures, to know when they occur .
7. To learn the basic concepts of immunotropic therapy: immunosuppression, immunostimulation, immunomodulation, immunotherapy, immune reconstruction, immunization, specific vaccination immunorehabilitation.
8. Know the groups of immunotropic drugs and basic commonly used drugs of these groups, their mechanisms of action and indications for use.
9. Be able to identify the clinical indications for the use of immunotropic therapy.

4. Materials: Equipment to run powerpoint presentation

Main books. Short information due to the topic.

5. Interdiscipline integration

Subjects	To know	To be able to
1	2	3
Biology	The evolution of the immune system of living organisms	_
Physiology	Principles of the thymus, adrenal system cytokines	Be able to identify Immunotropic preparations according to the rhythm of the main circulating endocrine glands and thymus. Specify the basic methods of evaluating immune system.
Anatomy	The bodies immune system	Specify the bodies immune system
Histology	Cells of the immune system	To distinguish microscopically between cells of the immune system
Pathophysiology	Immunological reactivity. Mechanisms of immunopathological processes. The concept of allergy.	Interpret changes in overall performance levels (leucogram, protein grams) under immunopathology. Simulate anaphylaxis.
Microbiology	Antagonists immunological protection	To explain the basic parameters characterizing immune Antagonists
Propaedeutic Faculty, Hospital Therapy	The course of systemic diseases, principles of therapy	Assess the patient's condition, identify systemic disease, prescribe treatment
Pediatrics	Classification of primary immunodeficiencies and secondary immunodeficiencies, their clinical signs	Determine the need for replacement of the immunostimulatory or immunosuppressive reconstructive therapy.
Overall, Departmental, Hospital Surgery	Postoperative immunodependent complications	Assess the patient's condition, to identify infectious complications and prescribe treatment

Pharmacology	The main groups of immunotropic drugs and mechanisms of action	Identify a group of drugs with Immunotropic action based on immunopathology. Calculate doses of medication, prescribe drugs/medications.
Reanimation	Multiorgan syndromes	Assess the patient's condition, prescribe treatment
Infectious Diseases	Type of vaccine	Vaccination, vaccination calendar
Epidemiology	The prevalence of infectious diseases. The modern epidemic.	Evaluate the effectiveness of vaccination to prevent the spread of infectious diseases

6. Student has to know:

1. Convince the need to maximize vaccination coverage for the benefit of society as a whole and each individual in particular.
2. Develop a sense of responsibility in students to conduct vaccination activities as a factor in preventing epidemics of certain infections.
3. Develop an understanding of the importance of a professional, to identify at-risk population and warning them of post complications.
4. Develop a sense of responsibility for determining the need of immunotropic therapy, timeliness of its purpose, side effects.
5. Interdisciplinary integration

7. Study questions.

1. Immunostimulation, immune modulation, immunosuppression definition.
2. Classification of immunotropic drugs.
3. Overview of antineoplastic agents
4. Immunosuppressants - clinical applications
5. Mechanisms of action of corticosteroids on the immune system, side effects .
6. Effects of Corticosteroids on the Immune System
7. Therapy with cytokines, a group of drugs, indications for use.
8. IVIG, the benefits of the use, indications, dosage.
7. Definition of vaccination. Vaccines, their types and applications.
8. Historical aspects of vaccination.
9. Peculiarities of vaccination by immunization schedule.
10. Formation of immune response and evaluation of vaccination.
11. Risk-group vaccination. Peculiarities of vaccination of patients with immune deficiencies
12. Contraindications to vaccination. Vaccination complications and their treatment

Main part

Immunotherapy is a medical term defined as the "treatment of disease by inducing, enhancing, or suppressing an immune response". Immunotherapies designed to elicit or amplify an immune response are classified as activation immunotherapies, while immunotherapies that reduce or suppress are classified as suppression immunotherapies.

Immunotherapy is a type of treatment that uses the body's immune system to combat disease. The therapy is administered through synthetic immune system proteins or by helping the body's immune system to work more efficiently. These forms of therapy are often used in addition to other treatments to help cancer patients.

The immune system is responsible for protecting the body against harmful germs. It is comprised of organs, cells, and substances that work together to achieve the goal of a healthy body. Cells in the immune system and the substances created by these cells move throughout the body, constantly protecting it from harmful germs.

What Is Immunomodulatory Therapy?

Immunomodulatory therapy consists of a series of three types of treatments for diseases that plague the human immune system, and it is more often referred to as just immunotherapy. The three types of immunomodulatory therapy strategies involve the use of immunosuppressant drugs to scale back the natural action of the immune system or the use of immunostimulant drugs to enhance its response, and the use of tolerogens which condition the immune system to tolerate tissue such as that of transplanted organs. Each class of treatments is designated for specific immune system problems. Immunosuppressant drugs and tolerogens are used together to treat autoimmune diseases like multiple sclerosis (MS) and organ transplants where the body is attacking its own tissue. Immunostimulant drugs are given to enhance the immune system in cases where it is weakened, such as with cancer, AIDS, and other life-threatening infections.

At times the ever-vigilant immune system is unable to identify cancer cells as foreign invaders. For this reason, cancers are able to slip through the line of defense and enter the body. Immunotherapy is one way that doctors try to help the body fight against cancer.

In order to help the body recognize cancer cells, doctors prescribe immunotherapy to some patients. Two principle types of immunotherapy exist: active and passive. Active immunotherapy uses the immune system to combat disease while passive immunotherapy uses synthetic substances to help the immune system.

What Is an Immunostimulant?

An immunostimulant triggers increased immune activity. Some, such as vaccines, target particular proteins; these are termed specific immunostimulants. Others are nonspecific and work on the immune system as a whole or general systems within it to increase the immune response. The body produces a number of these compounds naturally and they are also produced in synthetic settings and by some natural organisms.

The immune system includes a complex network of systems that function together to protect the body from infectious agents. Immunostimulants can trigger the immune

system to kick into action to respond to a threat. With vaccination, for example, the immune system learns to recognize specific proteins and attack them, thus ensuring that when a patient is exposed to an infectious agent, the immune system will act. Nonspecific immune stimulants can boost overall immune activity.

Vaccines are often given with a compound called an adjuvant. These act as immunostimulants, increasing the body's response to the vaccine. In addition to increasing the chances that the vaccine will be effective, the immunostimulant also reduces the amount of material needed in a vaccine, which makes it safer for the patient.

Immunostimulants are substances that modulate the immune system by stimulating the function of one or more of the system's components. There are two types. Specific immunostimulants, such as vaccines, stimulate an immune response to one or more specific antigenic types. In contrast, non-specific immunostimulants do not have any antigenic specificity but can act as general stimulants that enhance the function of certain types of immune cells.

In terms of immunostimulant substances used in the general human population, it is vaccines which are most commonly employed. Vaccines are used to stimulate a protective immune response to antigens from specific pathogens. The influenza vaccine, for example, uses several antigens from different strains of the flu virus. People who are vaccinated are then protected against infection from those particular strains.

Another type of immunostimulant called an adjuvant is often used in conjunction with vaccines. Adjuvants are a type of non-specific immunostimulant. Administering an adjuvant along with a vaccine helps generate a stronger protective response to the antigens in the vaccine, providing a better degree of protection against the pathogen. One example of an adjuvant is alum, which is often used in human vaccines. Alum is made from aluminum salts such as aluminum hydroxide and aluminum phosphate.

Antineoplastic chemotherapy is an important component of small animal practice and is routinely used for selected tumors of horses and cattle. Effective use of anti-neoplastic chemotherapy depends on an understanding of basic principles of cancer biology, drug actions and toxicities, and drug handling safety.

Tumor Growth and Response to Chemotherapy

The fundamental biochemical and genetic differences between cancer cells and normal cells are areas of active investigation as these divergences are not fully understood. None of the empirically developed conventional antineoplastic drugs appears to act on a process or component that is entirely unique to cancer cells. Newer therapies are evolving that specifically target markers or pathways that are unique to particular cancers. However, the mainstay of cancer therapy continues to be traditional chemotherapy. Clinically useful drugs achieve a degree of selectivity on the basis of certain characteristics of cancer cells that can be used as pharmacologic targets. These characteristics include rapid rate of division and growth, variations in the rate of drug uptake or in the sensitivity of different types of cells to particular drugs, and retention in the malignant cells of hormonal responses characteristic of the cells from which the cancer is derived, eg, estrogen responsiveness of certain breast carcinomas.

Aspects of normal cell growth and the cell cycle provide the rationale for the successful application of antineoplastic chemotherapy. In the S phase, DNA synthesis occurs; the M phase begins with mitosis and ends with cytokinesis; and the G_o phase is a dormant or nonproliferative phase of the cell cycle. Tumor doubling time is related to the length of the cell cycle and the growth fraction (the proportion of a population of cells undergoing cell division). Antineoplastic agents can be classified according to a number of schemes relative to effects at different stages of the cell cycle. In the simplest sense, cycle-nonspecific agents are considered to be lethal to cells in all phases of the cell cycle. Cells are killed exponentially with increasing drug levels, and the dose-response curves follow first-order kinetics. Phase-specific agents exert their lethal effects exclusively or primarily during one phase of the cell cycle, usually S or M; the greater the rate of cell division, the more effective the drug. The G_o phase of the cell cycle is important, not as a target for chemotherapeutic agents, but as a time during which dormant tumor cells can escape or repair the effects of drug therapy.

Principles of Antineoplastic Chemotherapy

The decision to use antineoplastic chemotherapy depends on the type of tumor to be treated, the stage of malignancy, the condition of the animal, and financial constraints. Chemotherapy can be used as an adjuvant to surgery and irradiation and can be administered immediately after or before the primary treatment. Neo-adjuvant therapy is administered before surgery or irradiation and is intended to improve the effectiveness of the primary therapy by possibly decreasing tumor size, stage of malignancy, or presence of micro-metastatic lesions. Responses to cancer chemotherapy can range from palliation (remission of secondary signs, generally without increase in survival time) to complete remission (in which clinically detectable tumor cells and all signs of malignancy are absent). The percentage and duration of complete remissions are criteria for the success of a particular chemotherapeutic protocol.

Effective clinical use of antineoplastic drugs depends on the ability to balance the killing of tumor cells against the inherent toxicity of many of these drugs to host cells. Because of their narrow therapeutic indices, dosages for antineoplastics are frequently calculated based on body surface area (BSA) rather than body mass. However, evidence suggests that small dogs and cats may best be treated based on body weight to avoid overdosage. This is especially true if the primary toxicity is bone marrow suppression. Apparently, BSA does not correlate well with either stem cell number in the bone marrow or resulting hematopoietic toxicity. Correlation is better between body weight and these toxicities. Antineoplastic agents can be administered by almost any route including PO, IV, SC, IM, topical, intra-cavitary, intralesional, intravesicular, intra-thecal, or intra-arterial. The route chosen depends on the individual agent and is determined by drug toxicity; location, size, and type of tumor; and physical constraints.

Antineoplastic agents are administered in various combinations of dosages and timing; the specific regimen is referred to as a protocol. A protocol may use 1 or as many as 5 or 6 different antineoplastic agents. Selection of an appropriate protocol should be

based on type of tumor, grade or degree of malignancy, stage of the disease, condition of the animal, and financial constraints. Preferences of individual clinicians for treatment of specific neoplastic conditions may also vary. Regardless of the protocol chosen, a thorough knowledge of the mechanism of action and toxicities of each individual therapeutic agent are essential.

Combination antineoplastic chemotherapy offers many advantages. Drugs with different target sites or mechanisms of action are used together to enhance destruction of tumor cells. If the adverse effects of the component agents are different, the combination may be no more toxic than the individual agents given separately. Combinations that include a cycle-nonspecific drug administered first, followed by a phase-specific drug, may offer the advantage that cells surviving treatment with the first drug are provoked into mitosis and, therefore, are more susceptible to the second drug. Another advantage of combination therapy is the decreased possibility of development of drug resistance.

Special considerations associated with administration of antineoplastic drugs include evaluation of the animal's quality of life, medical and nutritional support, control of pain, and psychological comfort for the owner. Many owners who choose to treat neoplasia in their pets have experienced cancer themselves or have been involved with individuals or family members who have had cancer. Discussion of neoplasia in pets should be handled tactfully and should provide the owners with appropriate information for decision-making.

Resistance to Antineoplastic Agents

Failure to respond, or resistance to antineoplastic agents, can be seen for several reasons. Pharmacokinetic resistance is seen when the concentration of a drug in the target cell is below that required to kill the cell. This may be due to altered rates of drug absorption, distribution, bio-transformation, or excretion. In addition, marginal blood flow to a tumor may not provide sufficient drug, resulting in inadequate therapeutic drug concentrations and the potential for creation of a population of quiescent, less susceptible cells. Cytokinetic resistance is seen when the tumor cell population is not completely eradicated; this may be a result of dormant tumor cells, dose-limiting host toxicity associated with drug therapy, or the inability to achieve a 100% kill rate even at therapeutic drug dosages. Resistance can also develop via biochemical mechanisms within the tumor cell itself that block transport mechanisms for drug uptake, alter target receptors or enzymes critical to drug action, increase concentrations of normal metabolites antagonized by the antineoplastic drug, or cause genetic changes that result in protective gene amplification or altered patterns of DNA repair. Acquired multidrug resistance can result from amplification and overexpression of a multidrug resistance gene. This gene encodes a cell transmembrane protein that effectively pumps a variety of structurally unrelated antineoplastic agents out of the cell. As intracellular drug concentrations decline, tumor cell survival and resistance to therapy increase.

Patterns of Toxicity

Antineoplastic agents that act primarily on rapidly dividing and growing cells produce multiple side effects or toxicities, including bone marrow or myelosuppression, GI complications, and immune suppression. Patterns of toxicity may be either acute or delayed. Acute vomiting may develop during the administration of an emetogenic drug or within 24 hr after the administration of chemotherapy, probably from direct stimulation of the chemoreceptor trigger zone. Several available drugs are aimed at preventing these toxicities, including dolasetron, ondansetron, and maropitant citrate. Dolasetron and ondansetron act as serotonin receptor antagonists that work centrally on the brain to prevent emesis. Maropitant citrate is an oral or subcutaneous FDA-approved medication for treatment of acute nausea or vomiting in dogs. It inhibits both central and peripheral vomiting pathways by blocking neurokinin-1 receptors to prevent the activation of the emetic center. Administration of oral antiemetics may be indicated for delayed GI toxicities that can occur 3–5 days after chemotherapy administration. Common antiemetic therapy includes metoclopramide, which functions through direct antagonism of central and peripheral dopamine receptors. Metoclopramide has the added benefit of stimulating motility of the upper GI tract without stimulating gastric, biliary, or pancreatic secretions. This can be useful in dogs that develop ileus secondary to vincristine administration. Neurokinin-1 receptor antagonists are routinely used in human oncology to treat delayed emesis, and they are under investigation for this purpose in veterinary oncology. Allergic reactions and anaphylaxis may also be of concern with selected drugs and can be treated with antihistamines or corticosteroids as needed. In more severe cases, epinephrine and IV fluids may be indicated. Other delayed toxicities may develop days to weeks after antineoplastic therapy. Myelosuppression, a common delayed toxicity, can be life-threatening due to the increased risk of infection associated with neutropenia. Less commonly, anemia and increased risk of bleeding associated with thrombocytopenia may be seen. Other important delayed toxicities include tissue damage associated with extravasation of selected drugs and alopecia caused by hair follicle damage, particularly in nonshedding breeds with continuous hair growth. Adverse effects on spermatogenesis and teratogenesis may be of concern in breeding animals. Chemotherapy-induced stomatitis or ulcerative enteritis are rare events in dogs and cats. Prevention and management of toxicities are crucial to successful antineoplastic therapy. Collection of an adequate database before treatment can identify potential problems so that contraindicated drugs can be avoided. Several antineoplastic agents should not be used in the presence of specific organ impairment. For example, doxorubicin should not be used in dogs with certain cardiac abnormalities that impair left ventricular function, and cisplatin is contraindicated in animals with impaired renal function. When a drug is chosen, supportive or preventive therapy aimed at reducing toxic side effects may be required. Potential myocardial damage from doxorubicin may be avoided with the administration of dexrazoxane, a free radical inhibitor. Active diuresis should accompany administration of nephrotoxic agents (eg, cisplatin). Administration or availability of appropriate antihistamines may be indicated with L-asparaginase and doxorubicin therapy. The availability of recombinant products is an additional resource for managing myelosuppression and immunosuppression induced

by antineoplastic chemotherapy. Recombinant human (rhG-CSF) and canine (rcG-CSF) granulocyte colony-stimulating factor have been used effectively in management of cytopenias induced by chemotherapy and radiation therapy. Administration of rcG-CSF results in a rapid, significant increase in neutrophil numbers that is sustainable as long as the factor is administered. Neutrophil counts drop quickly when therapy is discontinued. Neutrophil phagocytosis, superoxide generation, and antibody-dependent cellular cytoxicity all increase with G-CSF treatment. Longterm (>2–3 wk) or repeated use of recombinant human products should be avoided in dogs and cats as it can result in anti-factor antibody formation and a subsequent decline in targeted cell numbers. Prophylactic antibiotics have been shown to reduce hospitalization rates and death in human cancer patients receiving chemotherapy. These are occasionally used in veterinary medicine to reduce the occurrence or severity of hematologic and nonhematologic complications that can result from the administration of particular chemotherapy agents.

Biologic Response Modifiers in Cancer Therapy

In recent years, a number of alternative modes of cancer therapy have been investigated. Foremost among these has been the development of biologic response modifiers aimed at enhancing innate anti-tumor defense mechanisms of the host. Nonspecific immunomodulators, including intact bacteria or bacterial cell components, acemannan, IL-2 or IL-12, interferon alpha, levamisole, and cimetidine, have variable efficacy to enhance immune responsiveness and improve outcomes after surgery or antineoplastic chemotherapy. Liposome-encapsulated muramyl tripeptide phosphatidylethanolamine is perhaps the best studied nonspecific immunomodulator in veterinary medicine. This synthetic bacterial wall component has been used effectively with chemotherapy to increase survival in dogs with splenic hemangiosarcoma and osteosarcoma. Another class of biological response modifiers includes NSAID that may be either nonselective or selective cyclooxygenase-2 (COX-2) inhibitors, such as aspirin and piroxicam or deracoxib, firocoxib, and meloxicam, respectively. These drugs directly inhibit COX-2 enzyme activity, which is frequently over-expressed by many tumors. Piroxicam has been the drug most researched in dogs, but any of the newer NSAID with more COX-2 selective inhibition could yield equal or improved effects. These drugs may work by reducing cell proliferation, increasing apoptosis, inhibiting angiogenesis, and modulating immune function. The clinical usefulness has been demonstrated in canine transitional cell carcinoma and other tumor types in dogs and cats. A novel or nontraditional means of administering chemotherapy, known as metronomic or antiangiogenic dosing, consists of oral drugs that are administered at a continuous, often daily, low dose. This chemotherapy protocol affects the tumor vasculature and antitumor immunosuppression rather than the tumor itself. Preliminary studies suggest this may be a promising alternative to maximally tolerated doses of conventional chemotherapy administered on an episodic basis. The intended outcome of low-dose chemotherapy is disease stabilization. Limited side effects are an added benefit. The development of a therapeutic vaccine to stimulate active immunity against cancer has long been a goal in both human and veterinary oncology. This recently became a reality with the introduction of a canine

melanoma vaccine. The vaccine exploits the immune response induced by human tyrosinase, an enzyme in the pathway of melanin formation. The antibodies and T-cell responses produced by xenogeneic tyrosinase cross-react with the tyrosinase overexpressed on canine melanoma cells. Initial studies reported prolonged survival in dogs with advanced stage oral malignant melanoma treated with radiation therapy or surgery of the primary tumor, followed by vaccine administration. The vaccine is licensed by the USDA. Another investigational melanoma vaccine incorporates the administration of either nonviable canine melanoma cells or cells expressing a common melanoma antigen (hgp-100) with a gene that acts as a pleuripotent immune cytokine granulocyte-macrophage colony stimulating factor (GM-CSF). The goal of this therapy is to generate an immune response against the cancer cells. Development of lymphokines and cytokines (eg, interleukins, interferon, and tumor necrosis factor) for clinical use in cancer patients has long been an attractive goal. The potential of these potent immunomodulators has not been fully realized, largely due to their toxicity. These agents are not commonly used in veterinary medicine. Passive immunotherapy using mono-clonal antibodies has grown substantially in human oncology in recent years. Monoclonal antibodies may attach to specific antigens on cancer cells, thereby either marking the cancer cells for destruction by the immune system or impairing functional pathways within the neoplastic cells. Furthermore, monoclonal antibodies may be conjugated to other antineoplastic agents (such as chemotherapy agents, radionuclides, or other toxins) to allow for more targeted delivery of cytotoxic therapy to cancer cells while sparing normal tissues. However, such therapies are not currently marketed for veterinary use and may not have activity across different species. Beyond enhancement of immune recognition and control of neoplastic disease, new therapies attempt to exploit specific pathways that are aberrantly or overexpressed in neoplastic cells. Paramount among these is angiogenesis, because tumors must develop their own vascular supply if they are to grow beyond a few millimeters in diameter. Various drugs, such as angiostatin, thrombospondin-1, and matrix metalloproteinase inhibitors, have been investigated with varying results. Specific angiogenesis inhibitors for veterinary patients are not yet commercially available, although metronomic chemotherapy cocktails are the most practical approach to antiangiogenic therapy at this time. Targeting of specific pathways that are aberrant or dysregulated in cancers has yielded novel therapies in a variety of human cancers. An example of such a target is the receptor for tyrosine kinases, which mediate processes involved in tumor growth, progression, and metastasis. Toceranib recently received FDA approval for treatment of recurrent cutaneous mast cell tumors in dogs. Its primary action is inh⅓ ∼⅓ of these high-grade tumors. Because toceranib impacts multiple tyrosine kinase pathways, it may have activity against other tumor types.

Safe Handling of Antineoplastic Agents

Most antineoplastic chemotherapeutic agents are potentially toxic as mutagens, teratogens, or carcinogens. Handling of these agents can result in unhealthy personal or environmental exposure in a number of different ways. A common route of exposure is inhalation due to aerosolization during mixing or administration of cytotoxic drugs. This may occur when a needle is withdrawn from a pressurized drug container or upon

expulsion of air from a drug-filled syringe. Transferring drugs between containers, opening drug-filled glass ampules, or crushing or splitting oral medications may also aerosolize drug residues. The best way to avoid aerosolization is to prepare cytotoxic drugs in a biologic safety cabinet or hood; a Class II, type A vertical laminar air flow hood exhausted outside the building is recommended. If a hood is not available, drugs should be prepared in a specified low-traffic area with proper ventilation where no food, drink, or tobacco products are allowed. This area should be equipped with supplies needed for drug reconstitution, including a disposable, plastic-backed liner for the working surface; powder-free latex gloves; gown; goggles; and mask with a filter. Disposal of contaminated vials, syringes, needles, and gloves in this area should be anticipated, and the proper puncture-proof containers provided. Aerosol exposures can be further decreased through the use of chemotherapy-dispensing pins ("chemopins") or closed, dry-membrane dispensing systems. Another potential route of exposure to antineoplastic agents is by absorption of drug through the skin. This could occur during preparation or administration of drug, cleaning of the drug preparation area, or handling of excreta from animals that have received selected cytotoxic drugs. Most exposure of this type may be avoided by conscientious wearing of latex gloves and careful handling of drug-contaminated needles or catheters. Re-capping of needles containing drug residues is discouraged to avoid accidental self-inoculation. Antineoplastic agents can be inadvertently ingested if food, drink, or tobacco products are allowed in the vicinity of drug preparation areas, treatment areas, or kennels housing treated animals. Any ingestible materials should be restricted to a separate area that is far enough away to avoid any possible contamination with these agents. All personnel should handle antineoplastic agents with care. Women of child-bearing age should be particularly cautious, and pregnant women should not handle anti-neoplastic drugs. A source of exposure to cytotoxic drugs that is commonly overlooked is the handling of body fluids and excreta of treated patients. Uniform guidelines for handling of these potentially dangerous substances have not been published. Nevertheless, simple measures can be taken to minimize exposure to veterinary personnel and pet owners. Collection of biologic samples, such as blood, urine, or tissue, should be performed prior to chemotherapy administration. The duration and type of precautionary measures that should be taken after treatment depend on the half-life and routes of elimination of the drug administered. Pet owners and veterinary hospital personnel should be advised to allow dogs to urinate and defecate in a confined area outdoors, away from spaces where people may congregate or children play. A mask should be worn when cleaning a litterbox and the contents placed in a sealed plastic bag. Powder-free, disposable gloves should be worn when cleaning up urine, feces, or vomitus. Veterinarians are encouraged to contact their local Board of Health and other regulatory agencies for local regulations regarding disposal of hazardous waste.

Classification of Antineoplastic Chemotherapeutic Agents

Conventional cytotoxic antineoplastic agents can be grouped by biochemical mechanism of action into the following general categories: alkylating agents,

antimetabolites, mitotic inhibitors, antineoplastic antibiotics, hormonal agents, and miscellaneous.

Table 1
Mechanisms of Action, Indications, and Toxicities of Selected Antineoplastic Agents

Drug	Mechanism of Action	Major Indications	Toxicities
Alkylating Agents			
Cyclophosphamide	Undergoes hepatic biotransformation to active metabolites that alkylate DNA; alkylation leads to miscoding of DNA and cross-linking of DNA strands	Lymphoma, mammary adenocarcinoma, sarcomas, lymphocytic leukemia	Nausea, vomiting (infrequent), moderate to severe myelosuppression, sterile hemorrhagic cystitis
Melphalan	Alkylates DNA causing miscoding and cross-linking of DNA strands	Multiple myeloma	Nausea, vomiting, anorexia, moderate myelosuppression (may be more myelosuppressive in cats)
Chlorambucil	Alkylates DNA causing miscoding and cross-linking of DNA strands; slowest-acting alkylating agent	Chronic lymphocytic leukemia, small cell lymphoma	Nausea, vomiting, mild to moderate myelosuppression
Lomustine CCNU	Alkylates DNA causing miscoding and cross-linking of DNA strands; inhibits both DNA and RNA synthesis; not cross resistant with other alkylating agents	Lymphoma, mast cell tumor, histiocytic sarcoma, CNS neoplasias, multiple myeloma	Nausea, vomiting, moderate to severe myelosuppression (may be delayed for 4–6 wk), hepatotoxicity, nephrotoxicity, pulmonary toxicity
Streptozotocin	Inhibits DNA synthesis; high	Insulinoma	Severe, potentially fatal nephrotoxicity (if given without

	affinity for pancreatic β cells		diuresis) and hepatotoxicity, nausea (immediate and delayed), vomiting, mild myelosuppression
Dacarbazine (DTIC)	Undergoes hepatic biotransformation to active metabolites that alkylate DNA; inhibits RNA synthesis	Lymphoma, sarcomas	Severe acute nausea, vomiting, phlebitis, moderate myelosuppression, hepatotoxicity, anecdotal reports of pleural effusion in cats
Ifosfamide	Analog of cyclophosphamide; undergoes hepatic biotransformation to active metabolites that alkylate DNA; alkylation leads to miscoding of DNA and cross-linking of DNA strands	Various sarcomas	Nausea, vomiting, myelosuppression, sterile hemorrhagic cystitis, possible nephrotoxicity
Antimetabolites			
Methotrexate	Inhibition of dihydrofolate reductase that is required for formation of tetrahydrofolate, a necessary cofactor in thymidylate synthesis; thymidylate essential for DNA synthesis and repair	Lymphoma	Nausea, vomiting, moderate myelosuppression, GI ulceration, hepatoxicity, pulmonary toxicity
5-Fluorouracil	Pyrimidine analog; interferes with DNA synthesis and may be	Carcinomas (systemic); cutaneous carcinomas (topical)	Systemic: nausea, vomiting, moderate

	incorporated into RNA to cause toxic effects		myelosuppression, neurotoxicity, GI ulceration, neurotoxicity, hepatotoxicity Topical: local irritation, pain, hyperpigmentation Cannot be given to cats (fatal neurotoxicity)
Cytarabine	Pyrimidine analog; incorporates into DNA causing steric hindrance and inhibition of DNA synthesis	Lymphoma (including CNS), leukemias; No activity in solid tumors	Nausea, vomiting, moderate myelosuppression, nephrotoxicity, hepatotoxicity
Gemcitabine	Pyrimidine analog; incorporates into DNA causing steric hindrance and inhibition of DNA synthesis	Limited efficacy seen in lymphoma and various carcinomas	Mild nausea, vomiting, mild to moderate myelosuppression, pulmonary toxicity, nephrotoxicity
Antibiotic Antineoplastics			
Doxorubicin	Intercalates and binds to DNA, disrupting helical structure and DNA template; inhibits RNA and DNA polymerases; causes DNA topoisomerase-II-mediated chain scission; generates free radicals that cause DNA scission and cell membrane damage	Lymphoma, leukemias, multiple myeloma, osteo-sarcoma, hemangiosarcoma, and various other sarcomas and carcinomas	Nausea, vomiting, moderate myelosuppression, hemorrhagic colitis, severe cutaneous reactions if extravasated; red urine (not hematuria), transient ECG changes and arrhythmias, nephrotoxicity, anaphylactoid reactions

			Cumulative dose-related congestive heart failure in dogs; cumulative nephrotoxicity in cats
Mitoxantrone	Topoisomerase-II-mediated chain scission; DNA aggregation, oxidation, and strand breakage	Lymphoma, various carcinomas	Nausea, vomiting, moderate to severe myelosuppression, diarrhea, bluish discoloration to sclera; less severe adverse effects than others in this group
Bleomycin	Mixture of glycopeptides; generates oxygen radicals that cause chain scission and fragmentation of DNA	Carcinomas	Nausea, vomiting, myelosuppression, fever, allergic reactions including anaphylaxis, hyperpigmentation, skin ulceration, pneumonitis, pulmonary fibrosis
Dactinomycin (Actinomycin D)	Intercalates and binds to DNA, disrupting helical structure and DNA template; inhibits RNA and DNA polymerases; causes DNA topo-isomerase II-mediated chain scission; generates free radicals that cause DNA scission and cell membrane damage	Lymphoma, various sarcomas	Nausea, vomiting, moderate to severe myelosuppression, phlebitis; severe tissue reaction if extravasated

Mitotic Inhibitors

Vinblastine	Binds to tubulin, leading to disruption of mitotic spindle apparatus and arrest of cell cycle	Lymphoma and leukemias, mast cell tumors	Mild nausea, vomiting, severe myelosuppression, neurotoxicity with high doses, inappropriate secretion of anti-diuretic hormone
Vincristine	Binds to tubulin, leading to disruption of mitotic spindle apparatus and arrest of cell cycle	Lymphoma and leukemias, transmissible venereal cell tumors, various sarcomas	Mild to moderate nausea, vomiting, mild to moderate myelosuppression, severe tissue reaction if extravasated, cumulative peripheral neuropathy, constipation, paralytic ileus, inappropriate secretion of anti-diuretic hormone
Vinorelbine	Binds to tubulin, leading to disruption of mitotic spindle apparatus and arrest of cell cycle	Primary lung tumors, limited efficacy in mast cell tumors	Mild nausea, vomiting, myelosuppression
Miscellaneous			
Cisplatin	Reacts with proteins and nucleic acids; forms cross-links between DNA strands and between DNA and protein; disrupts DNA synthesis	Osteosarcoma, carcinomas and mesothelioma	Intense nausea, vomiting, mild to moderate myelosuppression; potentially fatal nephrotoxicity if not given with diuresis, anaphylaxis, ototoxicity, peripheral neuropathy,

			hyperuricemia, hypermagnesemia Cannot be given to cats (fulminant pulmonary edema)
Carboplatin	Reacts with proteins and nucleic acids; forms cross-links between DNA strands and between DNA and protein; disrupts DNA synthesis	Osteosarcoma, carcinomas	Mild nausea, vomiting, diarrhea, moderate to severe myelosuppression
L-Asparaginase	Inhibits protein synthesis by hydrolyzing tumor cell supply of asparagine	Acute lymphoid leukemias and lymphoma	Hypersensitivity reactions, anaphylaxis especially after repeated doses, alteration in coagulation parameters, hepatotoxicity, pancreatitis (humans), potential inhibition of immune responsiveness (B and T cells)
Mitotane	Destroys adrenal zona fasciculata and zona reticularis	Pituitary hyperadrenocorticism, palliation of adrenal cortical tumors	Nausea, vomiting, anorexia, diarrhea, adrenal insufficiency, CNS depression, dermatitis
Hydroxyurea	Inhibits conversion of ribonucleotides to deoxyribonucleotides by destroying ribonucleoside diphosphate reductase	Polycythemia vera, granulocytic and basophilic leukemia, thrombocythemia, investigational for meningiomas	Nausea, vomiting, mild myelosuppression, alopecia, sloughing of claws, dysuria

Procarbazine	Mechanism is unclear; inhibits DNA, RNA and protein synthesis, perhaps through alkylation	Lymphoma, as part of MOPP chemotherapy protocol; brain tumors	Nausea, vomiting, myelosuppression, diarrhea
Hormones			
Prednisone	Lympholytic; inhibits mitosis in lymphocytes	Lymphoma, mast cell tumors, multiple myeloma, palliative treatment of brain tumors	Sodium retention, GI ulceration, protein catabolism, muscle wasting, delayed wound healing, suppression of hypothalamic-pituitary-adrenal axis, immunosuppression

Immunosuppressants are used to control severe manifestations of allergic, autoimmune and transplant-related diseases. Some drugs have a diffuse effect on the immune system while others have specific targets. Drugs with diffuse effects are more likely to cause damaging adverse effects, but the effectiveness of the more specific drugs may be reduced if their action can be bypassed by alternative metabolic pathways. Treatment protocols therefore frequently use drug combinations to minimise adverse effects and to prevent resistance to treatment. Although protocols are essential to allow scientific evaluation, the clinician must be prepared to tailor treatment based on the ongoing assessment of drug effects, disease activity and the robustness of the individual patient. Many of the currently available immunosuppressants were developed for the use in oncology or transplantation. As this treatment is potentially life-saving desperate measures can be justified. However, there are now over 80 autoimmune diseases and several common allergic conditions in which immunosuppressants could play a role although they may not be life-saving.

Some immunosuppressants act through immunodepletion of effector cells, while others are predominantly immunomodulatory, affecting the activity of cells, usually through cytokine inhibition. Immunosuppressants can be categorised as glucocorticoids, small molecules or proteins.

Small molecules

The small molecule immunosuppressants include calcineurin inhibitors, such as cyclosporin, and antiproliferative drugs, such as sirolimus.

Calcineurin inhibitors

Since the 1980s, calcineurin inhibitors have been the main contributors to the success of solid organ transplantation, especially kidneys. By blocking interleukin-2 synthesis, they prevent activation of T-lymphocytes and are therefore useful in disorders of cell-mediated immunity. Calcineurin inhibitors have a proven role in the prevention of acute cellular rejection of transplanted organs, in psoriasis and in nephrotic syndrome.

Table 1

Small molecule immunosuppressant drugs in current use

Class of drug	Generic name	Potential clinical uses	Drug monitoring	Main adverse effects
Immunophilin-binding drugs				
Calcineurin inhibitors	cyclosporin	organ transplants, nephrotic syndrome, psoriasis, atopic eczema, rheumatoid arthritis	TDM(C_0orC_2), creatinine, potassium, magnesium, glucose, lipids	nephrotoxicity, hypertension, tremor, hirsutism, gum hypertrophy, diabetes, haemolytic uraemic syndrome
	tacrolimus	organ transplants	TDM(C_0), creatinine, potassium, magnesium, glucose, lipids	as for cyclosporin but more tremor, diabetes, less hypertension and fewer cosmetic effects
Mammalian target of rapamycin (m TOR) inhibitors	sirolimus everolimus	organ transplants	TDM(C_0), lipids, FBC, UA	delayed wound healing, mouth ulcers, acne, pancytopenia, hyperlipidaemia, interstitial pneumonitis, peripheral oedema, proteinuria
Inhibitors of nucleotide synthesis				
Purine synthesis inhibitors	mycophenolate mofetil mycophenolic acid	organ transplants, vasculitides, SLE	TDM not widely used, FBC	diarrhoea, dyspepsia, neutropenia, anaemia, viral infections
Purine analogue	azathioprine	organ transplants, rheumatoid arthritis, SLE, inflammatory bowel disease	FBC, LFTs	neutropenia, macrocytosis, liver dysfunction, skin cancers, interaction with allopurinol

Pyrimidine synthesis inhibitor	leflunomide	rheumatoid arthritis, organ transplants	FBC, LFTs	diarrhoea, nausea, rash, alopecia, hepatitis, pancytopenia
Antimetabolites				
Dihydrofolate reductase inhibitor	methotrexate	rheumatoid arthritis (may be used in parallel with TNF inhibitors or leflunomide), psoriasis, psoriatic arthritis, inflammatory bowel disease	FBC, LFTs	anaemia, neutropenia, nausea, hepatitis, pulmonary fibrosis
Alkylating drugs				
Prodrug of phosphoramide mustard	cyclophosphamide	systemic vasculitides, especially Wegener's granulomatosis, SLE, membranous glomerulonephritis	FBC, MSU, UA	neutropenia, anaemia, alopecia, haemorrhagic cystitis, sepsis, infertility, bladder cancer

TDM	therapeutic drug monitoring C_0= trough concentration C_2= concentration 2 hours after a dose	TNF	tumour necrosis factor
FBC	full blood count	MSU	midstream urine for microscopy and culture
UA	urinalysis		
SLE	systemic lupus erythematosus		
LFTs	liver function tests		

They have been used in many other autoimmune conditions but have a diminishing role in rheumatoid arthritis. While they are good at maintaining autoimmune diseases

in remission, withdrawal often leads to relapse. In solid organ transplantation, combinations of calcineurin inhibitors, mycophenolate mofetil and prednisone give better results than monotherapy. Ironically, calcineurin inhibitors are nephrotoxic and may contribute to long-term renal failure, both in transplanted organs and normal kidneys. They also aggravate hypertension and hyperlipidaemia thereby inducing an unfavourable cardiovascular profile. There is also an increased risk of diabetes.

Mycophenolate mofetil

Since it was introduced into Australia in 1996 mycophenolate mofetil has largely replaced azathioprine in organ transplantation. One advantage over azathioprine is that allopurinol can be used for gout prophylaxis without the need to reduce the dose of mycophenolate. Possibly because of its anti-B cell properties mycophenolate seems particularly effective in severe forms of systemic lupus erythematosus. It is also gaining favour as a steroid-sparing drug in the maintenance phase of a number of immune disorders, particularly the vasculitides. The main adverse effects are haematological and gastrointestinal. On higher doses a third of patients will develop diarrhoea. An enteric-coated formulation of mycophenolate has been developed to try and reduce gastrointestinal adverse effects. Therapeutic drug monitoring is available but not widely used.

Sirolimus and everolimus

These potent antiproliferative drugs have gained acceptance in renal transplantation as a strategy to minimise the use of calcineurin inhibitors in low immunological risk patients. They have a decreased likelihood of causing hypertension and glucose intolerance. Although these drugs are associated with less nephrotoxicity than calcineurin antagonists, they potentiate the renal toxicity of cyclosporin and regular monitoring of renal function is recommended. Sirolimus and everolimus are generally avoided perioperatively because they can severely delay wound healing. They are potent inhibitors of intimal hyperplasia in arteries, and sirolimus-eluting intra-arterial stents are now used to reduce re-stenosis rates. However, they can increase serum cholesterol and lipids. The balance of the harm and benefit of continued treatment should be re-evaluated in patients who develop severe refractory hyperlipidaemia. Therapeutic drug monitoring is essential because of the risk of toxicity such as anaemia, leucopenia and thrombocytopenia.

Cyclophosphamide

Cyclophosphamide is a cytotoxic drug. It is the drug of choice for Wegener's granulomatosis, but is also used in other vasculitides such as microscopic polyangiitis and systemic lupus erythematosus. Monthly intravenous pulses are as effective as daily oral use in systemic lupus erythematosus, but allow a reduced total dosage. Cyclophosphamide is also used to induce sustained remission in relapsing nephrotic syndrome. Marrow suppression with neutropenia is common after six weeks of

treatment and continuing more than six months runs the risk of gonadal suppression and infertility in both sexes.

Methotrexate

This antimetabolite is used in some autoimmune diseases including psoriasis, psoriatic arthritis, rheumatoid arthritis and Crohn's disease. As a disease-modifying antirheumatic drug, its use in combination with tumour necrosis factor inhibitors (such as infliximab or etanercept) or leflunomide has been shown to markedly improve symptoms in rheumatoid arthritis.

Proteins

Polyclonal antilymphocyte (antithymocyte) antibodies have been used in Australia since the 1960s. More recently, hybridoma technology has produced a plethora of monoclonal antibodies against molecules expressed by human immune effector cells. T-lymphocyte depleting antibodies such as muromonab-CD3 have been widely used to prevent or treat acute rejection of organ transplants. The main drawback is a 'cytokine storm' reaction to the first dose, which can cause life-threatening pulmonary oedema. Basiliximab and daclizumab are monoclonal antibodies against the interleukin-2 receptor (CD25). They are used as induction drugs in transplantation as they significantly reduce the acute rejection rate, with little or no increase in morbidity. They are not yet significantly used in autoimmune diseases. The anti-B cell antibody (anti-CD20), rituximab, is licensed for use against B-cell lymphomata, but there are now published anecdotal reports of its effectiveness in 29 different autoimmune diseases.7 Randomised controlled trials are proceeding in systemic lupus erythematosus, rheumatoid arthritis, dermatomyositis, antineutrophil cytoplasmic antibody (ANCA)-positive vasculitis and in renal transplantation of highly sensitised recipients. A new monoclonal antibody, alemtuzumab, is directed against a surface molecule (CD54), which is widely distributed on lymphocytes, macrophages and dendritic cells, thereby causing severe and long-lasting depletion of these cell lines. As a result, the risk of serious infection is increased. The use of this antibody is cautiously making the transition from immunoprophylaxis in transplant recipients to a wider use in immune diseases. Two monoclonal antibodies against tumour necrosis factor, infliximab and adalimumab, and etanercept which prevents tumour necrosis factor binding to its receptor, are licensed for use in rheumatoid arthritis. They are also being used in ankylosing spondylitis, psoriatic arthritis and inflammatory bowel disease. Infusion reactions are common. Pooled intravenous immunoglobulin was introduced to restore immunocompetence to patients with congenital acquired immune deficiency syndrome. Paradoxically, the discovery of its ability to inhibit the production and binding of auto-and allo-antibodies means that it is now more widely used as an immunomodulatory drug in the treatment of debilitating autoimmune diseases and antibody-mediated allograft rejection.The fact that immunoglobulin also provides passive immunity means that it is regarded as having a low risk of infectious complications compared to other immunosuppressants. Consequently, it has been used

in many conditions without good supportive evidence of efficacy, so the Australian National Blood Authority guidelines now restrict its use. Nevertheless, it is likely that immunoglobulin use will continue to rise as knowledge about its mechanisms of action accumulates.

Table 2

Protein-based immunosuppressant drugs in current use

Drug	Potential clinical uses	Adverse effects
Lymphocyte depleting antibodies		
Polyclonal antithymocyte globulin	prevention and treatment of allograft rejection treatment of moderate to severe aplastic anaemia	cytokine-release syndrome (fever, chills, hypotension), thrombocytopenia, leucopenia, serum sickness
Muromonab-CD3	prevention and treatment of allograft rejection in transplant patients	severe cytokine-release syndrome, pulmonary oedema, acute renal failure, gut upset, neurological disturbances
Alemtuzumab	treatment of B-cell chronic lymphocytic leukaemia, immunoprophylaxis for renal transplants, GVHD, multiple sclerosis, rheumatoid arthritis	mild cytokine-release syndrome, neutropenia, anaemia, pancytopenia, immune thrombocytopenia, thyroid disease
Rituximab	treatment of B-cell non-Hodgkin's lymphoma antibody-mediated transplant rejection, SLE, vasculitis	infusion reactions, hypersensitivity reactions (uncommon)
Non-depleting antibodies and fusion proteins		
Basiliximab Daclizumab	prevention of allograft rejection in transplant patients	hypersensitivity reactions (uncommon)
Belatacept (LEA29Y)	prevention and treatment of allograft rejection in transplant patients	clinical trials still in progress
Tumour necrosis factor inhibitors		
Etanercept Infliximab Adalimumab	rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, Crohn's disease	injection site and infusion reactions, heart failure, opportunistic infections including fungi and tuberculosis, lymphoproliferative disease, demyelinating disease - reactivation of multiple sclerosis, SLE-like illness
Pooled immunoglobulin		
Intravenous immunoglobulin	Kawasaki disease, CIDP, multiple sclerosis, Guillain-Barr?, ITP, bone marrow transplants, myeloma, chronic lymphocytic leukaemia with hypogammaglobulinaemia, transplant rejection	rash, headache, abdominal pain, haemolysis (especially in patients with blood groups A, AB), thromboses, liver dysfunction, aseptic meningitis, acute renal failure

GVHD	graft versus host disease
SLE	systemic lupus erythematosus
CIDP	chronic inflammatory demyelinating polyneuropathy
ITP	idiopathic thrombocytopenic purpura

Using immunosuppressants - strategies and protocols

Treatment protocols are designed to:

(a) remove/suppress the predominant immune effectors and/or

(b) resolve acute inflammation

(c) prevent relapse.

To achieve (a) and (b), high doses are often used initially ('induction phase'). To achieve (c), lower doses of safer drugs are often chosen for the longer term ('maintenance phase'). Withdrawal of therapy is usually only considered after achieving clinical and laboratory evidence of sustained remission. Drugs are withdrawn gradually, one at a time and in the case of corticosteroids only after a long taper.

Empiricism vs controlled trials

Many protocols have evolved empirically from an understanding of the putative immune mechanisms operating in a particular disease. Sometimes the protocols were derived from what had been seen to work in conditions with apparently similar immunopathology. Randomised controlled trials of immunosuppressive protocols are available in the more common conditions such as rheumatoid arthritis or organ transplantation, but as new drugs emerge, the combinations for comparison become bewildering. Today's 'gold standard' treatment can be very quickly outdated, perhaps even before it has been optimised. Tailoring of immunotherapy to the individual is desirable, but this approach makes protocol comparisons difficult. Similarly, the disease being treated may be so pleomorphic that finding like populations to compare in trials becomes very difficult. For example, lupus nephritis has five distinct histological subtypes, each with their own prognosis.

Choosing immunosuppressive regimens

In order to make sound judgements when choosing a treatment protocol the clinician has to consider the clinical trial evidence and then decide:

- Is the aim to pre-empt an anticipated immune response (for example, after organ transplantation) or to suppress an established immune-mediated inflammation (for example, acute glomerulonephritis)?

- In the case of an immune disease, how much immunosuppression will be required and for how long (that is, an assessment of disease activity)? Consider:
    - the natural history of the untreated disease
    - is the disease multiphasic (for example, polyarteritis nodosa) or 'single shot' (for example, microscopic polyangiitis)
    - the extent and severity of the disease in this particular patient
    - is the affected organ beyond recovery
    - the likelihood of relapse
    - the ability to monitor disease parameters long term
- Is this patient likely to withstand the treatment I will recommend (host fitness parameters)? Consider:
    - age (older patients are easier to immunosuppress but have a greater risk of infection)
    - sepsis risk
    - cancer risk
    - cardiovascular/diabetes risk
    - presence of comorbidities
    - patient compliance and availability for follow-up.

In choosing the dose and duration of immunosuppressive treatments, one must always weigh disease activity versus host fitness. For example, an elderly patient with perinuclear-ANCA positive microscopic polyangiitis, confined to the kidneys, with crescents in 10% of glomeruli, will not need as aggressive an approach as the same disease in a young patient, with 80% crescents, lung haemorrhage and mononeuritis multiplex.

Managing and monitoring patients taking immunosuppressants

Patients need to be under constant surveillance, usually by a partnership between the specialist and the general practitioner. Therapeutic drug monitoring is available now for a number of drugs, for example cyclosporin, tacrolimus, sirolimus and mycophenolate. This allows for 'concentration-controlled' regimens. Some common drugs, for example corticosteroids, still have no good measure of individual bioavailability.

Infection risk

Immunosuppression increases susceptibility to infections which can become life-threatening in a matter of hours. At first, common bacterial infections of wounds, chest or urine predominate, but after 1-2 months of therapy opportunistic infections emerge, particularly herpes viruses, pneumocystis pneumonia, fungi and atypical mycobacteria. Vaccinations against influenza (injected) and pneumococcus are recommended in chronically immunosuppressed patients. They are safe and reasonably effective when given in the stable maintenance phase. In general, live attenuated virus vaccines, such

as varicella or measles, should not be given to immunosuppressed patients (or to close family contacts).

Cancer risk

In patients taking immunosuppressants, early cancers are often viral induced. They include lymphoproliferative disorders and cervical cancer. In the long term, nearly all common cancers are increased, but particularly skin cancers. After 20 years of immunoprophylaxis following renal transplant, 80% of Australian patients will have developed skin cancer.

Table 3

Routine monitoring of patients taking immunosuppressant drugs

Monitoring of immune system	Acute phase reactants (e.g. C-reactive protein, erythrocyte sedimentation rate)
	Disease-specific auto-antibodies (e.g. antineutrophil cytoplasmic antibody, anti-double-stranded DNA antibody)
	Immunoglobulin and complement concentrations
	Organ function and histology
	Neutrophil and lymphocyte counts, and T-cell subsets
Monitoring of adverse effects	Haemoglobin, platelets, lipids, blood glucose
	Blood pressure
	Skin cancer surveillance, rectal examination, pap smear and possibly prostate specific antigen
	Bone densitometry
	Cataract screening
Therapeutic drug monitoring	Meeting therapeutic targets (where known)
	Defines poor absorption
	Helps assess compliance

Table 4

Common prophylactic treatments for patients taking immunosuppressant drugs

Infection prophylaxis	'Heavy' immunosuppression may warrant prophylaxis for cytomegalovirus (valganciclovir), *Pneumocystis jirovec*ipneumonia (cotrimoxazole) and candidiasis (oral nystatin) Influenza and pneumococcal vaccines
Anticoagulation	Immune diseases are frequently associated with thrombophilia requiring antiplatelet drugs or warfarin
Cardiovascular/diabetes risk	Corticosteroids, calcineurin inhibitors and mammalian target of rapamycin inhibitors all have adverse cardiovascular risk profiles. 'Statins' and antidiabetic drugs are often indicated
Bone preservation	May require calcium, vitamin D and bisphosphonate supplements
Ulcer prophylaxis	Consider H_2 antagonist or proton pump inhibitor especially with steroid use

Advances in our understanding of the immune aetiology of many debilitating diseases have resulted in wider use of immunosuppressant drugs in common clinical practice. The last two decades have seen the development of several useful small molecule drugs but also a profusion of monoclonal antibodies targeting the immune system. Increasingly, primary care physicians are involved in the supervision of patients taking these drugs. This task has been made easier and safer by the establishment of therapeutic targets for drug monitoring and the obligatory use of prophylactic drugs to prevent common adverse effects. Good clinical judgement, supported by laboratory investigations, is needed to differentiate the patients who are over-immunosuppressed (and therefore at risk of infections and cancer) from those experiencing relapse of their underlying disease.

Corticosteroids and Corticosteroid Replacement Therapy

Hydrocortisone (cortisol) is secreted by the adrenal cortex and has both glucocorticoid and mineralocorticoid effects. The term 'glucocorticoid' derives from the early discovery that these hormones were important in glucose metabolism. Since the 1940s synthetic glucocorticoids have been developed for their anti-inflammatory and

immunomodulatory effects. Attempts have been made to increase the beneficial effects and reduce the adverse effects by modifying the steroid nucleus and side groups.

Mode of action and relative strengths

Even an outline of complex steroid biosynthesis and physiology is helpful when considering the therapeutic benefits and adverse effects of the glucocorticoids.

- The adrenal cortex has 3 distinct anatomical zones. Glucocorticoids originate from the zona fasiculata (mineralocorticoids from the zona glomerulosa, and androgens from the zona reticularis).
- Regulation of glucocorticoid synthesis and release is complex. Glucocorticoids are not stored and must be synthesised when required. The mechanism involves:
  - The hypothalamo-pituitary axis. Feedback mechanism inhibits production.
  - Feedback via catecholamines from the adrenal medulla.
  - The autonomic nervous system.
- Glucocorticoids have anti-inflammatory and immunosuppressive effects important in natural immune responses. They are involved in the mobilisation of substrates for gluconeogenesis (amino acids, fatty acids, etc.) and maintenance of normal blood glucose by:
  - Stimulating gluconeogensis in the liver.
  - Mobilising amino acids from extrahepatic tissues.
  - Reducing glucose usage by inhibiting uptake in muscle and fat.
  - Stimulating fat breakdown.
- These effects are mediated through the glucocorticoid receptor (GR), an intracellular protein acting as a nuclear transcription factor regulating the expression of a diverse range of genes. This process involving mediation of the main metabolic and cardiovascular effects is called 'transactivation' and the inhibitory effect is called 'transrepression'.
- The basal daily rate of cortisol secretion is 6-8 mg per square metre, and this increases tenfold in acute stress. Physiological replacement requires 10-15 mg per square metre because of reduced bioavailability. The different natural and synthetic glucocorticoids have different potencies and pharmacodynamic properties according to:
  - Relative and absolute affinity for the GR and mineralocorticoid receptor (MR).
  - Affinity for the associated enzyme.
  - Ability to modulate the glucocorticoid responsive genes.
- The different glucocorticoids also have differing pharmacokinetic properties affecting:
  - Bioavailability
  - Plasma half life
  - Clearance rates
  - Water solubility, etc.

Steroids Compared

Type	Drug	Equivalent Doses
Short-acting. Biological half life*: 8-12 hours	Cortisol	20 mg
	Hydrocortisone	25 mg
Intermediate-acting. Biological half life: 18-36 hours	Prednisolone	5 mg
	Triamcinolone	4 mg
	Methylprednisolone	4 mg
Long-acting. Biological half life: 36-54 hours	Dexamethasone	0.75 mg
	Betamethasone	0.75 mg
Mineralocorticoids	Aldosterone	0.3 mg
	Fludrocortisone	2 mg

*Duration of adrenocorticotrophic hormone (ACTH) suppression after a single dose of the drug.

Indications and benefits of corticosteroids

- Corticosteroids can be life-saving and have dramatic benefits. However, their therapeutic use has to be balanced against the risks of serious adverse effects.
- Dose, route of administration, duration of treatment and choice of corticosteroid must be considered to maximise therapeutic benefit and minimise adverse effects.
- The list of conditions below clearly illustrates the diverse range of benefits that are possible.

Conditions where corticosteroids have been used with evidence based benefits include:

- Asthma
- Croup
- Crohn's disease
- Ulcerative colitis
- Temporal arteritis
- Polymyalgia rheumatica
- Rheumatoid arthritis
- Systemic lupus erythematosus
- Polyarteritis nodosa
- Wegener's granulomatosis
- Sarcoidosis
- Eczema
- Otitis externa
- Pemphigus
- Dermatomyositis
- Minimal change glomerulonephritis
- Acute leukaemia
- Acquired haemolytic anaemia
- Idiopathic thrombocytopenic purpura
- Cerebral oedema
- Cluster headache
- Congenital adrenal hyperplasia
- Anaphylaxis and allergic reactions

Drug initiation and choice of steroid

Benefits should be weighed against risks.

Every patient should be given a steroid card (see box below) as recommended by the Committee for Safety of Medicines (CSM) following concern about severe chickenpox associated with systemic steroids. Patients should also be given the manufacturer's patient information leaflet with their prescription.

Steroids are commonly used at high initial dose and then reduced to maintain remission.

The choice of steroid is made according to properties required. For example:

Hydrocortisone and cortisone have glucocorticoid effects but relatively high mineralocorticoid activity. They are therefore unsuitable for long-term use, but useful intravenously in emergency situations. Hydrocortisone can be used topically with less risk of side-effects as it is less potent.

Prednisolone has high glucocorticoid activity with less mineralocorticoid effect and is used for longer-term treatment.

Betamethasone and dexamethasone have even higher glucocorticoid activity and insignificant mineralocorticoid effect. They can thus be used when high dosages are required without effects such as fluid retention - for example, cerebral oedema from malignancy. They cross the placenta readily and should be avoided in pregnancy.

Long duration of action with betamethasone and dexamethasone makes them useful in conditions like congenital adrenal hyperplasia when suppression of corticotrophin must be maintained.

Local treatments should be used when possible in preference to systemic.

Adrenal suppression can be reduced by:

Morning dosage.

Alternate day dosing.

Intermittent courses of treatment.

Addition of small doses of immunosuppressive drug.
There is a wide range of adverse effects:

Cardiovascular: hypertension; congestive cardiac failure.

Central nervous system: mood disturbance (including mania), psychosis, sleep disturbance

Endocrine/metabolic: adrenal suppression, growth failure in children, insulin resistance, diabetes, disturbance of thyroid function, hypokalaemia, metabolic alkalosis.

Gastrointestinal: gastric effects (peptic ulceration, etc.), fatty liver.

Haematopoietic: leukocytosis and other effects (eg reduced eosinophils and monocytes).

Immune system:

Suppression type IV hypersensitivity (interferes with Mantoux' test).

Inhibitory effects (leukocytes, macrophages, cytokines).

Suppression of primary antigen response (important with vaccines).

Musculoskeletal system:

- Myopathy (especially proximal muscles).
- Osteoporosis.
- Avascular necrosis of bone.

Ophthalmic: cataracts (more common in children), elevation of intraocular pressure, glaucoma.

Skin and other systems: moon face, truncal obesity, dorsolumbar hump, acne, thin skin, skin striae (violaceous), impotence, irregular periods.

Scenarios

In addition to the disadvantages of longer-term treatment, there are several clinical scenarios worthy of special mention:

Corticosteroids and surgery

Adrenal suppression caused by steroid therapy may result in an inadequate adrenocortical response to surgery (acute adrenocortical insufficiency can precipitate hypotension and death). Therefore:

- Anaesthetists must be informed when patients have taken corticosteroids within 3 months of surgery (10 mg or more) so that:

- For minor surgery under general anaesthesia either the usual corticosteroid dose can be given orally, or 25-50 mg of hydrocortisone can be given intravenously (IV) at induction.
- For moderate/major surgery the usual oral dose is taken on the day of surgery with hydrocortisone as above at induction and the same IV dose three times daily for between 24 and 72 hours after surgery, depending on the extent of surgery. This is then followed by the usual oral dose.
- Patients on prolonged treatment with potent inhaled or nasal corticosteroids should have the same precautions taken as above before surgery.

Corticosteroids and live vaccines

Live vaccines should not be given within 3 months of:

- An adult receiving 40 mg/day of prednisolone or equivalent for more than a week.
- A child receiving either 2 mg/kg/day for 1 week or 1 mg/kg/day for 1 month.

Corticosteroids in pregnancy and breast-feeding

The 1997 review of the CSM looked at safety in pregnancy and lactation. This stressed again the importance of weighing risk and benefit and concluded:

- Corticosteroids vary in their ability to cross the placenta. Prednisolone is mostly (88%) inactivated as it crosses the placenta, whereas betamethasone and dexamethasone cross readily.
- Although corticosteroids can cause abnormalities in fetal development in animals, this has not been shown in humans (for example, cleft lip and palate).
- Prolonged or repeated corticosteroid administration in pregnancy increases the risk of intrauterine growth restriction (IUGR). Short-term treatment carries no such risk.
- The theoretical risk of adrenal suppression in neonates after prenatal exposure to corticosteroids is not clinically important and resolves spontaneously after birth.
- Prednisolone is excreted in small amounts in breast milk and is unlikely to cause systemic effects in the infant unless doses exceed 40 mg daily. Above this dose infants should be monitored for adrenal suppression. No data are available on other corticosteroids.

Corticosteroids and infection

Corticosteroids affect the severity and clinical presentation of infections as well as susceptibility to infections. For example:

- Ocular infections may be exacerbated (fungal and viral).
- Diagnosis of serious infection may be delayed (septicaemia, tuberculosis).
- Corticosteroids may activate or exacerbate infections (tuberculosis, amoebiasis, strongyloides).
- Corticosteroids predispose to fungal infections in chronic lung disease (pulmonary aspergillosis).
- Topical corticosteroids probably predispose to eczema herpeticum, although the association may not be as strong as is often suggested.

- Patients on corticosteroids should avoid exposure to measles and seek medical advice if exposed. Prophylaxis with human normal immunoglobulin may be given.

Corticosteroids and chickenpox

Patients on corticosteroids (systemic but not topical, rectal or inhaled) or who have used them within 3 months and are non-immune to varicella infection, are at risk of severe chickenpox. Infection can be severe (fulminant pneumonia, hepatitis and disseminated intravascular coagulation, often without prominent rash). Therefore:

- Exposed non-immune patients on or within 3 months of taking corticosteroids should be given passive immunisation with varicella-zoster immunoglobulin (within 3 days, and no later than 10 days, after exposure).
- Confirmed chickenpox in such patients warrants urgent referral and urgent treatment.

Corticosteroids and osteoporosis

Corticosteroid therapy is a major risk factor for osteoporosis. Prophylactic advice and drug treatment to prevent osteoporosis should be offered to:

- Any patient aged over 65 taking steroids for 3 months or more (any dose).
- Any patient aged under 65 with previous fragility fracture, taking steroids for 3 months or more (any dose).
- Any patient aged over 65 or with relevant fracture and taking intermittent corticosteroids should have bone mineral density measured. Advice should be given according to results. For example:
- If the T score is -1.5 or less consider treatment if there is a long wait for dual energy X-ray absorptiometry (DEXA) scanning.
- If the T score is between 0 and 1.5 no drug treatment is required but repeat DEXA scanning every 1 to 3 years whilst on steroids.

Steroids and the skin

Systemic and local side-effects can occur, particularly with moderate-strength steroids. The emphasis should be on appropriate use to produce benefit and to minimise side-effects. Once-daily applications are recommended by the National Institute for Health and Clinical Excellence (NICE).Information on use of steroid in dermatology can be found in the separate article Steroids and the Skin. Important areas for consideration include:

- Appropriate strength of steroids
- Use of emollients with steroids
- Clear instructions for patients
- Regular follow-up

Inhaled and nasal corticosteroids

The CSM advised that:

- Systemic effects can occur with prolonged high-dose therapy.
- Susceptibility to side-effects varies between individuals.

- Intranasal steroids are less likely than inhaled steroids to cause systemic side-effects.

5 main areas of concern are identified:

- Adrenal suppression.
- Osteoporosis and reduced bone mineral density.
- Growth restriction in children.
- Cataracts.
- Glaucoma.

Advice to reduce risk includes:

- Give the lowest dose to control asthma and rhinitis.
- The dose and duration of treatment, as well as strength of the steroid, should be monitored.
- The height of children on moderate-strength steroids should be monitored (reduce treatment and/or refer if growth is affected).
- Observe the precautions for surgery, as above, when patients are on prolonged high-dose steroids.

Key points from clinical scenarios:

- Ensure steroid cards are available and given out appropriately.
- Ensure patients at risk of osteoporosis are identified and treated.
- Ensure good instructions are given for topical steroid use.
- Identify patients on regular high-dose inhaled steroids at risk of systemic side-effects and in need of steroids before surgery.
- Ensure patients receiving live vaccines should be asked about corticosteroid usage.

Drug interactions

Important interactions include:

- Antagonism of antihypertensives.
- Exacerbation of gastrointestinal side-effects (eg non-steroidal anti-inflammatory drugs (NSAIDS) and peptic ulcer).
- Enhanced anticoagulant effects.
- Antagonism of diabetic drugs.
- Exacerbation of hypokalaemia with digoxin, diuretics, theophyllines and $beta_2$ agonists.
- Impaired immune response of vaccines.

Monitoring and stopping steroids

Monitoring may include the following (as well as monitoring of the disease being treated): The CSM has suggested that, in patients whose disease is unlikely to relapse, steroids should be reduced gradually when they have:

- Received repeated courses (especially courses lasting >3 weeks).[1]
- Taken a short course within 1 year of long-term corticosteroid therapy.
- Other possible causes of adrenal suppression.
- Received more than 40 mg daily of prednisolone or equivalent.
- Received more than 3 weeks of corticosteroid treatment.

The dose may be reduced rapidly to physiological doses of about 7.5 mg of prednisolone and then more slowly, at the same time ensuring that disease relapse does not occur.

Patients not in the groups above (eg who have received fewer than 3 weeks of corticosteroids) may have corticosteroids stopped abruptly.

Effects of Corticosteroids on the Immune System

Dexamethasone, like any typical glucocorticoid, is capable of causing immunosuppression, thereby inhibiting immune and inflammatory responses. Experimental studies have shown a pro-apoptotic effect of dexamethasone on T lymphocytes, suggesting that glucocorticoids may direct T-cell positive and negative selection in the thymus, limit activation-induced cell death during the contraction phase of an adaptive immune response and induce generalized thymocyte apoptosis after polyclonal T-cell activation. Dexamethasone is also capable of inducing a shift in an immune response towards a Th2 humoral response from a Th1 cellular response by influencing the levels of cytokines produced by the lymphocytes.Moreover, dexamethasone causes a reduction of splenic and lymph node B-cell numbers and attenuation of early B-cell progenitor proliferation. Glucocorticoids also enhance the activity of macrophages and induce tolerogenic dendritic cells, exerting a potent anti-inflammatory effect.

Cytokines and anti-cytokines as therapeutics

Cytokines are a large family of mainly soluble proteins and glycoproteins that function as key modulators of the immune system. They encompass interleukins (ILs), interferons (IFNs), growth factors, colony stimulating factors (CSFs), the tumour necrosis factor (TNF) family and chemokines (or chemotactic cytokines). These small signalling molecules (<30 kDa) are mostly secreted by leukocytes, but can also be produced by other cell types (e.g., endothelial cells, epithelial cells and fibroblasts). Cytokines can function in an autocrine, paracrine or endocrine manner to stimulate or suppress the activity of target cell populations. Signals conveyed by cytokines are essential for generation, survival and homeostasis of immune cells, as well as for the generation of immune responses upon external stimuli. Due to their natural role as immune modulators, many cytokines have been identified as suitable therapeutic candidates for the treatment of a number of infectious, inflammatory, autoimmune and malignant diseases.

The discovery of cytokines was guided by initial observations that soluble biological factors could mediate inflammation, cellular stimulation and anti-viral activities. For instance, the pro-inflammatory cytokine, interleukin-1 (IL-1), was initially described

as a pyrogenic factor isolated from cultured rabbit leukocyte supernatants. Similarly, reports of a T-cell growth factor present in mixed leukocyte cultures led to the identification of IL-2, a master regulator of T-cell function and development. Furthermore, IFNs were first reported as soluble secreted factors mediating viral interference in chick chorioallantoic membranes. In fact, pioneering cytokine immunotherapy in humans was performed with IFN-containing (<1%) protein fractions extracted from growth medium of virus-stimulated human leukocytes.

The emergence of recombinant DNA technology in the 1970s represented a turning point for exploiting the therapeutic potential of cytokines. Similarly important was the development of high performance liquid chromatography, which eased the laborious task of obtaining cytokine preparations pure enough for characterization. Using these techniques, the first cytokines to be cloned and synthesized in *E. coli* were IFN-α and IFN-β in 1980 and IL-2 in 1983. Initial regulatory approval of recombinant IFN-α for the treatment of hairy cell leukaemia was obtained in 1986, while recombinant IL-2 was approved for treating metastatic melanoma in 1992. Additional therapeutic applications for IFN-α, IL-2 and a number of other cytokines have since been approved or are currently under trial.

Cytokine Toxicity

Cytokine immunotherapy often results in the development of severe dose-limiting side effects. Two properties shared by most cytokines are thought to play a crucial role in the development of treatment-associated adverse effects. Firstly, cytokines are pleiotropic, meaning they are able to influence more than a single cell type. In fact, some cytokines are able to stimulate cell types that mediate opposing biological effects. Furthermore, cytokines have a short serum half-life and, thus, need to be administered at high doses to achieve their therapeutic effects. While effectively enhancing therapeutic efficacy, high doses exacerbate pleiotropic activities that manifest as adverse effects in patients. Molecular engineering of cytokines with prolonged half-life, enhanced specificity or localized activity is therefore required to enhance the pharmacological properties of these proteins. Engineered

A prominent example of cytokine-associated toxicity is the adverse effects observed in patients receiving high-dose (HD) IL-2 cancer immunotherapy. IL-2 stimulates the proliferation of cytotoxic $CD8^+$ T-cells and NK cells, which both act to promote tumour regression. Due to its anti-tumorigenic activity, HD IL-2 therapy has been approved for the treatment of metastatic melanoma and renal cancer, with overall objective responses observed in approximately 16–17% of patients. However, HD IL-2 therapy can cause excessive vascular permeability, which leads to an adverse state known as vascular leak syndrome (VLS). Symptoms arising from VLS can be life-threatening, making intensive patient management a requirement for the use of IL-2 immunotherapy.

Antibody-IL-2 Immune Complexes

Neutralizing monoclonal antibodies (mAbs) directed against cytokines are commonly used for blocking interactions between cytokines and their receptors.

However, several studies in the early 1990s showed dramatic increases in the biological activity of a number of cytokines when mice were administered with a mixture containing cytokine/anti-cytokine mAb at a 2:1 molar ratio. These observations suggest that some anti-cytokine mAbs act as cytokine carriers *in vivo*, identifying a potential use of antibody-cytokine immune complexes in therapeutic applications. Subsequent studies have extensively characterized IL-2/anti-IL-2 mAb complexes, demonstrating that these complexes not only display enhanced biological activity, but also modulate the pleiotropic effects of IL-2.

IL-2 is a member of the common γ chain (γ_c) family, which comprises cytokines that share the IL-2 receptor γ_c for signalling (IL-2, -4, -7, -9, -15 and -21). Structurally, IL-2 is classified as a four α-helix bundle cytokine together with several other interleukins, interferons and colony stimulating factors.

IL-2 is a major modulator of the immune system capable of stimulating cells bearing either dimeric or trimeric IL-2 receptors (IL-2Rs). The low-affinity dimeric IL-2R (K_D = 1 nM), composed of IL-2Rβ (CD122) and γ_c subunits, is found in $CD122^{high}$ populations, such as memory phenotype (MP) $CD8^+$ T-cells and NK cells. In contrast, the high-affinity (K_D = 10 pM) trimeric IL-2R consisting of IL-2Rα (CD25), CD122 and γ_c subunits is found in $CD25^{high}$ populations, such as activated T-cells, activated B-cells and regulatory T-cells (Tregs). Notably, signal transduction in both types of IL-2Rs is mediated by CD122 and γ_c, while the CD25 chain acts to confer high-affinity binding of IL-2 to trimeric IL-2Rs.

IL-2/mAb complexes formed using different mAbs have been shown to selectively stimulate cells depending on the type of IL-2R displayed. IL-2/mAb_{CD122} complexes mediate preferential expansion of $CD122^{high}$ cytotoxic $CD8^+$ T-cells and NK cells, resulting in the potent inhibition of tumour growth in various mouse models of cancer. By contrast, IL-2/mAb_{CD25} complexes selectively expand immunosuppressive $CD25^{high}$ Tregs, resulting in prevention of allogeneic pancreatic islet rejection, autoimmune disease and the reduction of inflammation.

Further to modulating IL-2 activity, IL-2/mAb complexes can also prolong the biological effects of IL-2. While reduced renal clearance is suggested to play a major role in the increased half-life observed in IL-2 complexes, additional mechanisms are thought to contribute to this feature. These mechanisms include reduced receptor-mediated endocytosis (for IL-2/mAb_{CD122} complexes) and indirect recycling of IL-2 by the neonatal Fc receptor (for IL-2/mAb_{CD25} complexes), which rescues IgG antibodies from cellular metabolism.

Based on the enhanced properties of IL-2/mAb complexes in animal models, the use of mAbs as cytokine carriers has emerged as a potential strategy for improving pharmacodynamics and pharmacokinetics of therapeutic cytokines. However, the therapeutic value of this approach is yet to be tested in clinical trials.

Cytokine Fusion Proteins

Genetic fusion to other proteins is a useful strategy for improving or modifying the biophysical properties of cytokine therapeutics. A large body of work has been directed towards the development of cytokine fusion proteins that display extended half-life

(e.g., fusion to Fc, albumin or transferrin), increased activity (e.g., fusion to cytokine agonists), enhanced cytotoxicity (e.g., fusion to bacterial toxins or lytic Fc) and localized delivery (e.g., fusion to antibodies or antibody fragments), with several of them currently undergoing clinical trials.

Fusion to Toxins

A number of cytokines have been fused to bacterial toxins in order to promote targeted cytotoxicity of specific cell subsets. Ontak® (denileukin diftitox) is a fusion protein composed of IL-2 and the enzymatically active portion of diphtheria toxin (DT) approved for the treatment of cutaneous T-cell lymphoma (CTCL). Malignant cells in CTCL lesions overexpress IL-2Rβγ and IL-2Rαβγ, with greater denileukin diftitox sensitivity observed in cells displaying the IL-2R heterotrimer. The IL-2 portion of denileukin diftitox directs DT to cells bearing IL-2Rs, ultimately resulting in cell death through inhibition of protein synthesis.

Partial and complete responses are observed in approximately 20% and 10% of CTCL patients after denileukin diftitox treatment, respectively. Nevertheless, treatment-associated toxicities are common and include the development of VLS (in ~26% of patients) and, in rare cases, visual loss. Although the mechanisms mediating the latter effect are unknown, a direct effect on Tregs—which express high levels of IL-2Rαβγ—has been proposed to promote autoimmune retinopathy and subsequent visual impairment.

Other cytokines that have been fused to toxins for targeted cell cytotoxicity include the functionally related cytokines, IL-4 and IL-13. Fusion proteins composed of either IL-4 or IL-13 and a truncated form of*Pseudomonas* exotoxin (PE) were developed to target the elimination of malignant cells within glioblastoma multiforme, a type of solid brain tumour that overexpresses IL-4 and IL-13 receptors. Notably, IL-13-PE (cintredekin besudotox) reached phase III clinical trials, where it mediated increased progression-free survival in patients receiving drug infusions within resected tumour regions, when compared to patients receiving Gliadel® (carmustine) wafers after tumour resection. However, no overall survival advantage was achieved with cintredekin besudotox treatment, which may have reflected sub-optimal drug delivery.

Fusion to Antibodies and Antibody Fragments (Immunocytokines)

The generation of cytokine-antibody fusion proteins is an elegant approach for engineering targeted cytokine activity. The rationale for the development of such fusion proteins, also known as immunocytokines, derives from the superior efficacy and reduced toxicity of localized over systemic administration of therapeutic cytokines. Since physical delivery of cytokines is not feasible in many instances, immunocytokines provide an alternative avenue for achieving localized cytokine action. As such, immunocytokines are designed to target disease antigens through their antibody moieties in order to induce (or potentiate) effector functions through their cytokine components. A number of immunocytokines are currently undergoing clinical trials, with many more being evaluated in pre-clinical studies. The vast number of engineered immunocytokines and their therapeutic potential has been recently reviewed in previous issues and elsewhere. A brief summary of the major factors

determining the therapeutic activity of specific immunocytokines, namely, commonly targeted antigens, antibody formats and employed cytokines, is presented below.

Target Antigens

Antibodies (or antigen-binding antibody fragments) mediate the targeted delivery of immunocytokines into disease environments and/or to specific cell subsets. Ideal target antigens should be, therefore, overexpressed in diseased tissues, while remaining at low levels elsewhere. Accordingly, a number of immunocytokines incorporate antigen-binding moieties that target antigens overexpressed on the surface of malignant cells (e.g., epithelial cell adhesion molecule, GD2 disialoganglioside, HER2/neu, CD20 and CD30), as well as targeting of neoangiogenic antigens found in tumours and chronic inflammation sites (e.g., fibronectin splice variants EDA/EDB and A1 domain of tenascin C). Furthermore, targeting of DNA/histone complexes exposed in necrotic tissue provides an additional strategy for directing immunocytokines into tumours and metastases.

Cytokine Partners

A number of cytokines, including IL-2, IL-7, IL-12, IL-15, GM-CSF, IFN-α, IFN-γ and members of the TNF-superfamily, have been utilized for the development of immunocytokines with potential for cancer immunotherapy. While targeted delivery of most of these cytokines into cancerous lesions aims for the recruitment and activation of cytotoxic immune cells, such as T-cells and NK cells; some others also promote anti-proliferative activities (IFN-α) or induce cell death upon binding to their cognate receptors (TNF-superfamily members). By contrast, the anti-inflammatory cytokine IL-10, which suppresses the expression of inflammatory mediators by immune cells, has been utilized for the development of immunocytokines targeting the neo-vasculature of chronic inflammation sites.

Cytokine Mutagenesis

Mutagenesis can be employed to engineer cytokines with enhanced stability, half-life, specificity and activity. In early studies, scanning and deletion mutagenesis allowed the development of cytokines with modified activities. These approaches have gradually been replaced by protein engineering techniques, such as rational design, computational modelling and directed evolution, which allow for more efficient cytokine optimization.

Modulation of Specificity

Modulating the specificity and activity of pleiotropic cytokines is necessary for increasing therapeutic efficacy and preventing the development of adverse effects. Accordingly, a number of cytokines have been engineered through rational design and directed evolution in order to mediate selective stimulation of specific cell subsets.

Generated by yeast display, variants of IL-2 with greater than 100-fold increases in affinity to IL-2Rα were shown to mediate enhanced expansion of T-cells *in vitro*. Further modification of these variants to disrupt binding to IL-2Rβ and IL2Rγ resulted

in the generation of signalling-deficient analogues that act as competitive antagonists of IL-2 in cells displaying IL-2Rαβγ. The therapeutic application of such IL-2 mutants is mainly directed towards the inhibition of immunosuppressive Tregs in malignant conditions.

Recently, an IL-2 variant displaying ~250-fold increased affinity to IL-2Rβ was developed through *in vitro*evolution using yeast display. Interestingly, most of the five mutations resided in the core of IL-2 and resulted in a conformational change similar to the one induced after IL-2Rα binding. This mutant, also known as super-2, displayed increased ability to stimulate cells displaying IL-2Rβγ, when compared to unmodified IL-2. Accordingly, super-2 mediated more potent expansion of MP $CD8^+$ T-cells and NK cells, which correlated with increased anti-tumour activity in mice. By shifting the balance of IL-2 stimulation towards cells displaying IL-2Rβγ, super-2 is predicted to mediate tumour regression with a reduced incidence of treatment-associated adverse effects.

Modulation of the activity of another common γ_c cytokine, IL-4, was achieved by using a combination of rational design and directed evolution. IL-4 is a pleiotropic cytokine that can signal through either type I or type II (also responsive to IL-13) IL-4Rs. Importantly, regulatory functions of IL-4 are mostly mediated by cells displaying type I IL-4Rs (e.g., B-cells and T-cells) and drive the development of humoral immune responses (T_H2 responses). In contrast, effector functions of IL-4 are largely mediated by non-hematopoietic cells expressing the type II IL-4Rs and promote the development of allergic inflammation.

Interestingly, both types of IL-4Rs display the high affinity (K_D = 100 pM) IL-4Rα subunit, differing only in the signalling subunit they incorporate, namely γ_c in type I and IL-13Rα in type II IL-4Rs. Increasing the affinity of IL-4 for γ_c or IL-13Rα by mutagenesis produced a number of IL-4 variants that mediated selective*in vitro* expansion of cells bearing either type I or type II IL-4Rs, respectively. In particular, IL-4 variants with enhanced selectivity for cell subsets displaying type I receptors show therapeutic potential for immune stimulation with reduced effector functions.

Development of Cytokine Antagonists

Disruption of cytokine binding to signalling receptor sub-units is a useful approach for the development of antagonists with therapeutic potential. An IL-4 variant (pitrakinra) that was rationally designed to have reduced binding to γ_c and IL-13Rα, but unimpaired binding to IL-4Rα, is currently undergoing phase II clinical trials for the treatment of allergic asthma and atopic eczema. Pitrakinra effectively targets cells bearing type I and II IL-4Rs without inducing signalling, thereby acting as a dual competitive inhibitor of IL-4 and IL-13. Similarly, by mutating residues in IL-6 to disrupt binding to gp130, the signalling sub-unit of IL-6R, but not affecting the binding to the IL-6Rα chain, resulted in the development of an IL-6R antagonist with therapeutic potential for the treatment of multiple myeloma and lung fibrosis.

Concluding Remarks

The immunomodulatory properties of cytokines provide exceptional potential for the treatment of several conditions. However, sub-optimal pharmacokinetics and toxicity have limited their therapeutic potential, as reflected by the reduced number of cytokines currently approved for clinical use. The therapeutic shortcomings displayed by most cytokines have motivated the optimization of these proteins through a number of molecular engineering strategies, including chemical conjugation (e.g., PEGylation), immunocomplexing, development of cytokine fusion proteins and mutagenesis. These strategies have been mainly directed towards the generation of cytokines with prolonged half-life, enhanced specificity/activity and localized action, but also to take advantage of the targeting properties of some cytokines (e.g., cytokine-toxin fusion proteins) and for the generation of cytokine antagonists.

The large number of engineered cytokines currently undergoing pre-clinical and clinical evaluation are likely to constitute the next generation of cytokine therapeutics displaying increased efficacy and reduced incidence of adverse effects. As research continues in this field, we anticipate that optimally designed cytokines will be developed utilizing a combination of molecular engineering strategies in order to simultaneously enhance multiple pharmacokinetic and pharmacodynamic parameters.

Intravenous immunoglobulin (IVIG) therapy

Immune globulin products from human plasma were first used in 1952 to treat immune deficiency. Intravenous immunoglobulin (IVIG) contains the pooled immunoglobulin G (IgG) immunoglobulins from the plasma of approximately a thousand or more blood donors. Initially, immune globulin products were administered by intramuscular injection.

One of biggest advances with IVIG in recent years has been the use of sorbitol-based formulations as opposed to sucrose-based formulations.

IVIG was initially shown to be effective in autoimmune idiopathic thrombocytopenic purpura (ITP) in 1981. IVIGs are sterile, purified IgG products manufactured from pooled human plasma and typically contain more than 95% unmodified IgG, which has intact Fc-dependent effector functions and only trace amounts of immunoglobulin A (IgA) or immunoglobulin M (IgM).

Schematic representation of an immunoglobulin G molecule. CH indicates constant region of heavy chain; CL, constant region of light chain; VH, variable region of heavy chain; and VL, variable region of light chain.

IVIG is an immunomodulating agent that has multiple activities. These include modulation of complement activation; suppression of idiotypic antibodies; saturation of Fc receptors on macrophages; and suppression of various inflammatory mediators, including cytokines, chemokines, and metalloproteinases. The Fc region of IgG facilitates interaction with and signaling through Fc receptors on phagocytes, B cells, and other cells and with Fc-binding plasma proteins (eg, components of the complement system).

Blockade of macrophage Fc receptors is considered the primary mechanism of action of immune globulin in persons with ITP and other autoantibody-mediated cytopenias. In persons with Kawasaki disease and dermatomyositis, IVIG is thought to inhibit the generation of membrane attack complexes (C5b-C9) and subsequent complement-mediated tissue damage by binding the activated components C3b and C4b, thus preventing their deposition on target surfaces. In persons with dermatomyositis, IVIG induces a decrease in plasma levels of membrane attack complex and a substantial decrease in the amounts of C3b and membrane attack complex deposited in endomysial capillaries. The high content of anti-idiotypes against autoantibodies in IVIG facilitates its ability to neutralize autoantibodies, as is shown in patients with acquired hemophilia due to autoantibodies against factor VIII.

Specific effects of IVIG have been described. The results of in vitro C3 uptake studies and the effect of IVIG on the clearance of preopsonized cells suggest that IVIG produces a kinetic depression of C3 uptake and modifies the process of complement fragment deposition on erythrocytes.

Normal serum contains IgG, IgM, and IgA antibodies, which are referred to as natural antibodies because they are induced without deliberate immunization and are independent of antigenic exposure. They are considered key to the immunoregulatory effects of immune globulin in immune-mediated disorders. Natural autoantibodies appear to be more polyreactive than immune antibodies; natural antibodies can frequently bind to different antigens. Natural autoantibodies can: (1) bind to pathogens; (2) help remove senescent or altered molecules, cells, and tumors; (3) induce remyelination; and (4) inhibit the growth of autoreactive B-cell clones. In the multifocal motor neuropathy disease state, IVIG intercedes to stop complement deposition that is triggered by anti-GM1 antibodies.

IVIG contains cytokines, antibodies of unclear clinical significance, perhaps neutralizing; interestingly, antibodies against granulocyte macrophage colony-stimulating factor, interferon, interleukin 1, and interleukin 6 in immune globulin have biologic activity in vivo. IVIG contains natural antibodies, accounting for some of its effects.

The broad range of applications of IVIG shows the importance of immunoglobulins in the immune homeostasis in healthy people.

A liquid, pasteurized, 10% concentrated intravenous gammaglobulin preparation is as effective as a 5% concentrated preparation.

IVIG replacement prevents severe and lower respiratory tract infections, but not upper respiratory tract and nonrespiratory infections in persons with common variable immune deficiency.

IVIG is used to treat various autoimmune, infectious, and idiopathic diseases. IVIG is an approved treatment for graft versus host disease and ITP. It is accepted for use in persons with Kawasaki disease, Guillain-Barre syndrome, andpolymyositis/dermatomyositis. The beneficial effects of an intramuscular injection

of immune globulin for the prophylactic treatment of patients with primary immunodeficiency syndromes are well established.

It does not work for all diseases; for example, a Korean study of 63 patients (ie, folliculitis decalvans hidradenitis suppurativa, folliculitis, furunculosis) with recalcitrant suppurative skin diseases reported that it helped 59% of patients, but with only a 20% success rate in treating hidradenitis.

Diseases that are purely of hematological or clotting factor defects such as Degos disease or paroxysmal nocturnal hemoglobinuria do not respond to IVIG.

The approved the use of IVIG for the following conditions:

- Allogeneic bone marrow transplantation
- Chronic lymphocytic leukemia
- Common variable immunodeficiency (CVID) - A group of approximately 150 primary immunodeficiencies (PIDs) that have a common set of features (including hypogammaglobulinemia) but that have different underlying causes
- Chronic inflammatory demyelinating polyneuropathy (CIDP) - Solely Gamunex
- Kidney transplantation with a high antibody recipient or with an ABO incompatible donor
- Primary immunodeficiency disorders associated with defects in humoral immunity.
- Immune-mediated thrombocytopenia
- Kawasaki disease (see the Kawasaki Disease Diagnostic Criteria calculator)
- Hematopoietic stem cell transplantation in patients older than 20 years (Gamimune-N only)
- Chronic B-cell lymphocytic leukemia
- Pediatric HIV type 1 infection

Its reported uses have been outlined by the National Guideline Clearinghouse, including the following (off-label) applications:

- Hematology
  - Aplastic anemia
  - Pure red cell aplasia
  - Diamond-Blackfan anemia
  - Autoimmune hemolytic anemia
  - Hemolytic disease of the newborn
  - Acquired factor VIII inhibitors
  - Acquired von Willebrand disease
  - Immune-mediated neutropenia
  - Refractoriness to platelet transfusion
  - Neonatal alloimmune/autoimmune thrombocytopenia
  - Posttransfusion purpura
  - Thrombotic thrombocytopenia purpura/hemolytic uremic syndrome
- Infectious diseases: Conditions in which acquiring an infectious disease could be deleterious include low birth weight (ie, < 1500 g), solid organ transplantation, surgery, trauma, burns, and HIV infection.

- Neurology
  - Epilepsy and pediatric intractable Guillain-Barr? syndrome
  - Chronic inflammatory demyelinating polyneuropathy
  - Myasthenia gravis: IVIG may improves the quality of life in patients; sometimes it is combined with plasmapheresis. A 2014 report noted that IVIG may act as prophylaxis against acute exacerbations.
  - Lambert-Eaton myasthenic syndrome
  - Multifocal motor neuropathy
  - Multiple sclerosis
- Obstetrics: IVIG may be helpful for recurrent pregnancy loss.
- Pulmonology
  - Asthma
  - Chronic chest symptoms
- Rheumatology
  - Rheumatoid arthritis (adult and juvenile)
  - Systemic lupus erythematosus
  - Lupus nephritis
  - Systemic vasculitides
  - Dermatomyositis, polymyositis
  - Inclusion-body myositis
- Wegener granulomatosis: Successful remission induction with the use of IVIG and steroids alone has been described in a woman diagnosed with de novo Wegener granulomatosis during the first trimester of pregnancy. Another report noted that high-dose IVIG could treat severe, corticosteroid-resistant extensive, GCSF-induced Sweet syndrome.
- Miscellaneous
  - Adrenoleukodystrophy
  - Amyotrophic lateral sclerosis
  - Behchet syndrome
  - Acute cardiomyopathy
  - Chronic fatigue syndrome
  - Congenital heart block
  - Cystic fibrosis
  - Autoimmune blistering dermatosis
  - Diabetes mellitus
  - Acute idiopathic dysautonomia
  - Acute disseminated encephalomyelitis
  - Endotoxemia
  - Hemolytic transfusion reaction
  - Hemophagocytic syndrome
  - Acute lymphoblastic leukemia
  - Lower motor neuron syndrome
  - Multiple myeloma
  - Human T-cell lymphotrophic virus-1–associated myelopathy
  - Nephritic syndrome

- Membranous nephropathy
- Nephrotic syndrome
- Euthyroid ophthalmopathy
- Opsoclonus-myoclonus
- Recurrent otitis media
- Paraneoplastic cerebellar degeneration
- Paraproteinemic neuropathy
- Parvovirus infection (general)
- Polyneuropathy, organomegaly, endocrinopathy, M-protein, and skin changes (POEMS) syndrome
- Progressive lumbosacral plexopathy
- Lyme radiculoneuritis
- Rasmussen syndrome
- Reiter syndrome
- Acute renal failure
- Thrombocytopenia (nonimmune)
- Streptococcal toxic shock syndrome
- Uveitis
- Vogt-Koyanagi-Harada syndrome

- In febrile ulceronecrotic pityriasis lichenoides, high-dose IVIG combined with extracorporeal photochemotherapy can be an effective treatment.
- Peripheral polyneuropathy linked to Churg-Strauss syndrome was improved by 6 rounds of high-dose IVIG.

Adverse Effects

Undesirable effects from IVIG occur in less than 5% of patients. The most common adverse effects occur soon after infusions and can include headache, flushing, chills, myalgia, wheezing, tachycardia, lower back pain, nausea, and hypotension. If this happens during an infusion, the infusion should be slowed or stopped. If symptoms are anticipated, a patient can be premedicated with antihistamines and intravenous hydrocortisone.

- IVIG can induce reactions in patients with IgA deficiency. This occurs in 1 in 500-1000 patients. Serious anaphylactoid reactions occur soon after the administration of IVIG. Anaphylaxis associated with sensitization to IgA in patients with IgA deficiency can be prevented by using IgA-depleted immune globulin. The presence of IgG anti-IgA antibodies is not always associated with severe adverse reactions to IVIG.
- Pompholyx (dyshidrotic eczema) and eczematous reactions have been linked to IVIG therapy.
- An uncommon but potentially irreversible adverse event is acute renal failure. Acute renal failure with IVIG therapy occurs with the sucrose-stabilized formulation, but not with the D-sorbitol–stabilized formulation.

- IVIG is associated with rare cases of thrombosis. It has caused disseminated intravascular coagulation, transient serum sickness, and transient neutropenia.
- One study reported 7 patients who had thromboembolic events while being treated with IVIG. Four patients had strokes or transient ischemic attacks, 1 had an inferior wall myocardial infarction, 1 developed deep venous thrombosis, and 1 had a retinal artery infarct. The age range of the patients was 57-81 years, and most had underlying risk factors such as hypertension, hypercholesterolemia, atrial fibrillation, history of vascular disease and stroke, and deep venous thrombosis. Three patients received multiple IVIG infusions before developing the thromboembolic complications. Therefore, clinicians should be vigilant about the possibility of thromboembolic complications with each IVIG infusion and should be especially judicious with the use of IVIG in patients with underlying risk factors.
- Life-threatening human parvovirus B19 infection and hepatitis C have been transmitted by IVIG.
- Severe cutaneous vasculitis has been reported following an intravenous infusion of gammaglobulin in a patient with type II mixed cryoglobulinemia.
- IVIG can precipitate acute myocardial infarction.
- Aseptic meningitis is a rare but well-recognized complication of IVIG therapy. It manifests as fever, neck stiffness, headache, confusion, nausea, and vomiting.
- IVIG therapy can result in postinfusion hyperproteinemia, increased serum viscosity, and pseudohyponatremia.
- IVIG should not be given to patients with sensitivity to thimerosal.
- IVIG has caused eczematous dermatitis and alopecia.
- Complement consumption associated with an eczematous cutaneous reaction has been noted during infusions of high doses of IVIG.
- Orbach et al noted encouraging reports on the efficacy of IVIG in different types of glomerulonephritis (mainly lupus nephritis) resistant to conventional therapy, but the exact success rate and clinical indications remain undetermined. However, the issue of IVIG treatment and renal function is a 2-edged sword because nephrotoxicity can be a serious rare complication of IVIG therapy. Products containing sucrose as a stabilizer are mainly associated with such injury through the mechanism of osmotic nephrosis. Preexisting renal disease, volume depletion, and old age are risk factors for such toxicity.
- It is important the using high-dose (2 mg/kg) IVIG to desensitize transplantation patients who are broadly sensitized to HLA antigens.
    - Since 2000, 57 broadly sensitized patients (19 with cadaver donor kidneys and 38 with living donor kidneys) have been evaluated and subsequently undergone transplantation following IVIG treatment. The incidence of allograft rejection was 38.5%, and 4-year patient and graft survival rates were 96.5% and 82.5%, respectively.
    - IVIG has also been used in combination with pulse steroids to treat antibody-mediated rejection episodes in 18 patients with C4d deposition in rejection biopsy specimens. Thirteen responded to treatment and 5 grafts were lost in this group with severe antibody-mediated rejections.

- These results suggest that in many cases, high-dose IVIG treatment can neutralize or mitigate antibody responses to eliminate positive donor-specific crossmatches and permit transplantation of broadly sensitized patients, and they suggest a means to successfully treat antibody-mediated rejection.

Vaccination is the administration of antigenic material (a vaccine) to stimulate an individual's immune system to develop adaptiveimmunity to a pathogen. Vaccines can prevent or ameliorate morbidity from infection. The effectiveness of vaccination has been widely studied and verified; for example, the influenza vaccine, the HPV vaccine, and the chicken pox vaccine. Vaccination is the most effective method of preventing infectious diseases; widespread immunity due to vaccination is largely responsible for theworldwide eradication of smallpox and the restriction of diseases such as polio, measles, and tetanus from much of the world.

The active agent of a vaccine may be intact but inactivated (non-infective) or attenuated (with reduced infectivity) forms of the causative pathogens, or purified components of the pathogen that have been found to be highly immunogenic (e.g., outer coatproteins of a virus). Toxoids are produced for immunization against toxin-based diseases, such as the modification of tetanospasmintoxin of tetanus to remove its toxic effect but retain its immunogenic effect.

Smallpox was probably the first disease people tried to prevent by inoculating themselves and was the first disease for which a vaccine was produced. The smallpox vaccine was designed in 1796 by the British physician Edward Jenner, although at least six people had used the same principles years earlier. Louis Pasteur furthered the concept through his work in microbiology. The immunization was called *vaccination* because it was derived from a virus affecting cows (Latin: *vacca—cow*). Smallpox was a contagious and deadly disease, causing the deaths of 20–60% of infected adults and over 80% of infected children. When smallpox was finally eradicated in 1979, it had already killed an estimated 300–500 million people in the 20th century.

In common speech, 'vaccination' and 'immunization' have a similar meaning. This distinguishes it from inoculation, which uses unweakened live pathogens, although in common usage either can refer to an immunization. Vaccination efforts have been met with some controversy on scientific, ethical, political, medical safety, and religious grounds. In rare cases, vaccinations can injure people and, in the United States, they may receive compensation for those injuries under the National Vaccine Injury Compensation Program. Early success and compulsion brought widespread acceptance, and mass vaccination campaigns have greatly reduced the incidence of many diseases in numerous geographic regions.

Different Types of Vaccines

The first human vaccines against viruses were based using weaker or attenuated viruses to generate immunity. The smallpox vaccine used cowpox, a poxvirus that was similar enough to smallpox to protect against it but usually didn’t serious illness. Rabies was the first virus attenuated in a lab to create a vaccine for humans.

Vaccines are made using several different processes. They may contain live viruses that have been attenuated (weakened or altered so as not to cause illness); inactivated or killed organisms or viruses; inactivated toxins (for bacterial diseases where toxins generated by the bacteria, and not the bacteria themselves, cause illness); or merely segments of the pathogen (this includes both subunit and conjugate vaccines).

Vaccine type	**Vaccines of this type on U.S. / Europe Recommended Childhood (ages 0-6) Immunization Schedule**
Live, attenuated	Measles, mumps, rubella (MMR combined vaccine) Varicella (chickenpox) Influenza (nasal spray) Rotavirus
Inactivated/Killed	Polio (IPV) Hepatitis A
Toxoid (inactivated toxin)	Diphtheria, tetanus (part of DTaP combined immunization)
Subunit/conjugate	Hepatitis B Influenza (injection) *Haemophilus influenza* type b (Hib) Pertussis (part of DTaP combined immunization) Pneumococcal Meningococcal

Vaccine type	**Other available vaccines**
Live, attenuated	Zoster (shingles) Yellow fever
Inactivated/Killed	Rabies
Subunit/conjugate	Human papillomavirus (HPV)

Live, attenuated vaccines currently recommended as part of the U.S. Childhood Immunization Schedule include those against measles, mumps, and rubella (via the combined MMR vaccine), varicella (chickenpox), and influenza (in the nasal spray version of the seasonal flu vaccine). In addition to live, attenuated vaccines, the immunization schedule includes vaccines of every other major type—see the table above for a breakdown of the vaccine types on the recommended childhood schedule.

The different vaccine types each require different development techniques. Each section below addresses one of the vaccine types.

Live, Attenuated Vaccines

Attenuated vaccines can be made in several different ways. Some of the most common methods involve passing the disease-causing virus through a series of cell cultures or animal embryos (typically chick embryos). Using chick embryos as an example, the virus is grown in different embryos in a series. With each passage, the virus becomes better at replicating in chick cells, but loses its ability to replicate in human cells. A virus targeted for use in a vaccine may be grown through—"passaged" through—upwards of 200 different embryos or cell cultures. Eventually, the attenuated virus will be unable to replicate well (or at all) in human cells, and can be used in a vaccine. All of the methods that involve passing a virus through a non-human host produce a version of the virus that can still be recognized by the human immune system, but cannot replicate well in a human host.

When the resulting vaccine virus is given to a human, it will be unable to replicate enough to cause illness, but will still provoke an immune response that can protect against future infection.

One concern that must be considered is the potential for the vaccine virus to revert to a form capable of causing disease. Mutations that can occur when the vaccine virus replicates in the body may result in more a virulent strain. This is very unlikely, as the vaccine virus's ability to replicate at all is limited; however, it is taken into consideration when developing an attenuated vaccine. It is worth noting that mutations *are* somewhat common with the oral polio vaccine (OPV), a live vaccine that is ingested instead of injected. The vaccine virus can mutate into a virulent form and result in rare cases of paralytic polio. For this reason, OPV is no longer used in the United States, and has been replaced on the Recommended Childhood Immunization Schedule by the inactivated polio vaccine (IPV).
Protection from a live, attenuated vaccine typically outlasts that provided by a killed or inactivated vaccine.

Killed or Inactivated Vaccines

One alternative to attenuated vaccines is a killed or inactivated vaccine. Vaccines of this type are created by inactivating a pathogen, typically using heat or chemicals such as formaldehyde or formalin. This destroys the pathogen's ability to replicate, but keeps it "intact" so that the immune system can still recognize it. ("Inactivated" is generally used rather than "killed" to refer to viral vaccines of this type, as viruses are generally not considered to be alive.)

Because killed or inactivated pathogens can't replicate at all, they can't revert to a more virulent form capable of causing disease (as discussed above with live, attenuated vaccines). However, they tend to provide a shorter length of protection than live vaccines, and are more likely to require boosters to create long-term immunity. Killed or inactivated vaccines on the U.S. Recommended Childhood Immunization Schedule include the inactivated polio vaccine and the seasonal influenza vaccine (in shot form).

Toxoids

Some bacterial diseases are not directly caused by a bacterium itself, but by a toxin produced by the bacterium. One example is tetanus: its symptoms are not caused by the *Clostridium tetani* bacterium, but by a neurotoxin it produces (tetanospasmin). Immunizations for this type of pathogen can be made by inactivating the toxin that causes disease symptoms. As with organisms or viruses used in killed or inactivated vaccines, this can be done via treatment with a chemical such as formalin, or by using heat or other methods.

Immunizations created using inactivated toxins are called *toxoids*. Toxoids can actually be considered killed or inactivated vaccines, but are sometimes given their own category to highlight the fact that they contain an inactivated toxin, and not an inactivated form of bacteria.

Toxoid immunizations on the U.S. Recommended Childhood Immunization schedule include the tetanus and diphtheria immunizations, which are available in a combined form.

Subunit and Conjugate Vaccines

Both subunit and conjugate vaccines contain only pieces of the pathogens they protect against. Subunit vaccines use only part of a target pathogen to provoke a response from the immune system. This may be done by isolating a specific protein from a pathogen and presenting it as an antigen on its own. The acellular pertussis vaccine and influenza vaccine (in shot form) are examples of subunit vaccines. Another type of subunit vaccine can be created via genetic engineering. A gene coding for a vaccine protein is inserted into another virus, or into producer cells in culture. When the carrier virus reproduces, or when the producer cell metabolizes, the vaccine protein is also created. The end result of this approach is a recombinant vaccine: the immune system will recognize the expressed protein and provide future protection against the target virus. The Hepatitis B vaccine currently used in the United States is a recombinant vaccine. Another vaccine made using genetic engineering is the human papillomavirus (HPV) vaccine. Two types of HPV vaccine are available—one provides protection against two strains of HPV, the other four—but both are made in the same way: for each strain, a single viral protein is isolated. When these proteins are expressed, virus-like particles (VLPs) are created. These VLPs contain no genetic material from the viruses and can't cause illness, but prompt an immune response that provides future protection against HPV. Conjugate vaccines are somewhat similar to recombinant vaccines: they're made using a combination of two different components. Conjugate vaccines, however, are made using pieces from the coats of bacteria. These coats are chemically linked to a carrier protein, and the combination is used as a vaccine. Conjugate vaccines are used to create a more powerful, combined immune response: typically the "piece" of bacteria being presented would not generate a strong immune response on its own, while the carrier protein would. The piece of bacteria can't cause illness, but combined with a carrier protein, it can generate immunity against future infection. The vaccines currently in use for children against pneumococcal bacterial infections are made using this technique.

The Future of Immunization.

Vaccines have been a part of the human fight against disease for more than 200 years. The worldwide vaccination campaign eradicated smallpox and immunization has eliminated polio in all but a handful of countries. Childhood vaccination has substantially reduced the morbidity and mortality from infectious diseases in much of the developed world, and yearly influenza vaccination is a commonly accepted practice worldwide to reduce the impact of the seasonal influenza infection.

While we can attribute many public health successes to vaccination, the future presents continued challenges. Diseases remain for which researchers have been unable to find effective vaccines (such as HIV/AIDS, Malaria, and Leishmaniasis) or that flourish in areas of the world where infrastructures for vaccination are poor or nonexistent and even the currently available vaccines cannot be delivered. In other cases, the cost of vaccines is too high for poorer countries to afford, even though this is often where they are most needed. And, of course, although many of the current vaccines are highly effective, efforts continue to develop vaccines that are more effective than those available today. Thus, researchers continue to explore new possibilities. Higher effectiveness, lower cost, and convenient delivery are some of the main goals.

New Development Techniques

The first vaccine—the smallpox vaccine—consisted of a live, attenuated virus. "Attenuation" means weakening a virus to the point where it can still provoke an immune response, but doesn't cause illness in a human host. Many of the vaccines used today, including those for measles and some influenza vaccines, use live, attenuated viruses. Others used killed forms of viruses, pieces of bacteria, or inactivated forms of toxins that the bacteria create. Killed viruses, pieces of bacteria and inactivated toxins can't cause illness, but can still provoke an immune response that protects against future infection.

New techniques are also being employed, however, to create different types of vaccines. Some of these new types include:

- Live recombinant vaccines
- DNA vaccines

Live recombinant vaccines use attenuated viruses (or bacterial strains) as vectors: a virus or bacterium from one disease essentially acts as a delivery device for an immunogenic protein from another infectious agent. In some cases this approach is used to enhance the immune response; in others, it is used when giving the actual agent as a vaccine would cause disease. For example, HIV cannot be attenuated enough to be given as a vaccine in humans—it could cause AIDS. Starting with a complete virus, researchers identify a section of the virus's DNA that is not necessary for replication. One or more genes that code for immunogens of other pathogens are then inserted into this region. (Each gene essentially contains instructions that tell the body how to make

a certain protein. In this case, researchers select genes that code for a protein specific to the target pathogen: an immunogen that will generate an immune response to that pathogen.) For example, a baculovirus (a virus that only infects insects) can be used as a vector and the gene for a particular immunogenic surface protein of an influenza virus may be inserted. When the modified virus is introduced into a person's body, the immunogen is expressed and presented, generating an immune response against the immunogen—and, as a result, against the pathogen it originates from. In addition to insect viruses, human adenoviruses have been considered as potential vectors for use in recombinant vaccines, particularly against diseases such as AIDS. The vaccinia virus, which is the basis for the smallpox vaccine, was the first used in live recombinant vaccine approaches. Experimental recombinant vaccinia strains have been designed to deliver protection against influenza, rabies, and hepatitis B, among other diseases.
DNA vaccines consist of DNA coding for a particular antigen, which is directly injected into the muscle. The DNA itself inserts into the individual's cells, which then produce the antigen from the infectious agent. Since this antigen is foreign, it generates an immune response. This type of vaccine has the benefit of being relatively easy to produce, since DNA is very stable and easy to manufacture, but is still experimental because no DNA-based vaccines have been shown to elicit the substantial immune response required to prevent infection. Researchers are hopeful, however, that DNA vaccines may be able to generate immunity against parasitic diseases such as malaria—currently, there is no human vaccine in use against a parasite.

VACCINATIONS: KNOW THE RISKS

Like the first vaccine for smallpox, every vaccine recommended today by government health officials and medical trade associations carries a risk for complications, such as brain inflammation, which can lead to chronic brain and immune system damage or death.

There is a wide spectrum of vaccine complications, which have been identified and acknowledged in the medical literature and by the Institute of Medicine (IOM), National Academy of Sciences, icluding:

- Brain Inflammation/Acute Encephalopathy
- Chronic Nervous System Dysfunction
- Anaphylaxis
- Febrile Seizures
- Guillain Barre Syndrome (GBS)
- Brachial Neuritis;
- Acute and Chronic Arthritis
- Thrombocytopenia
- Smallpox, polio, measles and varicella zoster vaccine strain infection
- Death (smallpox, polio and measles vaccine)
- Shock and "unusual shock-like state"
- Protracted, inconsolable crying
- Syncope

- Deltoid Bursitis

Outstanding Questions About Vaccines & Chronic Illness

Due to a lack of enough methodologically sound studies conducted and published in the medical literature, the IOM Committee examining the safety of the current childhood vaccine schedule was unable to determine if the schedule *is or is not* associated with the development of the following chronic brain and immune disorders and disabilities in children:

- asthma;
- atopy;
- allergy;
- autoimmunity;
- autism;
- learning disorders;
- communication disorders;
- developmental disorders;
- intellectual disability;
- attention deficit disorder;
- disruptive behavior disorder;
- tics and Tourette's syndrome;
- seizures;
- febrile seizures and
- epilepsy.

Identifying Symptoms of Vaccine Reactions

Not every serious health problem that occurs after vaccination is caused by a vaccine or vaccinations recently received. Different vaccines are associated with different vaccine reaction signs and symptoms that occur within different time periods following vaccination.

If symptoms listed below occur in the hours, days or weeks following vaccination, it is very important to immediately contact a doctor:

- pronounced swelling redness, heat or hardness at injection site that continues for days or weeks;
- body rash or hives;
- shock/collapse;
- unresponsiveness, prolonged deep sleep;
- high pitched screaming (may include arching of back);
- hours of persistent, inconsolable crying;
- high fever (over 103 F)

- respiratory distress (difficulty breathing);
- twitching or jerking of the body, arm, leg or head;
- rolling or crossing of eyes;
- severe head or neck pain;
- joint pain or muscle weakness;
- disabling fatigue;
- loss of memory and mental skills;
- paralysis of any part of body;
- changes in sleep/wake pattern and dramatic personality changes;
- lack of eye contact or social withdrawal
- loss of ability to roll over, sit up or stand up
- head banging or unusual flapping, rubbing, rocking, spinning;
- onset of chronic ear or respiratory problems (including asthma);
- severe/persistent diarrhea or chronic constipation;
- excessive bruising, bleeding or anemia
- other serious loss of physical, mental or emotional wellness

Serious complications of vaccination can lead to permanent injury or death. Make sure that all health problems, hospitalizations and injuries that occur after vaccination are entered into permanent written and electronic medical records and written copies are kept by the person vaccinated or parent/guardian of that person.

Prevenring vaccine reactions: the "first, do no harm" approach to vaccination changed after Congress passed the National Childhood Vaccine Injury Act shielding doctors and vaccine manufacturers from vaccine injury lawsuits. After the law was passed, there was less emphasis on vaccine reaction prevention as public health officials and medical trade associations narrowed the definition of what constitutes a serious vaccine reaction - like convulsions/seizures, high pitched screaming and collapse/shock (hypotonic hyporesponsive episodes) associated with pertussis-containing vaccines.
However, it is very important to read the vaccine manufacturer product information statements to learn more about results of pre-licensure clinical trials and post-marketing vaccine reaction reports, as well as what the vaccine manufacturer considers a contraindication (reason not vaccinate) to use of a particular vaccine.

3. Final part Test-control
Initial knowledge level

1. A potential disadvantage of immunological protection using passive transfer of horse globulins is:
a. Serum sickness
a. Irreversible protection
b. Lack of antibody-mediated immune response
c. Type IV hypersensitivity reactions
d. Immunodeficiency

2. Monoclonal antibodies are a type of vaccine.
a. False
b. True
c. Unknown
d. Not applicable
e. Monoclonal antibodies are not used in oncology.

3.Which of the following types of targeted therapies are usually given to patients orally?
a. Monoclonal antibodies
b. Small molecules
c. Vaccines
d. Unknown
e. Not applicable

4.Which types of therapies can cause the immune system to attack cancer cells?
a. Small molecules
b. Monoclonal antibodies
c. Both Monoclonal antibodies and Vaccines
d. Vaccines
e. Not applicable

5.Which types of therapies can be used to directly target molecules inside the cell?
a. Vaccines
b. Small molecules
c. NSAID's
d. Unknown
e. Not applicable

6. BCG is used to protect against:
a. Tuberculosis
b. Rabies
c. Hepatitis B
d. Influenza
e. Pertussis

7. How do cancer cells evade apoptosis?
a. Reduce the activity of proteins that detect DNA damage
b. Mutation of proteins that induce apoptosis
c. Increase the activity of proteins that prevent apoptosis
d. All of the above
e. Not applicable

8. Angiogenesis inhibitors will prevent existing blood vessels from delivering oxygen and nutrients to normal cells.
a. True
b. False
c. Unknown
d. Not applicable
e. Angiogenesis inhibitors are not used in oncology.

9. There are the next types of immunotherapy:
a. cytokines
b. vaccines
c. bacillus Calmette-Guerin (BCG)
d. some monoclonal antibodies
e. All of the above

10. Monoclonal antibodies can be used:
a. alone directly to cancer cells
b. to carry drugs directly to cancer cells
c. to carry toxins directly to cancer cells
d. to carry radioactive substances directly to cancer cells
e. All of the above

11. Which of the following statements about clinical features and factors affecting management of hand osteoarthritis is most likely correct?
a. Hand osteoarthritis is not associated with significant disability
b. Hand osteoarthritis is limited to the articular cartilage only
c. Lipid abnormalities have not been reported as a comorbidity
d. Pain, occupation, multisite involvement, illness perceptions, and impact should form part of a biopsychosocial assessment of hand osteoarthritis, as these may affect management
e. All of the above

12. Your patient is a 74-year-old male thought to have hand osteoarthritis. Which of the following statements about diagnosis of hand osteoarthritis is most likely correct?
a. Imaging shows that erosive osteoarthritis is a less severe form of hand osteoarthritis characterized by marginal erosions

b. Radiographic metacarpophalangeal joint osteoarthritis has a similar pattern to interphalangeal and thumb base osteoarthritis
c. Magnetic resonance imaging (MRI) may offer more insight into disease progression from onset to joint failure and risk factors associated with disease progression
d. The diagnosis of generalized osteoarthritis does not require hand osteoarthritis
e. Not applicable

13. Which of the following statements about treatment of hand osteoarthritis would most likely be correct?
a. Systemic treatments are preferred over nonpharmacological approaches
b. Oral nonsteroidal anti-inflammatory drugs (NSAIDS) are first-line pharmacotherapy
c. Advice and education are recommended, emphasizing joint protection education and hand exercises
d. No guidelines are currently available for the diagnosis and management of hand osteoarthritis
e. Not applicable

14. You are seeing a 63-year-old woman with a history of rheumatoid arthritis (RA) and a 7-day history of cough and fever. What should you consider regarding how RA affects the risk of infection?
a. Anti-tumor necrosis factor (TNF) therapies generally reduce the risk of infection
b. Patients who are at increased risk of tuberculosis should receive disease-modifying anti rheumatic drug (DMARD) therapy with infliximab
c. The lung is the most common site of infection among patients with RA
d. The pneumococcal vaccine is ineffective among patients taking methotrexate
e. Not applicable

15. As the patient is being prepared for discharge, what should you consider regarding the effects of her RA treatment on her risk of concurrent disease?
a. Anti-TNF agents can reduce the progression of ILD
b. The incidence of methotrexate-induced pneumonitis is increasing
c. Anti-TNF agents improve bone density
d. Methotrexate promotes a higher risk of cardiac events
e. Not applicable

Final knowledge level

1. Tumor necrosis factor (TNF) inhibitors have wide-ranging effects, including acting on which of the following pathogenetic mechanism in psoriatic arthritis (PsA)?
   a. Polymorphonuclear cells
   b. E-selectin
   c. T helper 17 (T_h17) cells
   d. Interleukin (IL)-8
   e. Not applicable

2. Which disease domains of PsA are disease-modifying antirheumatic drugs (DMARDs) such as methotrexate and sulfasalazine believed to benefit?
   a. Peripheral arthritis, skin/nail disease, axial disease, dactylitis, enthesitis
   b. Skin/nail disease, axial disease, dactylitis, enthesitis
   c. Axial disease, dactylitis, enthesitis
   d. Peripheral arthritis, skin/nail disease
   e. All of the above

3. Glucocorticoid injections are recommended as adjunctive therapy. Which of the following events has been reported with corticosteroid taper?
   a. Enthesitis
   b. Dactylitis
   c. Skin flares
   d. Single joint flares
   e. All of the above

4. The first production of live but non-virulent forms of chicken cholera bacillus was achieved by:
a. Pasteur.
b. Salk
c. Jenner
d. Montague
e. Sabin

5. The term variolation refers to:
a. The generation of antibody variable regions.
b. The attenuation of virulent organisms
c. Innoculation of scab material into small skin wounds
d. The removal of scab material
e. A type of gene therapy from an individual with smallpox

6. The circulation of a two month old breast-fed baby will contain maternal:
a. IgA
b. IgD
c. IgE

d. IgG
e. IgM

7. What is the minimum percentage of children which needs to be vaccinated successfully in order to achieve herd immunity to diphtheria;
a. 100%
b. 10%
c. 50%
d. 75%
e. Herd immunity cannot be achieved against diphtheria

8. For vaccination against mycobacterial diseases such as tuberculosis, the most important facet of the immune response to be stimulated is:
a. A high titer of antibody
b. Macrophage-activating cell-mediated
c. Cytotoxic T-cells immunity
d. Antibody in the gut lumen
e. Neutrophils

9. Which one of the following diseases has been completely eradicated world-wide?
a. Measles
b. Smallpox
c. Tuberculosis
d. Cowpox
e. Psittacosis

10. A protein that is part of a growth signaling pathway inside the cell is mutated, causing it to become continually active and resulting in the formation of a tumor. What type of targeted therapy might be effective?
a. Monoclonal antibody that prevents growth factors from interacting with the receptor
b. Monoclonal antibody that holds the growth factor receptor in the "OFF" position
c. Small molecule that selectively binds to the mutated protein
d. Monoclonal antibody that selectively binds to the mutated protein
e. Not applicable

11. A small protein subunit used in a vaccine may fail to stimulate T-cell immunity because of:
a. Lack of glycosylation
b. Lack of conformation
c. Lack of carrier determinants
d. HLA-related
e. Inherently insufficient antigen concentration unresponsiveness

12.Which of the following can baculovirus vectors produced in cell lines be derived from?

e. Moth

b.Chicken bursa

c. Chinese hamster ovary

d. Tobacco

e. Green monkey kidney

13.DNA vaccines:

a. Are relatively poor at stimulating cytotoxic T lymphocyte responses in mice

b. Must be administered on gold particles if they are to be effective

c. Are only effective if followed by a protein boost

d. May have distinct advantages when preparing subunit vaccines against viruses which frequently alter their antigens

e. Require cold storage in tropical countries

14. A peptide immunogen:

a. Adopts a single rigid structure in solution

b. Can mimic a part of a discontinuous epitope

c. Can elicit potent antibody responses in the absence of T-cell help

d. Can be used to stimulate B-cell but not T-cell responses

e. Lack contact residues capable of interacting with the antigen receptor on the relevant lymphocytes

15.Tetanus toxoid is usually given to humans:

a. Absorbed to aluminum hydroxide

b. With complete Freund's adjuvant

c. Without the addition of any other agent

d. Together with the toxin

e. Only as a therapeutic agent, not prophylactically

CORRECT ANSWERS:

Initial knowledge level:

1.A; 2.A; 3.B; 4.C; 5.B; 6.A; 7.D; 8. B; 9.E; 10.E; 11.D; 12.C; 13.C; 14. C; 15.C

Final knowledge level:

1.C; 2.D; 3. C; 4.A; 5.C; 6.D; 7.D; 8.B; 9.B; 10.C; 11.D; 12.A; 13.D; 14.B; 15.A

Control questions:

1. Kinds of immunotropic therapy, definition.
2. Classification of immunotropic drugs, mechanism of action, side effects.
3. The principles of clinical application of immunotropic preparations, indications and contraindications.
4. Immunological control for the therapeutic effectiveness: immunosuppressive drugs; drugs of the immune correction; blockers of mediators of the immune reactions; anti-inflammatory drugs; Replacement therapy; cytokine therapy, anti-receptors drugs, etc..
5. Basic principles of immunoprophylaxis of bacterial and viral infections.
6. The main types of immunorehabilitation, its strategy, tactics and basic principles.
7. How do vaccines work? Do they work against viruses and bacteria?
8. Why aren't all vaccines 100% effective?
9. Why are there so many vaccines?
10. Is natural immunity better than vaccine acquired immunity?
11. Why do some vaccines require boosters?
12. I was invited to a chicken pox party. Would it be better for my child to get the chicken pox this way? Why do we vaccinate against a mild disease like chicken pox?
13. Can you get a disease from the vaccine that's supposed to prevent it? And why do some vaccines have live pathogens but others have killed pathogens?
14. Can babies' immune systems handle so many vaccines?
15. Why is there a new flu vaccine every year?
16. What is herd immunity? Is it real? Does it work?
17. Why is allergy to eggs a contraindication to getting some vaccines?
18. Do vaccines cause autism?
19. People say that vaccines are linked to long-term health problems such as multiple sclerosis, diabetes, and autism. Is that true?
20. The vaccine information sheet for my child's recent vaccination listed lots of potential side effects. Why is vaccination recommended if it can cause all of these side effects?
21. Do we do enough safety testing with vaccines?
22. Do vaccines have aborted fetal tissue?
23. Isn't it true that better hygiene and nutrition were responsible for decreases in deaths and disease rates, rather than vaccines?
24. Why can't we eradicate other diseases, as we did with smallpox?
25. Is the polio vaccine linked to HIV?
26. Is the polio vaccine linked with cancer?

Practical skills and abilities

1. Know immunotropic main groups of drugs and mechanisms of action.
2. To be able to correctly assign Immunotropic therapy.
3. To be able to examine the person who vaktsynuyetsya to prevent the possible occurrence of post-vaccination complications.
4. To be able to evaluate the effectiveness of the vaccination.

5. To be able to make immunization schedule in respect of a particular person.

Structure of class.

Plan and organizational structure of the lesson:

Preparatory stage: organization of lesson and test control of incoming level of knowledge (5 academic hours or 225 minutes)

№	Content	Level of learning	Methods of the learning control	Materials of the methodological support	Time, min.
1.	The preparatory phase Organisation of classes Educational objectives	I	1. Front rapid survey	Tests. Scheme.	25
2.	The main stage Control input level of knowledge, skills: - The history of vaccination; - Types of vaccines; - Immune response after vaccination. The term "immune modulation", its synonyms; concept of immunization, immunization activities; indications for immunosuppressive therapy; main groups of drugs with immunosuppressive action; replacement therapy; therapy with cytokines; immunostimulation, fashion, drugs; immune reconstruction.	 II II II II II II-III II-III III III III	1. Front rapid survey. 2. Individual oral examination. 3. Test control.	1. Tables. 2. Folio-grams. 3. Tests I-III 1. Scheme. 2. Slideshow.	180
3.	The final stage Monitoring and adjustment of professional knowledge, skills and abilities: basic principles-purpose immunotropic therapy; - Vaccination complications, their diagnosis; - Prospects and problems of vaccination.	III III III	1. Testing. 2. Solving custom applications. 3. Oral examination.	1. Tests III-IV levels. 2. Situational problems.	15
4.	To sum up the lessons. Homework for the next topic.				2 3

Task for homework

Remember the structure and functions of certain links of immunity, normal laboratory parameters, classification of immunotropic means.

Individual work:

Make independently a clinical examination of the patient (anamnesis, examination, work with medical documents) and based on obtained the data to decide whether the dysfunction of immunity occurs in these pateints, and what methods of immunocorrection must be used in this case.

Recommended Literature:

1. Abbas A. Cellular and Molecular Immunology. – 6th Edition. – 2009. – 576 p.
2. Male D., Brostoff J., Roth D., Roitt I. Immunology. – 7th Edition. – 2006. – 564 p.
3. Peakman M., Vergani D. Basic and Clinical Immunology. – 2nd Edition. – 2009. – 352 p.
4. Abbas A., Lichtman A. Basic Immunology: Functions and Disorders of the Immune System. – 3rd Edition. – 2008. – 320 p.
5. Nairn R., Helbert M. Immunology for Medical Students. – 2nd Edition. – 2007. – 320 p.
6. Volcheck P., Gerald W. Clinical allergy: diagnosis and management. – Humana Press Inc., 2009. – 491 p.

METHODICAL INSTRUCTION

Practical class №4

1. THEME. IMMUNODIAGNOSTICS AND THERAPY IN IMMUNOONCOLOGY. IMMUNODIAGNOSTICS AND THERAPY IN IMMUNOTRANSPLANTATION (5 academic hours).

2. Background: due to the increasing incidence of tumors in Ukraine over last 5 years (25% cancer), immunology has occupied a special place among the other branches of fundamental immunology. In relation to the relentless rise of diseases that are not amenable to conservative treatment, the attention of clinicians and researchers is increasingly attracted by transplantation as one of the promising areas of practical medicine and clinical immunology in particular.

3 Aim:

- ***Study:*** Students need to learn the basic, know the current views on the characteristics of transplantation immunity;
be able to diagnose based on clinical, laboratory studies, determine the main manifestations of graft rejection;
be able to administer the treatment of transplant disease;
students should learn the basic pro- and anticancer factors of immunity and directions of modern cancer immunotherapy.

- ***Professionally oriented:*** Students should know the basic stages of the immune response and the types of their regulation;
students must learn to use data of immunological laboratory studies for diagnosing cancer pathology, substantiate the feasibility of consultation of cancer-stricken patients with immunologist.
- ***Educational:*** to shape students' sense of responsibility for the timeliness and appropriateness of professional activities;
form a sense of accountability for timeliness and correctness of professional actions.

4. Materials: Equipment to run powerpoint presentation
Main books. Short information on the topic.

5. Interdiscipline integration

5.1. Interdisciplinary integration: the topic of this practical class is connected with topics of General Immunology: factors of innate immunity in anti-tumor protection, tumor antigens and antitumor antibodies, especially immune responses in tumors, the concept of monoclonal antibody as an anti-tumor agent

Out-disciplinary integration (tumor immunology)

Discipline	To Know	To Be Able To
1	2	3
Pathoanatomy	Structural change of a conditions of tumor processes	Microscopically evaluate morphological signs of cancer pathology
Pathophysiology	Definition of "tumor" and "tumor process, chemical, physical, viral carcinogenesis, interaction of tumors and the body	Slight changes of laboratory indicators of the development of tumor processes
Propedeutic training of therapy	Approaches to the examination of organs and systems	Perform palpation of organs, percussions
Oncology	Peculiarities of the physical examination of patients with suspected cancer pathology	Evaluate the results of the clinical, laboratory and instrumental methods of examination of patients with suspected cancer pathology
Pharmacology	The main groups of medications with cytotoxic, cytostatic, imunodepressive action	Justify the indication and write prescribe drugs

A. Out-disciplinary integration (immunology of transplantation)

Subject	**To know**	**Be able**
1	**2**	**3**
Biology	Cell structure, the properties of the cell membrane structure of cell receptors	Recognize under the microscope nucleus, cytoplasm, the cell membrane
Anatomy	The body’s immune system	Specify the body’s immune system
Histology	Cells of the immune system	Microscopically distinguish between cells of the immune system
Physiology	The main function of the immune system.	Specify the main methods for evaluating the immune system
Patanatomy	Structural changes of the immune system	Microscopically assess the morphological features of immunopathology

Pathophysiology	Immunological reactivity. Fundamentals of immunopathological processes.	Interpret changes in overall performance levels under conditions of immunopathology
Propaedeutic therapy	Features of the examination of the immune system	Identify the blood group by ABO System and Rh. To define a group, individual, biological compatibilities in blood transfusions
General Surgery	Fundamentals of hemotransfusion	Perform blood group ABO system and Rh, individual and biological compatibilities
Pharmacology	The main groups of immunosuppressive drugs and their mechanism of action	Prescribe immunosuppressants

6. Student has to know:

1. Types of tumor antigens.
2. Mechanism of tumor antigenesis
3. Classification of tumor antigens.
4. Importance of tumor antigens.
5. Paradigms for the role of innate immune cells in cancer innate cell polarization.
6. Management of the patients with cancer.
7. Monoclonal antibody (mAb) in cancer: advances and challenges.
8. Types of transplants
9. Breeding pairs of donor-recipient
10. Types of allograft rejection
11. Mechanisms of rejection
12. Immunological monitoring of patients after transplantation
13. Postoperative complications
14. Transplant disease

Main part

Tumor antigen is an antigenic substance produced in tumor cells, i.e., it triggers an immune response in the host. Tumor antigens are useful tumor markers in identifying tumor cells with diagnostic tests and are potential candidates for use in cancer therapy.

Mechanism of tumor antigenesis

Normal proteins in the body are not antigenic because of self-tolerance, a process in which self-reacting cytotoxic T lymphocytes (CTLs) and autoantibody-producing B lymphocytes are culled "centrally" in primary lymphatic tissue (BM) and "peripherally" in secondary lymphatic tissue (mostly thymus for T-cells and spleen/lymph nodes for B cells). Thus any protein that is not exposed to the immune system triggers an immune response. This may include normal proteins that are well sequestered from the immune system, proteins that are normally produced in extremely small quantities, proteins that are normally produced only in certain stages of development, or proteins whose structure is modified due to mutation.

Classification of tumor antigens

Initially tumor antigens were broadly classified into two categories based on their pattern of expression: Tumor-Specific Antigens (TSA), which are present only on tumor cells and not on any other cell and Tumor-Associated Antigens (TAA), which are present on some tumor cells and also some normal cells.

This classification, however,is imperfect because many antigens thought to be tumor-specific turned out to be expressed on some normal cells as well. The modern classification of tumor antigens is based on their molecular structure and source.

Accordingly they can be classified as;

- Products of Mutated Oncogenes and Tumor Suppressor Genes
- Products of Other Mutated Genes
- Overexpressed or Aberrantly Expressed Cellular Proteins
- Tumor Antigens Produced by Oncogenic Viruses
- Oncofetal Antigens
- Altered Cell Surface Glycolipids and Glycoproteins
- Cell Type-Specific Differentiation Antigens

Types

Any protein produced in a tumor cell that has an abnormal structure due to mutation can act as a tumor antigen. Such abnormal proteins are produced due to mutation of the concerned gene. Mutation of protooncogenes and tumor suppressors which lead to abnormal protein production are the cause of the tumor and

thus such abnormal proteins are called tumor-specific antigens. Examples of tumor-specific antigens include the abnormal products of ras and p53 genes. In contrast, mutation of other genes unrelated to the tumor formation may lead to synthesis of abnormal proteins which are called tumor-associated antigens.

Proteins that are normally produced in very low quantities but whose production is dramatically increased in tumor cells, trigger an immune response. An example of such a protein is the enzyme tyrosinase, which is required for melanin production. Normally tyrosinase is produced in minute quantities but its levels are very much elevated in melanoma cells.

Oncofetal antigens are another important class of tumor antigens. Examples are alphafetoprotein (AFP) and carcinoembryonic antigen (CEA). These proteins are normally produced in the early stages of embryonic development and disappear by the time the immune system is fully developed. Thus self-tolerance does not develop against these antigens.

Abnormal proteins are also produced by cells infected with oncoviruses, e.g. EBV and HPV. Cells infected by these viruses contain latent viral DNA which is transcribed and the resulting protein produces an immune response.

In addition to proteins, other substances like cell surface glycolipids and glycoproteins may also have an abnormal structure in tumor cells and could thus be targets of the immune system.

Importance of tumor antigens

Tumor antigens, because of their relative abundance in tumor cells are useful in identifying specific tumor cells. Certain tumors have certain tumor antigens in abundance.

Tumor antigen	Tumor in which it is found	Remarks
Alphafetoprotein (AFP)	Germ cell tumors Hepatocellular carcinoma	
Carcinoembryonic antigen (CEA)	Bowel cancers	Occasional lung or breast cancer
CA-125	Ovarian cancer	
MUC-1	Breast cancer	
Epithelial tumor antigen (ETA)	Breast cancer	

Tyrosinase	Malignant melanoma	Normally present in minute quantities; greatly elevated levels in melanoma
Melanoma-associated antigen (MAGE)	Malignant melanoma	Also normally present in the testis
Abnormal products of ras, p53	Various tumors	

Certain tumor antigens are thus used as tumor markers. More importantly, tumor antigens can be used in cancer therapy as tumor antigen vaccines.

Paradigms for the role of innate immune cells in cancer innate cell polarization.

Macrophages and neutrophils exist in a range of activation states that reflect their environment, and they can be polarized towards functional subclasses depending on the nature of the stress afflicting the tissue. One polarizing extreme is induced by IFN-γ–producing T_H1 cells and the engagement of TLRs by bacterial products, as in the case of bacterial or viral infection, generating conventionally activated macrophages (M1), neutrophils (N1), and myeloid progenitor cells. In contrast, responses to parasitic infections and wound healing involve the production of type II cytokines such as IL-4, IL-13, and TGF-β, which drive "alternative" (M2/N2) cell activation. These polarized activation states are contrasting driving forces in tumorigenesis, whereas M1/N1 cells are tumoricidal and destructive to the tissue, M2/N2 cells account for the production of growth factors (e.g., EGF, FGF-2), angiogenic factors (e.g., VEGF, MMP-9), and inflammatory cytokines (e.g., TNF-α, IL-1), which collectively promote tumor initiation, progression, and metastasis. Extreme stress conditions such as hypoxia, necrosis, and injury are hallmarks of the tumor microenvironment and are exploited by the tumor to recruit and polarize inflammatory myeloid cells towards the tumor-promoting state.

Control of tissue regeneration and integrity.

There is a network between different cells of the innate immune system that controls not only the adaptive immune response, but also the microflora, tissue regeneration, and carcinogenesis, particularly in the intestine. One example for such a complex regulatory mechanism is the control of IL-22 by DCs in the intestine. IL-22, a member of the IL-10 cytokine family can be produced by ILC3 and T_H17 cells. IL-22 has a protective function during the early phase of tissue damage, whereby it promotes the activation of intestinal stem cells and in turn the wound healing of the intestine. However, a high concentration of free IL-22 over a long period of time can be detrimental. IL-22 has been shown to promote colitis, which is associated with mucosal hyperplasia. Furthermore, results from several recent articles have shown that IL-22 can also promote tumorigenesis in the intestine. Accordingly, IL-22 needs to be carefully controlled, and this regulation can be exerted by DCs in the intestine by at least two mechanisms. First, DCs have the capacity to promote the expression of IL-

22 through the production of IL-23. DCs in the intestine participate in immune surveillance for possible pathogenic invasion. For example, microbial penetration and the presence of flagellin promote the activation of a particular subset of DCs, which are present in the lamina propria and which coexpress CD11b and CD103. Upon activation via TLR-5, these DCs produce a large amount of IL-23, which in turn boosts the production of IL-22.
DCs can also control IL-22 via the production of a soluble IL-22 receptor [IL-22 binding protein (IL22BP, IL22Ra2)]. IL-22BP is produced by DCs in steady-state conditions. Upon sensing tissue damage, the inflammasome shuts down the expression of IL-22BP via IL-18 activation, thereby allowing IL-22 to exert its protective effect. This is one example for a complex mechanism by which DCs regulate the availability and production of one cytokine to control homeostasis in the intestine. Perturbation of this coordinated crosstalk between microflora, DCs, and ILC3 at any stage may alter the balance in the intestine and result in increased tumorigenesis.

Immunosurveillance and immunoediting.

The capacity of DCs to prime $CD8^+$ T cells and trigger their cytotoxic activity against neoplastic cells is considered to be one of the key mechanisms by which the immune system can monitor and control the growth of tumor. In 1957, Sir Macfarlane Brunet and Lewis Thomas proposed for the first time the hypothesis of "cancer immunosurveillance". The hypothesis, initially vague, has been validated by recent data, which clearly show that the immune system can interact with neoplastic cells and DCs are one of the pivotal components in this immunosurveillance. DCs can present tumor antigens to the adaptive immune system, which in turn is instructed to control the growth of transformed cells. Mice that lack RAG-1 or RAG-2 cannot produce lymphocytes and they have higher susceptibility to develop tumors. One of the most convincing lines of evidence supporting the hypothesis of tumor immunosurveillance is provided by humans afflicted by paraneoplastic syndromes, which are neurologic disorders that arise as a consequence of an antitumor immune response. The same antigens, which are normally expressed in neurons, can be expressed in breast cancer cells and some other carcinomas. Some patients with paraneoplastic syndromes develop a strong antigen-specific $CD8^+$ T-cell–mediated response that efficiently controls tumor expansion, but that concomitantly results in autoimmune cerebellar degeneration. Immunosurveillance may be defined as the first stage of a continual "immunoediting" process in which tumor cells circumvent antitumor T-cell activity by the emergence of less immunogenic cells within the tumor. Moreover, the tumor microenvironment can block tumor-specific T-cell responses by converting myeloid cells into potent immunosuppressive cells.

Cancer management.

Cancer can be treated by surgery, chemotherapy, radiation therapy, immunotherapy, and monoclonal antibody therapy. The choice of therapy depends upon the location and grade of the tumor and the stage of the disease, as well as the general state of the (performance status). A number of experimental cancer treatments are also under development. Complete removal of the cancer without damage to the rest of the body

is the goal of treatment. Sometimes this can be accomplished by surgery, but the propensity of cancers to invade adjacent tissue or to spread to distant sites by microscopic metastasis often limits its effectiveness; chemotherapy and radiotherapy can unfortunately have a negative effect on normal cells. Because "cancer" refers to a class of diseases, it is unlikely that there will ever be a single "cure for cancer" any more than there will be a single treatment for all infectious diseases. Angiogenesis inhibitors were once thought to have potential as a "silver bullet" treatment applicable to many types of cancer, but this has not been the case in practice.

Monoclonal antibody (mAb) in cancer: advances and challenges.

Despite major advances in our understanding of cancer biology and technological advances in cancer diagnosis and therapy over past three decades, cancer is a global health problem and a major cause of death worldwide. In the UK, there were over 320 500 new cancer cases and cancer was responsible for 157 250 deaths in 2011. Worldwide, there were an estimated 12.7 million new cancer cases and 7.6 million cancer deaths in 2013. At present, there are three major approaches for reversing the worldwide increase in cancer incidence and mortality. The first and simplest approach is preventive measures such as reducing exposure to known carcinogenic agents (e.g. smoking, chemicals, infectious agents), healthier diets and the development of prophylactic cancer vaccines (e.g. HPV vaccines Gardasil and Cervarix). The second approach is detecting cancer at an earlier stage of the disease. This in turn would require the identification of more reliable tumour biomarkers and simpler screening methods. The third, and most expensive, approach is by developing more effective, tumour specific and consequently less toxic and more affordable anti-cancer drugs. In this review, we discuss and highlight some of the advances, current challenges and future opportunities for targeted therapy of human cancers using monoclonal antibody (mAb)-based products.

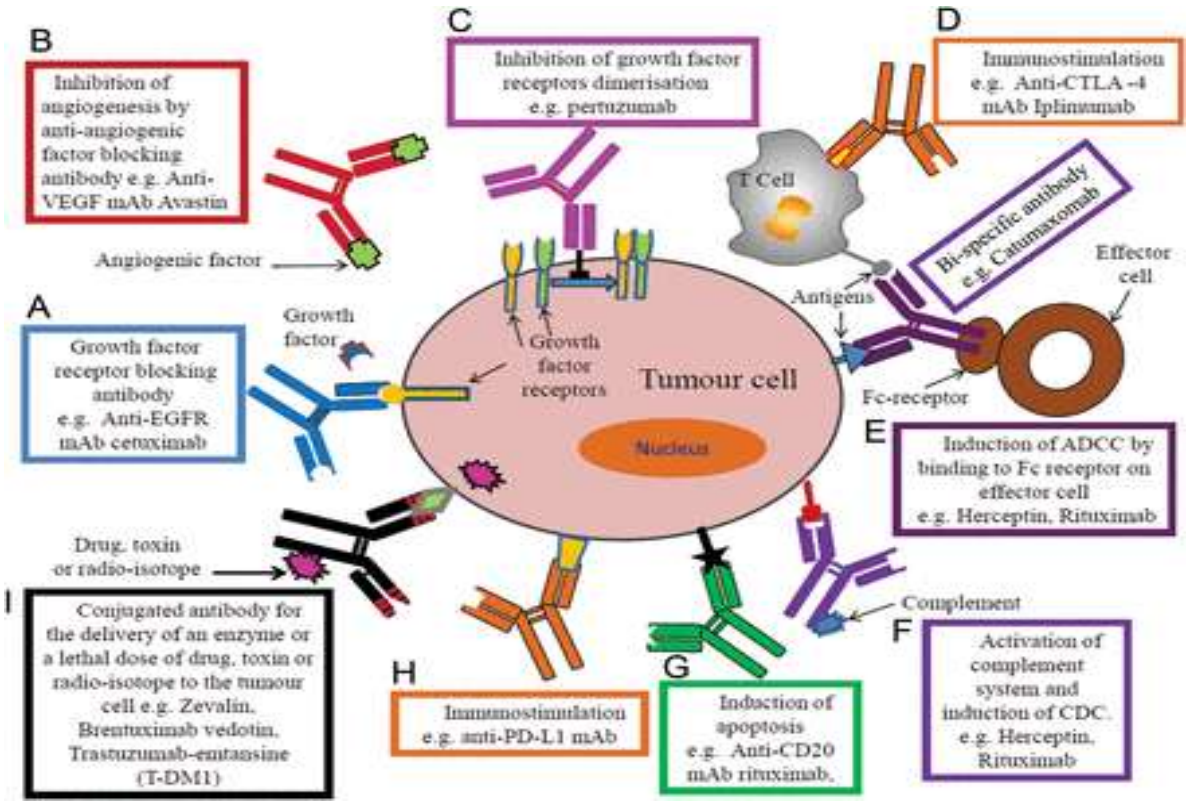
Advances in targeted therapy of human cancers using mAbs

The advent of hybridoma technology by Kohler and Milstein in 1975 for which they were awarded the Nobel prize in medicine in 1984 revolutionized many areas of biological and medical research.Prior to hybridoma technology, antibodies were generated by the repeated immunization of animals with the antigen of interest and then sera from these animals were used for many applications, including therapy. Unfortunately, the administration of crude preparations of sera, which contained other animal proteins and a mixture of antibodies, produced allergic reactions and no clinical benefit in many patients. In contrast, the advent of hybridoma technology has allowed scientists, for the first time, to produce unlimited quantities of a specific type of antibody (i.e. mAb) against a particular antigen by immortalizing the antibody-producing B lymphocytes from the spleen of immunized mice.

mAbs have become essential tools in unravelling the role of many genes and their protein products in tumour pathogenesis and other pathological conditions; in the discovery of novel and overexpressed cell surface antigens and in the diagnosis and therapy of many diseases including human cancers.However, most mouse mAbs,

produced using the traditional hybridoma technology, are highly immunogenic in cancer patients leading to the generation of human anti-mouse antibody (HAMA) response, resulting in their rapid clearance from the patients' serum. Following technological advances in genetic engineering in the 1980s and 1990s, it has been possible to reduce the immunogenicity and to increase the serum half-life of some rodent antibodies in humans by producing chimeric and humanized versions of such antibodies. Alternatively, using either transgenic mice containing human immunoglobulin gene or phage display technology, it has been possible to develop fully human mAbs against human antigens. Further biomedical and technological advances have facilitated the development of bispecific mAbs, antibody–drug conjugates as well as smaller antibody fragments for use in therapeutic applications and cancer imaging.

mAbs can induce their anti-tumour activities by several mechanisms. These are dependent on (i) the subclass of the antibodies and whether they are capable of binding to, and activating, the Fc receptors on host immune effector cells by inducing antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), (ii) the target antigen and (iii) whether the antibodies are conjugated to a lethal drug, toxin or therapeutic radioisotope. In general, an ideal antigen for mAb-based targeted therapy of human cancers should have the following characteristics. First, the antigen should be overexpressed on the tumour cell surface and therefore be accessible to the therapeutic antibody for inducing ADCC and CDC, with no or a low level of expression in normal cells. Secondly, there should be homogenous expression of such antigens on tumour cells, but limited, or no shedding of antigens (e.g. growth factor receptor extracellular domains such as EGFR, HER-2) or other factors in patients' sera, which could trap or compete with the administered therapeutic antibody for binding to the target antigen on tumour cells. Thirdly, the antigen should also play an important role in cancer progression and contribute to the hallmarks of cancer such as sustained proliferating signals, increased angiogenesis, migration and invasion, resistance to apoptosis or induction of immunosuppression.Because of their specificity and high affinity towards particular antigens, mAb-based products and antibody fragments account for around 30% of all new biotechnology drugs in development and hundreds of these antibodies are currently at different stages of clinical trials.



The mechanisms by which mAb-based drugs can induce their therapeutic effects. Unconjugated antibodies can induce their therapeutic effect by (A) blocking the binding of growth factors to growth factor receptor and subsequent cell signalling pathways essential for cell proliferation such as anti-EGFR mAb cetuximab, (B) blocking and trapping an angiogenic factor such as anti-VEGF mAb Avastin, (C) preventing growth factor receptor–receptor dimerization and subsequent signal transduction pathways such as anti-HER mAb pertuzumab, (D) by blocking a key negative regulator of immune activity on T cells such as anti-CTLA-4 mAb ipilimumab, (E) binding to Fc receptors on effector cells (e.g. NK cells, macrophages, dendritic cells) and inducing ADCC such as anti-CD20 rituximab, (F) activating the complement system and inducing complement-mediated cytotoxicity CDC, (G) inducing apoptosis via upregulation of pro-apoptotic factors and downregulation of anti-apoptotic factors. (H) Blocking a key suppressor of the immune system expressed on tumour cells such as anti-programmed cell death 1 ligand 1 (PD-L1) antibody. In addition, mAbs can be conjugated to a therapeutic radio-isotope, drug or toxin for delivering a lethal dose of such agents to cancer cells (I).

There are currently several mAb-based products which have been approved by the US Food and Drug Administration (FDA) and/or the European Union Health Authorities for the treatment of a wide range of diseases including autoimmunity, organ transplant rejection, inflammation, infection as well as human cancers. Of these, 14 antibody-based products have been approved for the treatment of patients with haematological malignancies and a wide range of solid cancers. In the following sections, the characteristic features of some of the antibodies and their target antigens are discussed.

Table 1

Therapeutic mAbs approved in the USA and European Union for use in cancer management.

Antibody name generic/trade	Antibody format	Target antigen	Therapeutic area	Approved year
Rituximab/Rituxan	Chimeric IgG1	CD20	B-cell lymphoma, NHL	1997
			Chronic lymphocytic leukemia	2010
Trastuzumab/Herceptin	Humanized IgG1	HER-2	Metastatic breast cancer	1998
			Early stage breast cancer	2006
			Metastatic stomach cancer	2010
Gentuzumab Ozogamicin/Mylotarg	Humanized IgG1	CD33	Acute myeloid leukaemia	2000
Alezumtumab/Campath	Humanized IgG1	CD52	Chronic myeloid leukaemia	2001
Ibritumomab Tiuxetan/Zevalin	Mouse IgG1 conjugated to ^{90}Y)	CD20	NHL	2002
Tositumomab/Bexxar	Mouse IgG1 conjugated to ^{131}I	CD20	NHL	2003
Bevacizumab/Avastin	Humanized IgG1	VEGF	Metastatic colorectal cancer	2004
			Non-small-cell lung cancer	2006
			Metastatic renal cancer	2009
			GBM	2009
			Ovarian cancer (in Europe only)	2011
Cetuximab/Erbitux	Chimeric IgG1	EGFR	Metastatic colorectal cancer	2004
			Head and neck cancer	2006
			Metastatic colorectal cancer	2012

Antibody name generic/trade	Antibody format	Target antigen	Therapeutic area	Approved year
			(first-line treatment)	
Panitumumab/Vectibix	Human IgG2	EGFR	Metastatic colorectal cancer	2006
Ofatumumab/Arezera	Human IgG1	CD20	Chronic lymphocytic leukaemia	2009
Removab®	Bi-sepecific mouse/rat Hrbrid IgG	EpCAM X CD3	Patients with malignant ascites (in Europe)	2009
Ipilimumab/Yervoy	Human IgG1	CTLA-4	Metastatic melanoma	2011
Brentuximab/Adcetris	Chimeric IgG1	CD30	ALCL and Hodgkin lymphoma	2011
Pertuzumab/Perjecta	Humanized IgG1	HER2	Metastatic breast cancer	2012

Unconjugated and conjugated mAbs for the treatment of haematological cancers

Anti-CD20 mAbs

The first mAb that was approved by the FDA for the treatment of cancer was rituximab (Rituxan). Rituximab is a chimeric mAb directed against B-lymphocyte-restricted differentiation antigen CD20. CD20 antigen is expressed on the surface of >90% of B cell non-Hodgkin's lymphomas (NHLs), on pre-B lymphocytes and on mature lymphocytes. However, it is not expressed on stem cells, plasma cells and other normal tissues. B-cell lymphoma accounts for 95% of all lymphomas and the binding of rituximab to CD20 antigen in NHL patients causes B cell death by inducing ADCC, CDC and apoptosis. In addition to its use in the treatment of NHL, rituximab has also been approved for the treatment of rheumatoid arthritis symptoms and disease progression in 2008 and 2009, chronic lymphocytic leukaemia in 2010; Wegener's granulomatosis and microscopic polyangiitis in 2012 and it was the best selling anti-cancer drug in the USA in 2012.

Despite the success with rituximab, about half of the patients with NHLs do not respond to treatment or acquire resistance to therapy and this may be reduced by the usage of other anti-CD20 mAbs.In addition to rituximab, three other anti-CD20 mAbs have been approved by the FDA for the treatment of patients with haematological malignancies. Of these, ibritumomab tiuxetan was the first radioimmunotherapy (RIT) agent to gain the FDA approval for the treatment of cancer (NHL) in 2002.

Ibritumomab tiuxetan is a mouse anti-CD20 mAb conjugated to Y radioisotope (Table 1). Tosituzumab was the second mouse anti-CD20 mAb conjugated to I which gained FDA approval for the treatment of patients with refractory NHL in 2003. The goal of RIT is to deliver cytotoxic radiation from therapeutic radioisotopes to tumours using the antibody molecule as a 'guided missile'. While RIT has the advantage of killing adjacent antigen-negative tumour cells and therefore increasing the overall percentage of cell kill, this cross-fire effect can cause toxicity to normal host tissues due to the killing of normal cells. To reduce the total body irradiation and to facilitate the rapid clearance of radiolabelled mAbs, both ibritumomab tiuxetan and Tosituzumab are of mouse origin, and require pre-infusion with unconjugated rituximab and murine tositumomab to 'de-bulk' the body of normal B cells that would otherwise compete for localization to the tumour. Such treatments prolong survival rates in rituximab-refractory NHL patients. The fourth anti-CD20 mAb to gain FDA approval is Arzerra. Unlike the other three anti-CD20 mAbs, Arzerra is a fully human anti-CD20 mAb and binds to a different distinct epitopes on CD20 from the previously discussed mAbs. In 2009, it was approved for the treatment of patients with chronic lymphocytic leukaemia who were refractory to Fludarabine and anti-CD52 mAb Aemtuzumab.

Anti-CD33, CD52 and CD30 mAbs

Gemtuzumab ozogamicin (Mylotarg) was the first toxin-linked antibody to be approved for therapy. In May 2000 under the FDA's accelerated approval programme, it was approved to treat patients with acute myleogenous leukaemia (AML). Gemtuzumab ozogamicin is a humanized anti-CD33 mAb attached to the cytotoxic anti-tumour antibiotic, calicheamicin. The binding of this immunotoxin to the CD33 antigen on AML cells results in the internalization of the immunotoxin and dissociation of calicheamicin, its transport into the nucleus, and the degradation of the DNA leads ultimately to cell death. It has been approved by the US FDA as a single agent for the treatment of patients with CD33-positive acute myeloid leukaemia at the first relapse, who are over 60 years and not suitable for therapy with conventional cytotoxic drugs. However, a confirmatory, post-approval clinical trial was stopped early when there was no improvement in clinical benefit and greater toxicity in the group of patients who received Mylotarg compared with those receiving chemotherapy alone. The drug was voluntarily withdrawn from the market in June 2010.

Alemtuzumab (Camplt-1H) is a humanized mAb directed against another cluster of differentiation antigens named CD52. While CD52 is absent on haematopoietic stem cells, it is present on normal T and B lymphocytes and a high proportion of lymphoid cancers. The original rat anti-CD52 mAb was developed in 1980 and was the first antibody to be humanized. The humanized version of this antibody was approved for the treatment of patients with B-cell chronic lymphocytic leukaemia in 2001. Recent studies also suggest its potential in the management of patients with T-cell prolymphocytic leukaemia (T-PLL), multiple sclerosis and graft-versus-host disease. This antibody can induce its anti-tumour activity by inducing ADCC, activating

complement and CDC. However, as a result of depletion of normal B- and T-lymphocytes, almetuzumab can cause immunosuppression and there is an increased risk of opportunistic infections in such patients.

Another important therapeutic target in patients with haematological malignancies is CD30, a member of the tumour necrosis factor receptor superfamily. CD30 was found to be overexpressed on the surface of tumour cells in patients with Hodgkin's lymphoma (HL) and systemic anaplastic large cell lymphoma which is an aggressive, but rare, type of NHL. Brentuximab Vedotin (Adcetris) is a chimeric anti-CD30 antibody IgG1 antibody (i.e. mAb cAC10) conjugated to four molecules of the microtubule-disrupting agent monomethyl auristatin E by a protease cleavable covalent linker. Under the FDA's accelerated approval programme, this antibody–drug conjugate has been approved for the treatment of patients with HL who have failed autologous stem cell transplant (ASCT) or who are not candidates for ASCT and who had had failed at least two prior combination chemotherapy (FDA labelling information, dcetris, 2011). In a single trial involving 102 HL patients, 73% of the patients had either a complete or partial response and on average the response to therapy lasted for 6.7 months. This drug has also been approved for the treatment of patients with systemic anaplastic large cell lymphoma (ALCL) who failed at least one prior multi-agent chemotherapy. In a single clinical trial involving 58 patients, 86% of the patients receiving this drug had a complete or partial response and the median response duration was 12.6 months (FDA labelling information, Adcetris, 2011). The most common side effects associated with this drug were neutropenia, peripheral sensory neuropathy, fatigue, nausea, upper respiratory tract infections, diarrhoea and thrombocytopenia.

mABS FOR THE TREATMENT OF SOLID TUMOURS

Anti-HER antibodies

The HER [also called erbB or epidermal growth factor receptor (EGFR)] family of receptors is one of the best characterized growth factor receptor family with tyrosine kinase activity. It consists of four family members namely, EGFR (HER-1), ErbB2 (HER-2), ErbB3 (HER-3) and ErbB4 (HER-4). Since the early 1980s, aberrant expression and activation of the HER family members, in particular EGFR and HER-2, have been reported in a wide range of epithelial cancers and in some cases have been associated with a poor prognosis. The biological consequences of EGFR and HER-2 activation in human malignancies include increased cell proliferation, reduced apoptosis, increased angiogenesis, increased motility, invasion and metastasis which are some of the hallmarks of human cancers. These observations have led to the strategic development of several mAbs, four of which have already been approved by the FDA for the treatment of cancer patients in combination with chemotherapy or radiotherapy.

Of the HER inhibitors, Herceptin (trastuzumab) was the first humanized anti-HER-2 mAb approved by the FDA in 1998 for the treatment of HER-2 over-expressing metastatic breast cancer. Although treatment with Herceptin can induce clinical benefit in 30% of HER-2 positive breast cancer patients, the duration of response can be limited and many patients acquire Herceptin resistance and disease progression within 1 year of treatment. It is therefore very important to identify molecular markers that are responsible for the poor response or development of resistance to therapy with HER-2 inhibitors. In some studies, however, the expression of other members of the EGFR family (e.g. EGFR and HER-3) or other growth factor receptors (e.g. IGF-IR), production of EGFR ligands and expression of truncated forms of HER-2 have been associated with the development of Herceptin-resistant breast cancer. At present, several clinical trials are underway examining the therapeutic advantage of trastuzumab when used in combination with other therapeutic strategies to circumvent this resistance for longer. Like Herceptin, pertuzumab is a humanized antibody but binds to a different distinct epitopes on the extracellular domain of HER-2, thereby blocking the heterodimerization of HER-2 with other members of the HER family. Indeed, this pivotal study showed that the combination of two mAbs which are directed against two distinct epitopes on a single target can prolong median progression free survival in cancer patients. In addition, the efficacy and safety of neoadjuvant with pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer in a randomized multicentre, open-label, phase II trial (NeoSphere) has been reported recently. The combination of pertuzumab and trastuzumab, without chemotherapy, had a favourable safety profile and eradicated tumours in a proportion of these patients.In addition to the two anti-HER-2 mAbs, two antibodies against the EGFR have been approved for the treatment of cancer patients. Of these, cetuximab is a chimeric (IgG1) antibody which has gained FDA approval for the treatment of metastatic colorectal cancer in combination with fluoropyrimidine-based chemotherapy. It has also been approved for the treatment of head and neck cancer patients in combination with radiotherapy. The second anti-EGFR mAb panitumumab is the first fully human mAb (IgG2) which has gained FDA approval for the treatment of patients with metastatic colorectal cancer. Three challenges with the routine use of anti-EGFR mAbs in the clinic are the lack of reliable markers for the identification of patients who gain benefit from therapy with anti-EGFR mAbs, the short duration of response in many patients and the high cost of the antibodies. At present, there are conflicting data in the literature on the importance of EGFR as a predictive biomarker for response to therapy with anti-EGFR mAbs. The results of ongoing clinical studies should unravel the importance of relative expression levels of various forms of the EGFR (e.g. membranous, cytoplasmic, nuclear, wild type, mutated forms) as predictive markers for response to therapy with anti-EGFR mAbs as well as their prognostic significance. On 6 July 2012, the FDA approved cetuximab for use in combination with folinic acid, fluorouracil and irinotecan (FOLFIRI) for first-line treatment of patients with *K-ras* mutation-negative (wild-type), EGFR-expressing metastatic colorectal cancer as determined by FDA-approved tests. They also included a new limitation for its use stating that it is not indicated for the treatment of colorectal cancer which carries the*K-ras* mutation. Concurrent with this, the FDA also approved

the companion Therascreen® KRAS RGQ PCR Kit (Qiagen Manchester Ltd) for analysis of Kras status of colorectal tumour.

Anti-VEGF mAb Avastin

One of the key hallmarks of human cancer is angiogenesis, which is the formation of new blood vessels, essential for the local growth of tumour cells. Bevacizumab (Avastin) was the first anti-angiogenic factor approved in 2004 as a first-line treatment for patients with metastatic colorectal cancer in combination with chemotherapy. Since angiogenesis also plays an important role in the growth and spread of other solid tumours, the anti-tumour activity of bevacizumab has also been investigated in other types of cancers. In 2006, bevacizumab gained the FDA approval for its use as a first-line treatment for patients with advanced non-squamous non-small cell lung cancer in combination with carboplatin and paclitaxel. In February 2008, the FDA also granted accelerated approval for bevacizumab, subject to further studies, for its use in combination with paclitaxel for the first-line treatment of HER-2 negative, locally recurrent or metastatic breast cancer, based on statistically significant progression-fee survival, but not overall survival, in patients receiving this combination. However, in December 2011, the FDA rescinded this decision as additional clinical studies showed no increase in overall survival or improvement in patients' quality of life. In 2009, bevacizumab gained FDA approval for the treatment of patients with metastatic renal cancer. Bevacizumab also improved progression-free survival in glioblastoma multiforme (GBM) and remains as the only FDA-approved molecular-targeted drug in patients with GBM. In 2011, bevacizumab in combination with carboplatin and paclitaxel chemotherapy was approved by the EMA as a first-line treatment for women with newly diagnosed advanced ovarian cancer based on the results of two-phase III clinical trials (i.e. GOG218 and ICON7). This is the first break-through treatment that has shown a survival advantage in advanced ovarian cancer patients, for over 20 years.

Anti-CTLA-4 and other promising immune-stimulating antibodies

Cytotoxic T-lymphocyte antigen-4, also known as CD152, is a member of the immunoglobulin superfamily and when expressed on the surface of cytotoxic T lymphocytes (CTLs) and regulatory T suppressor cells can result in downregulation of the immune response. In contrast to CD28, CTLA-4 has a higher affinity for binding to B7-1 (CD80)/B7-2(CD86) expressed on the surface of antigen-presenting cells resulting in T cell anergy and immunosupression. Ipilimumab is a fully human anti-CTLA-4 mAb (IgG1). CTLA-4 blockade by ipilimumab stimulates T cell activation and proliferation of cancer specific T cells, leading to stronger anti-tumour responses in several preclinical and clinical studies. Indeed, it was the first agent to show improved overall survival in patients with advanced or metastatic melanoma. In March 2011, it gained FDA approval for the treatment of patients with advanced melanoma. Adverse effects included autoimmune-related toxicities and further clinical trials are currently underway in other tumour types. In addition, encouraging results have been reported recently with antibodies against other inhibitory receptors

expressed by T cells such as mAb BMS-936558, which is directed against programmed death 1 (PD-1) protein and mAb against PD-1 ligand (PD-L1) expressed on tumour cells. The results of ongoing clinical trials should unravel the full potential of this class of antigens (i.e. the negative regulators of the immune system) as therapeutic targets for mAb-based directed therapy.

Tri-functional anti-EpCAM X anti-CD3 mAb Catumaxomab

Catumaxomab is another type of antibody-based product that was approved by the EMA in April 2009 for the intraperitoneal treatment of malignant ascites in patients whose tumours are epithelial cell adhesion molecule (EpCAM, also called CD326) positive and for whom the standard therapy in not feasible or available. EpCAM is a transmembrane glycoprotein that plays an important role in preventing cell–cell adhesion, tumour cell migration and proliferation. Catumaxomab is a bispecific rat/mouse hybrid antibody containing two different antigen-binding specificities with the mouse Fab binding to EpCAM antigen on tumour cells and the rat Fab binding to CD3 antigen on T cells. In addition, the Fc hybrid region of Catumaxomab binds to the Fcγ receptors on effector cells such as NK cells, macrophages and dendritic cells. Consequently, catumaxomab is a tri-functional antibody as it can induce its anti-tumour activity via T-cell mediated tumour lysis and induction of ADCC and phagocytosis via the activation of FcR-positive effector cells. In a Phase II/III trial involving 258 patients with malignant ascites, catumaxomab showed a clear clinical benefit compared with paracentesis and had an acceptable safety profile. Since catumaxomab is a mouse/rat hybrid antibody, recent studies suggest that the development of HAMAs, 8 days after the fourth antibody infusion, could be a useful predictive biomarker for response to therapy with this antibody. In patients with malignant ascites, a greater benefit is seen with catumaxomab therapy in those patients who developed HAMAs sooner. This could be due to the production of anti-idiotypic antibody (Ab2) against the administered therapeutic antibody (Ab1). Catumaxomab is the first tri-functional antibody and also the first drug to be approved for the treatment of malignant ascites, and clinical trials are currently underway for other indications including ovarian and gastric cancer as well as an open label, dose-escalating study to determine the safety and tolerability of ascending intravenous doses of this antibody in patients with epithelial cancers. In addition, the approval of catumaxomab as well as the two mouse antibodies described earlier (i.e. ibritumomab tiuxetan, tosituzumab) suggest that humanization of all rodent antibodies is not essential and that therapeutic benefits can also be gained by cancer patients using various forms (i.e. both conjugated and unconjugated) of rodent mAbs.

Challenges and future opportunities in targeted therapy of cancer using mAbs

One major limitation of cytotoxic drugs and radiation in the treatment of cancer patients is their inability to discriminate between malignant and normal tissues. This in turn prevents the delivery of the optimal (therapeutic) dose of such agents to malignant tissues for their eradication. Thanks to the advent of hybridoma technology, and subsequent advances in genetic engineering and our understanding of cancer biology, mAb-based products are a well-established treatment modality for a wide variety of solid tumours and haematological malignancies, when used alone or in combination with chemotherapy or radiotherapy. However, there are several outstanding challenges with the routine application of mAb-based products for use in oncology. First, there is currently no reliable biomarker for the identification of patients who are most likely to benefit from the antibody therapy for some of the antibodies. Secondly, it is clear that acquired resistance is not unique to cytotoxic drugs and does also occur following treatment with repeated doses of antibody-based drugs. Downregulation of the target antigen and the heterogeneous nature of human malignancies could be some of the contributing factors for the short duration of response (i.e. less than a year) to antibody-based drugs in some patients. Thirdly, none of the currently approved mAbs are directed against a cancer specific antigen. These antigens are often expressed at lower levels on normal cells in the epithelial tissue, stroma or on normal white blood cells. As a result they would contribute to some common, but not life threatening, side effects, that occur with some of these antibodies (e.g. allergic reactions, diarrhoea, skin rashes, flu-like symptoms). However, in comparison with chemotherapy, serious side effects are not common with mAb-based products. Side effects, such as allergic reactions, can often be prevented by a slow infusion rate and prophylactic premedication with intravenous antihistamine therapy prior to the antibody infusion. Specific side-effects such as the facial rash associated with cetuximab are often effectively controlled by the concurrent use of doxycycline and skin emollients.

Further studies of tumour cells, the tumour microenvironment (e.g. normal cells, cancer stem cells) and the heterogeneous molecular pathways which are involved in the development of human cancers could help in the identification of additional cell surface antigens (overexpressed or tumour specific) in cancer cells, and provide additional therapeutic targets for the development of novel and more effective mAb-based products for use in cancer therapy. This is currently an area of active research in many academic laboratories, including our laboratory, and many pharmaceutical companies. Finally, the treatment of cancer patients with mAb-based drugs is very expensive. This together with the increasing demand for various forms of antibodies for use in the diagnosis and treatment of cancer and other human diseases would dictate the development of alternative strategies and cheaper manufacturing facilities for the production of therapeutic antibodies. Two alternative strategies are the usage of transgenic plants and transgenic farm animals (e.g. cow, goat, chicken). For example, genetically engineered hens have the potential to lay eggs containing high levels of therapeutic antibodies. Further technological advances and optimization of transgenic plants and transgenic animals (e.g. for the proper folding, glycoslyation and stability of antibody, antibody secretion, purification, expression yield) would help to meet the increasing demand for the antibody-based products and ultimately to reduce the

antibody production cost. These together with better companion diagnostic and predictive tests to exclude patients who will not benefit from these treatments, and more definitive selection of patients who should benefit, will in the future, lead to the production of more effective and affordable batches of mAb-based products for therapeutic application in oncology and other pathological conditions. These would also help to prevent the exclusion of all patients from receiving these new therapies, based on the argument of high cost alone.

3. Final part Test-control
Initial knowledge level

1. Gemtuzumab is simply used CD33 to localize a cytotoxic drug which acts on the cancer cell. The primary mechanism by which Gemtuzumab kills cancer cells is the toxic effect of the drug that is conjugated to the antibody. Question: The primary mechanism by which Gemtuzumab kills cancer cells is blocking CD33 signaling.

- True
- False
- Unknown
- Not applicable
- Gentuzumab is not connected with cancer cells.

- Monoclonal antibodies are a type of vaccine.
  - True
  - False
  - Unknown
  - Not applicable
  - Monoclonal antibodies are not used in oncology.
- Which of the following types of targeted therapies are usually given to patients orally?

f. Small molecules
g. Monoclonal antibodies
h. Vaccines
i. Unknown
j. Not applicable

- Which types of therapies can cause the immune system to attack cancer cells?

f. Small molecules
g. Monoclonal antibodies
h. Vaccines
i. Both Monoclonal antibodies and Vaccines
j. Not applicable

- Which types of therapies can be used to directly target molecules inside the cell?

f. Small molecules
g. Vaccines
h. NSAID’s

i. Unknown
j. Not applicable

- A protein that is a part of a growth signaling pathway inside the cell is mutated, causing it to become continually active and resulting in the formation of a tumor. What type of targeted therapy might be effective?

f. Monoclonal antibody that prevents growth factors from interacting with the receptor

g. Monoclonal antibody that holds the growth factor receptor in the "OFF" position

h. Small molecule that selectively binds to the mutated protein

i. Monoclonal antibody that selectively binds to the mutated protein

j. Not applicable

- How do cancer cells evade apoptosis?
  - Reduce the activity of proteins that detect DNA damage
  - Mutation of proteins that induce apoptosis
  - Increase the activity of proteins that prevent apoptosis
  - All of the above
  - Not applicable

- Angiogenesis inhibitors will prevent existing blood vessels from delivering oxygen and nutrients to normal cells.
  - True
  - False
  - Unknown
  - Not applicable
  - Angiogenesis inhibitors are not used in oncology.
- There are the following types of immunotherapy:
  - cytokines
  - vaccines
  - bacillus Calmette-Guerin (BCG)
  - some monoclonal antibodies
  - All of the above

- Monoclonal antibodies can be used:
  - alone directly to cancer cells
  - to carry drugs directly to cancer cells

- to carry toxins directly to cancer cells
- to carry radioactive substances directly to cancer cells
- All of the above

- Which of the following statements about clinical features and factors affecting management of hand osteoarthritis is most likely correct?

f. Hand osteoarthritis is not associated with significant disability
g. Hand osteoarthritis is limited to the articular cartilage only
h. Lipid abnormalities have not been reported as a comorbidity
i. Pain, occupation, multisite involvement, illness perceptions, and impact should form part of a biopsychosocial assessment of hand osteoarthritis, as these may affect management
j. All of the above

- Your patient is a 74-year-old male thought to have hand osteoarthritis. Which of the following statements about diagnosis of hand osteoarthritis is most likely correct?

f. Imaging shows that erosive osteoarthritis is a less severe form of hand osteoarthritis characterized by marginal erosions
g. Radiographic metacarpophalangeal joint osteoarthritis has a similar pattern to interphalangeal and thumb base osteoarthritis
h. Magnetic resonance imaging (MRI) may offer more insight into disease progression from onset to joint failure and risk factors associated with disease progression
i. The diagnosis of generalized osteoarthritis does not require hand osteoarthritis
j. Not applicable

- Which of the following statements about treatment of hand osteoarthritis would most likely be correct?

f. Systemic treatments are preferred over nonpharmacological approaches
g. Oral nonsteroidal anti-inflammatory drugs (NSAIDS) are first-line pharmacotherapy
h. No guidelines are currently available for the diagnosis and management of hand osteoarthritis
i. Advice and education are recommended, emphasizing joint protection education and hand exercises
j. Not applicable

- Your patient is a 63-year-old woman with a history of rheumatoid arthritis (RA) and a 7-day history of cough and fever. What should you suggest regarding how RA affects the risk of infection?

f. Anti-tumor necrosis factor (TNF) therapies generally reduce the risk of infection
g. Patients who are at increased risk of tuberculosis should receive disease-modifying anti rheumatic drug (DMARD) therapy with infliximab
h. The lung is the most common site of infection among patients with RA
i. The pneumococcal vaccine is ineffective among patients taking methotrexate
j. Not applicable

- As the patient is being prepared for being discharged, what should you consider regarding the effects of her RA treatment on her risk of concurrent disease?

f. Anti-TNF agents can reduce the progression of ILD
g. The incidence of methotrexate-induced pneumonitis is increasing
h. Methotrexate promotes a higher risk of cardiac events
i. Anti-TNF agents improve bone density
j. Not applicable

Final knowledge level

1. Tumor necrosis factor (TNF) inhibitors have wide-ranging effects, including acting on which of the following pathogenetic mechanism in psoriatic arthritis (PsA)?

f. Polymorphonuclear cells
g. E-selectin
h. T helper 17 (T_h17) cells
i. Interleukin (IL)-8
j. Not applicable

2. Disease-modifying antirheumatic drugs (DMARDs) such as methotrexate and sulfasalazine are believed to benefit which disease domains of psoriatic arthritis (PsA)?

f. Peripheral arthritis, skin/nail disease, axial disease, dactylitis, enthesitis
g. Skin/nail disease, axial disease, dactylitis, enthesitis
h. Axial disease, dactylitis, enthesitis
i. Peripheral arthritis, skin/nail disease
j. All of the above

3. Glucocorticoid injections are recommended as adjunctive therapy, but which of the following events has been reported with corticosteroid taper?

f. Enthesitis
g. Dactylitis
h. Skin flares
i. Single joint flares
j. All of the above

4. The single best chance of a tissue graft being accepted in a human recipient is possible:

a. An isograft
b. An allograft given under the cover of potent immunosuppression
c. A xenograft from a pig in which the gene for alpha-1,3-galactosyltransferase has been 'knocked-out' and therefore the Galα-1,3-Gal epitope eliminated
d. Given to a recipient that is treated with anti-CD3
e. From a living sibling donor

5. The single best defining description of the murine MHC is that it:

- Is involved in skin transplant rejection
- Is encoded on chromosome 17
- Is called H-2
- Is polymorphic
- Encodes a heterodimeric cell surface molecule

6. Out of the following, the cadaveric organ with the best survival 10-years post-transplant is:

a. Lung
b. Pancreas
c. Liver
d. Intestine
e. Kidney

7. An example of a known oncogenic virus is:

a. Herpes zoster
b. HIV-2.
c. Epstein-Barr virus.
d. Vesicular stomatitis virus.
e. Proteus mirabilis.

8. Colleagues showed that syngeneic transplantable tumors which mutate such as those that express strong transplantation antigens are rejected. They called these options:

a. tum⁻
b. Xenogeneic.

c. tum +
d. MCA.
e. Non-immunogenic.

9. In pancreatic carcinoma the *ras* gene:
   a. Is absent.
   b. Is normal but is overexpressed.
   c. Has a large deletion.
   d. Contains a single point mutation, always at the same position.
   e. Contains a single point mutation, but not always at the same position.

10. Wheat germ agglutinin binds strongly to:
a. Surface lipoproteins on resting T-cells.
b. Surface glycoproteins on resting T-cells.
c. Surface glycoproteins on resting B-cells
d. Surface glycoproteins on activated T- and B-cells
e. Surface lipoproteins on activated T- and B-cells.

11. Strongly immunogenic tumors appear:
   a. Virtually in all cancers.
   b. Only in lymphoma and leukemia.
   c. In immunosuppressed patients
   d. Only in experimental animals.
   e. Only in elderly patients.

12. Which of the following is most commonly seen in African children with Burkitt's lymphoma:
   a. Absence of EBV.
   b. T-cell neoplasia.
   c. Deletion of the *c-myc* gene.
   d. Chromosome 8q24 to Chromosome 14q32 translocation
   e. Chromosome 8q24 to Chromosome 2p12 translocation.

13. Pre-B acute lymphoblastic leukemia (Pre-B ALL) cells lack:
   a. Expression of TdT.
   b. HLA-DR.
   c. Cytoplasmic μ heavy chain.
   d. CD5.
   e. CD10.
14. Chronic lymphocytic leukemia:
   a. Usually has a very poor prognosis.
   b. Has a good prognosis only in those patients with circulating monoclonal immunoglobulin.
   c. Is mostly a disease of childhood.

d. Is usually found in people over 50 years of age

e. Is a leukemia where both kappa and lambda light chains are found on the surface of the malignant cell.

15. Non-Hodgkin lymphomas:

a. Will be positive when stained with antibodies to cytokeratin

b. Are usually of T-cell origin but sometimes of B-cell origin.

c. Can be differentiated from carcinoma using antibodies to CD45 (leukocyte common antigen).

d. Are reactive B-cell hyperplasias.

e. Have a good prognosis.

CORRECT ANSWERS:

Initial knowledge level:

1.B; 2.B; 3.A; 4.D; 5.A; 6.C; 7.D; 8. B; 9.E; 10.E; 11.D; 12.C; 13.D; 14. C; 15.D

Final knowledge level:

1.C; 2.D; 3. C; 4.A; 5.C; 6.C; 7.C; 8.A; 9.E; 10.D; 11.C; 12.D; 13.D; 14.D; 15.C

Control questions.

1. Are different viruses associated with cancer?
2. Are other microbes associated with cancer?
3. How are cancer treatment vaccines designed to work?
4. What types of vaccines are being studied in clinical trials?
5. How are cancer vaccines made?
6. Can researchers add ingredients to cancer vaccines to make them work better?
7. Do cancer vaccines have side effects?
8. How can cancer treatment vaccines be combined with other types of cancer therapy?
9. What additional research is under way - the identification of novel cancer-associated antigens that may prove more effective in stimulating immune responses than the already known antigens and the development of methods to enhance the ability of cancer-associated antigens to stimulate the immune system.
10. Relationship Between Donor and Recipient
11. The Role of the Immune Response in Allograft Rejection
12. Clinical Characteristics of Allograft Rejection (Hyperacute Rejection, Acute Rejection,Chronic Rejection)
13. Histocompatibility Antigens
14. MHC Class I and Class II Molecules as Targets inAllograft Rejection
15. Xenogeneic Transplantation
16. Tests for Histocompatibility Antigens
17. Serologic Detection of Transplantation Antigens
18. Genotyping of Transplantation Epitopes
19. Detection of Transplantation Antigens by MixedLeukocyte Reaction
20. Prolongation of Allograft Survival
21. Cyclosporine, FK-506 (Tacrolimus), and Rapamycin (Sirolimus)
22. Antibody Therapy and Blocking of Costimulatory Molecules
23. Bone Marrow Transplantation
24. Graft-Versus-Host Reactions

Structure of class.

Plan and organizational structure of the lesson:

Preparatory stage: organization of lesson and test control of incoming level of knowledge (1 academic hour or 45 minutes – immunooncology and 4 academic hours or 180 minutes – immunology of transplantation)

№	Content	Level of learning	Methods of the learning control	Materials of the methodological support	Time, min.
1.	The preparatory phase Control input level of knowledge, skills.	I	1. Front rapid survey	Tests. Scheme.	25
2.	The main stage - Control the output level of knowledge, skills: • factors of innate immunity in antitumor protection • tumor antigens and antitumor antibodies Formation of professional knowledge, skills and abilities: describe the types of tumor antigens - identify the basic mechanisms of antitumor immune defense - determine major factors immunological resistance tumors - apply the basic principles of immunodiagnostics of tumors - form the main directions of the immunotherapy of tumors	II II II II II II II-III III	2. Individual oral examination. 3. Test control.	1.Tables. 2. Folio-grams. 3. Tests I-III 1. 2. Slideshow.	30
	- Mechanism of graft rejection - Immunological monitoring of patients - Examine patients who are preparing for a kidney transplant - Examination of the patient's medical history or analysis - Syndrome of kidney rejection • - Appointment of immunosuppressive therapy after transplantation	II II-III II-III III III	2. Individual oral examination. 4. Test control.	1.Tables. 2. Folio-grams. 3. Tests I-III 1. 2. Slideshow.	150
3.	The final stage Monitoring and adjustment of professional knowledge, skills and abilities: basic principles-purpose of immunotropic therapy in	III III III	1. Testing. 2. Solving custom applications. 5. Oral	1. Tests III-IV levels. 2. Situational problems.	15

	patients with primary and secondary immunodeficiencies, HIV/AIDS;		examination.		
4.	To sum up the lessons. Homework for the next topic.				2 3

List of practical skills.

1. Perform examination of immune response in patients after liver transplantation.
2. Key points of the immune response to transplanted tissue.
3. Perform immunogram examination of patients with primary pancreatic cancer.

Self student's work (reproductive immunology):

The formation of professional knowledge, skills and abilities:

1. Antigamete immunity
2. Intrauterine infections
3. Immunological conflict of pregnancy
4. Immunology of pregnancy
5. Immune infertility, its diagnosis.
6. Immunopathology in obstetric practice
7. Establishing of the understanding of the immune response characteristics during the pregnancy.
8. The basic immunological causes of sterility.
9. Antisperm Antibody. How Does This Happen in Men? How Does This Happen in Women?
10. Immunological testing and treatment in reproduction

References:

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2. Abul K. Abbas, Andrew H. H. Lichtman, Shiv Pillai Cellular and Molecular Immunology. - Saunders; 7 edition (2011). – 560 pages

3. Roitt's Essential Immunology, Includes Desktop Edition. Peter J. Delves, Seamus J. Martin, Dennis R. Burton, Ivan M. Roitt. Wiley-Blackwell; 12 edition (2011). – 560 pages

4. How the Immune System Works, Includes Desktop Edition. Lauren M. Sompayrac. Wiley-Blackwell; 4 edition (2012). – 152 pages

5. Lecture Notes: Immunology, 6th Edition. Ian Todd, Gavin Spickett. Wiley-Blackwell (2011). – 480 pages

6. Essentials of Clinical Immunology, 6th Edition. by Helen Chapel, Mansel Haeney, Siraj Misbah, Neil Snowden. Wiley-Blackwell (2014). – 376 pages

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METHODICAL INSTRUCTION

Practical class №5

1.THEME. PRIMARY AND SECONDARY IMMUNODEFICIENCY. HIV/SAID-INFECTION (5 academic hours).

2. **Background:** Immune deficiencies are categorized as primary immune deficiencies or secondary immune deficiencies. Primary immune deficiencies are considered to be "primary" because the immune system is the primary cause and other factors are genetic defects that may be inherited. Secondary immune deficiencies are called so because they have been caused by other conditions.

Secondary immune deficiencies are common and can occur as part of another disease or as a side-effect of certain medications. The most common secondary immune deficiencies are caused by aging, malnutrition, certain medications and some infections, such as HIV.

The most common medications associated with secondary immune deficiencies are chemotherapy agents and immune suppressive medications, cancer, transplanted organ rejection or autoimmune diseases. Other secondary immune deficiencies include protein losses in the intestines or the kidneys. When proteins are lost, antibodies are also lost, leading to low immune globulins or low antibody levels. These conditions are important to recognize because, if the underlying cause can be corrected, the function of the immune system can be improved and/or restored.

Regardless of the root cause, recognition of the secondary immune deficiency and provision of immunologic support can be helpful. The types of support offered are comparable to those used for primary immune deficiencies.

The primary immunodeficiency diseases are a group of disorders caused by basic defects in immune function that are intrinsic to, or inherent in, the cells and proteins of the immune system. There are more than 200 primary immunodeficiency diseases. Some are relatively common, while others are quite rare. Some affect a single cell or protein of the immune system and others may affect two or more components of the immune system.

Although primary immunodeficiency diseases may differ from one another in many ways, they share one important feature. They all result from a defect in one or more of the elements or functions of the normal immune system such as T-cells, B-cells, NK cells, neutrophils, monocytes, antibodies, cytokines or the complement system. Most of them are inherited diseases and may run in families, such as X-Linked Agammaglobulinemia (XLA) or Severe Combined Immune Deficiency (SCID). Other primary immunodeficiencies, such as Common Variable Immune Deficiency (CVID) and Selective IgA Deficiency are not always inherited in a clear-cut or predictable fashion. In these disorders, the cause is unknown, but it is believed that the interaction of genetic and environmental factors may play a role in their causation.

Because the most important function of the immune system is to protect against infection, people with primary immunodeficiency diseases have an increased susceptibility to infection. This may include too many infections, infections that are difficult to cure, unusually severe infections, or infections with unusual organisms. The infections may be located anywhere in the body. Common sites are the sinuses (sinusitis), the bronchi (bronchitis), the lung (pneumonia) or the intestinal tract (infectious diarrhea).

Another function of the immune system is to discriminate between the healthy tissue ("self") and foreign material ("non-self"). Examples of foreign material can be microorganisms, pollen or even a transplanted kidney from another individual. In some immunodeficiency diseases, the immune system is unable to discriminate between self and non-self. In these cases, in addition to an increased susceptibility to infection, people with primary immunodeficiencies may also have autoimmune diseases in which the immune system attacks their own cells or tissues as if these cells were foreign, or non-self.

There are also a few types of primary immunodeficiencies in which the ability to respond to an infection is largely intact, but the ability to regulate that response is abnormal. Examples of this are autoimmune lymphoproliferative syndrome (ALPS) and IPEX (an X-linked syndrome of immunodeficiency, polyendocrinopathy and enteropathy).

Primary immunodeficiency diseases can occur in individuals of any age. The original descriptions of these diseases were in children. However, as medical experience has grown, many adolescents and adults have been diagnosed with primary immunodeficiency diseases. This is partly due to the fact that some of the disorders, such as CVID and Selective IgA Deficiency, may have their initial clinical presentation in adult life. Effective therapy exists for several of the primary immunodeficiencies, and many people with these disorders can live relatively normal lives.

Primary immunodeficiency diseases were initially felt to be very rare. However, recent research has indicated that as a group they are more common than originally thought. It is estimated that as many as 1 in every 1,200–2,000 people may have some form of primary immunodeficiency.

3. Aim:

- ***Study:*** Students need to learn the basic modern data of the primary and secondary immunodeficiencies, HIV;
- ***Professionally oriented:*** Students should know the basic stages of the immune response and the types of regulation in patients with primary and secondary immunodeficiencies, HIV/AIDS;
- ***Educational:*** to shape students' sense of responsibility for the timeliness and appropriateness of professional activities.

4.Materials: Equipment to run powerpoint presentation
Main books. Short information due to the topic.

5. Interdiscipline integration

5.1. *Interdiscipline integration*: The theme of this practical lesson is related to the topics: general immunology, infections, primary and secondary immunodeficiencies, HIV/AIDS;

Subject	Know	Can
Anatomy	Haemopoietic organs of immune system	Name primary and secondary lymphoid organs, lymphatic ducts
Biology, histology, embryology.	Immunity as mechanism of functional and structure homeostasis. Essentials of immuno-embryogenesis.	Distinguish basic phylogenetic levels of immunity evolution, primitive cell-mediated immunity and integral cell-mediated and humoral immunity
Biochemistry	Chemical structure and biological action of mediators of cell-mediated immunity	Immunophoretic determination of immunoglobulins.
Pathophysiology	Phagocytosis in the focus of inflammation, immunological tolerance, reactivity, allergy.	Immunocompetent cells, participating in defense reactions of cell-mediated and humoral immunity
Normal physiology	Interaction of nervous, endocrine and immune system for functional and structure homeostasis maintaining	On practical classes determine interaction of nervous, endocrine and immune system.
Pathological anatomy	Role of immune disorders in mesenchymal and protein dystrophies, mucous and fibrinoid degeneration if connective tissue	Determine mesenchymal and protein dystrophies, mucous and fibrinoid degeneration if connective tissue. Types of tissue reactions in collagenoses.
Microbiology, virusology	Specific and non-specific mechanisms of defense. Types of antigenes, immunity, allergies.	Perform agglutination test, Coombs' test, precipitation. Titration of complement, bacteriolysis and haemolysis tests, immunofluorescence test
Propedeutics of internal diseases	Role of immune system in pathogenesis of different diseases.	Interpretation of rosette test, Manchini immunoglobulins, phagocytic activity of blood.

6. Student has to know:

1. Define “primary and secondary immunodeficiency”.
2. Main data about immune system structure and function in patients with different types of primary immunodeficiencies.
4. Characteristics of phagocytosis, complement system, T- and B-lymphocyte systems, scheme of main stages of immune response.

Study questions.

1. Why are these diseases called *primary* immunodeficiency disorders?
2. How did the child get a primary immunodeficiency disorder?
3. How are primary immunodeficiency disorders diagnosed?
4. Why should the child see a multidisciplinary team for pediatric immune disorders?
5. Will the child be cured of the disease?
6. What is the prognosis for children with primary immunodeficiencies?

Main part

Primary immunodeficiency

Introduction

Primary immunodeficiency disorder (PID) refers to a heterogeneous group of disorders characterized by poor or absent function in one or more components of the immune system. Over 130 different disorders have been identified to date, with new disorders continually being recognized. Most PIDs result from inherited defects in immune system development and/or function; however, acquired forms have also been described. It is important to note that PIDs are distinct from secondary immunodeficiencies that may result from other causes, such as viral or bacterial infections, malnutrition, or treatment with drugs that induce immunosuppression.

With the exception of immunoglobulin A (IgA) deficiency, PIDs are rare; the estimated prevalence of these disorders in the Europe, United States is approximately 1 in 1200 live births. IgA deficiency is the most common PID, occurring in approximately 1 in 300 to 1 in 500 persons.

The clinical presentation of PIDs is highly variable; however, most disorders involve increased susceptibility to infection. In fact, many PIDs present as “routine” infections (often of the sinuses, ears and lungs) and, therefore, may go undetected in the primary-care setting. The accurate and timely diagnosis of these disorders requires a high index of suspicion and specialized testing. Therefore, consultation with a clinical immunologist who is experienced in the evaluation and management of immunodeficiencies is essential, since early diagnosis and treatment are critical for preventing significant disease-associated morbidity and improving patient outcomes. This article provides an overview of the major categories of PIDs as well as strategies for the timely identification, diagnosis and management of these disorders.

Classification

PIDs are broadly classified according to the component of the immune system that is primarily disrupted: adaptive or innate immunity.

Classification of PIDs: examples and typical clinical presentations

Classification and examples	Clinical presentation

Disorders of adaptive immunity

T-cell (cellular) ımmunodeficiency	Atypical mycobacterial and salmonella infections
► IFN-γ/IL-12	Mucocutaneous candidiasis (thrush) and ıtoimmune
► AIRE mutations	Endocrinopathy
B-cell (antibody-mediated) ımmunodeficiency	Recurrent sinopulmonary infections with encapsulated bacteria
► XLA	Autoimmune disease and increased risk of alignancy in CVID
► CVID	
► Selective IgA deficiency	
► Specific antibody deficiency	
► IgG subclass deficiency	
	Thrombocytopenia with bleeding and bruising; :zema;
CID	recurrent bacterial and viral infections; autoimmune
► Wiskott-Aldrich syndrome	disease
	Chronic sinopulmonary disease; cerebellar ataxia (difficulty with control of movement); small, dilated ood
► Ataxia telangiectasia	vessels of the eyes and skin; malignancy
	Hypoparathyroidism; seizures; cardiac ɔnormalities;
► DiGeorge syndrome	abnormal facies; infection
► SCID • T^- , B^+ – γc deficiency – JAK3 deficiency • T^- , B^-	Severe, recurrent opportunistic infections; failure to thrive; diarrhea; rash

– ADA deficiency
– RAG 1/2 deficiency

Disorders of innate immunity	
Phagocyte defects	Severe infection; abscesses with granuloma ɪrmation
► Chronic granulomatous sease	Chronic dermatitis; recurrent, severe lung ɔns; skin infections; bone fragility; failure to shed ɪimary teeth
► Hyper IgE syndrome	Recurrent, severe bacterial infections; poor wound ealing;
► Leukocyte adhesion eficiency	delayed separation of the umbilical cord
Complement defects	
► Deficiency in early ɔmplement pathway components C1q, C1r, C2, C4)	SLE–like syndrome, rheumatoid disease, multiple autoimmune diseases, infections
► Deficiency in late ɔmplement pathway components (C5, C6, 7, C8, C9)	Neisserial infections, SLE-like syndrome
► C3 and regulatory ɔmponents	Recurrent infections with encapsulated bacteria

AIRE, autoimmune regulator; CVID, common variable immunodeficiency; IgG, ɪmunoglobulin G; IgE, immunoglobulin E; IgA, immunoglobulin A; IFNγ, interferon-ɪmma; IL, interleukin; CID, combined immunodeficiency; SCID, severe combined ɪmunodeficiency; XLA, X-linked agammaglobulinemia; SLE: systemic lupus ʏthematosus; JAK3, Janus kinase 3; ADA, adenosine deaminase; RAG, recombination ɛtivating gene

Disorders of adaptive immunity

T cells and B cells are the primary cells of the adaptive immune system. B cells mediate antibody production and, therefore, play a major role in antibody-mediated (humoral) immunity. T cells, on the other hand, govern cell-mediated immune responses. Defects occurring at any stage of T-cell development, differentiation and maturation lead to T-cell (cellular) immunodeficiency disorders, while defects relating to B-cell development and/or maturation result in B-cell (antibody-deficiency) disorders. Since B-cell-mediated antibody production requires intact T-cell function, most T-cell defects lead to combined (B- and T-cell) immunodeficiency disorders (CIDs).

Disorders of innate immunity

Innate immune responses represent the first line of defense against potentially invading organisms. Appropriate recognition of threats and induction of the inflammatory cascade are essential steps in the removal of these organisms from the system. Failure of the innate system to identify pathogens delays the induction of the immune response and may worsen outcomes of infection.

Numerous cells and proteins are involved in the innate immune response including phagocytes (neutrophils and macrophages), dendritic cells, and complement proteins. Phagocytes are primarily responsible for phagocytosis, the process by which cells engulf and eliminate invading pathogens. Complement proteins function to identify and opsonize (coat) foreign antigens, rendering them susceptible to phagocytosis. Defects in the development and function of any of these elements of innate immunity may lead to PIDs.

Clinical presentation

T-cell and combined immunodeficiencies

The clinical manifestations of T-cell (cellular) disorders and CIDs will vary depending on the specific underlying defect in the adaptive immune response. Therefore, clinical suspicion is important for timely diagnosis of these disorders. Patients with specific T-cell defects may be lymphopenic (i.e., have abnormally low levels of lymphocytes) and neutropenic (i.e., have abnormally low levels of neutrophils). In the most severe forms of CID (also known as severe combined immunodeficiency [SCID]), there is a virtual lack of functional T cells and immune function. These disorders are rare and are generally categorized into whether there is an absence of T cells, but presence of B cells (T^- , B^+), or an absence of both T and B cells (T^- , B^-) (see Table 1). Natural killer (NK) cell numbers are also informative for determining the genetic phenotype of SCID. However, normal T-cell numbers do not exclude the possibility of T-cell defects, and in patients with clinical presentations consistent with immunodeficiency, further investigations of T-cell function are warranted.

Patients with SCID usually present within the first year of life with chronic diarrhea and failure to thrive; severe, recurrent infections with opportunistic pathogens (e.g., *Candida albicans* [thrush], *Pneumocystis jiroveci*, or cytomegalovirus); and skin rashes. Some patients may also have associated neurological defects. SCID is a

pediatric emergency since infection often leads to death and bone marrow transplantation (BMT) can be curative.

Other, less severe CIDs that do not characteristically lead to early mortality include Wiskott-Aldrich syndrome, DiGeorge syndrome, ataxia-telangiectasia, and X-linked lymphoproliferative disease. Patients with these disorders often present later in childhood with recurrent infections and clinical findings that vary depending on the specific syndrome. Autoimmunity and immune dysregulation are also frequent complications associated with these CIDs.

B-cell immunodeficiencies

B-cell (antibody-deficiency) disorders are the most common type of immunodeficiencies, accounting for approximately 50% of all PID diagnoses. They comprise a heterogeneous group of disorders characterized by an increased susceptibility to respiratory tract infections with bacteria, particularly *Streptococcus pneumoniae* and *Haemophilus influenzae.*

Patients usually present after 6 months of age with recurrent, and often severe, sinopulmonary infections such as otitis media, sinusitis, and pneumonia. Diarrhea, fatigue, autoimmune manifestations (particularly autoimmune cytopenias), and sensorineural hearing loss are also common. Patients with humoral deficiency often have reduced or absent serum immunoglobulin levels, but may also show normal or increased serum immunoglobulin levels with abnormal function.

More than 20 antibody-deficiency disorders have been defined to date, however, many remain undefined. Typical disorders that fall into this category include: X-linked agammaglobulinemia (XLA; also known as Bruton's agammaglobulinemia), common variable immunodeficiency (CVID), and selective IgA deficiency. XLA results from a mutation in the Bruton's tyrosine kinase (Btk) gene, which is responsible for mediating B-cell development and maturation. The disorder is characterized by markedly reduced levels of circulating B-cells and serum IgG, IgA and IgM. Affected males usually present within the first 2 years of life with recurrent sinopulmonary infections and absent lymph nodes and tonsils. CVID is a heterogeneous disorder characterized by markedly reduced serum concentrations of IgG, low levels of IgA and/or IgM, and poor or absent responses to immunization. The disorder affects males and females equally, and usually has a later age of onset than other antibody-deficiency disorders (i.e., > 10 years of age). It is associated with recurrent sinopulmonary infections, autoimmune and granulomatous disease, gastrointestinal complications and an enhanced risk of malignancy (e.g., lymphoma and gastric carcinoma). Some patients may also present with bronchiectasis (irreversible widening of portions of the bronchi resulting from damage to the airway wall), which is a common cause of morbidity and mortality in these patients.

Milder antibody-deficiency disorders, such as selective IgA deficiency, are associated with variably low serum levels of an immunoglobulin class or subclass and, in some cases, impairments in specific antibody formation. IgA deficiency, for example, is characterized by low or absent levels of serum IgA in the presence of normal levels of IgG and IgM. Only about one-third of these patients are particularly prone to infection.

Innate immunodeficiencies

Patients with innate immunodeficiency disorders may present at any age, often with unusual or difficult to eradicate infections. The typical signs and symptoms of phagocyte disorders are severe pyogenic (puss-like) bacterial and fungal infections of the skin, respiratory tract, and internal organs, as well as painful sores around the mouth. Chronic granulomatous disease (CGD) is a common phagocyte defect. Hyper-IgE syndrome is another phagocyte disorder characterized by Staphylococcal infections of the skin, bone, and lungs, bony abnormalities and high IgE levels. It has recently been found to result from a mutation in signal transducer and activator of transcription 3 (STAT3) which affects phagocytic cell recognition of *Staphylococcus* as well as osteoclast function involved in bone remodelling.

Of all the PIDs, complement deficiencies account for less than 1% of identified cases. Patients with these disorders tend to present with systemic autoimmune disease that resembles lupus erythematosus or with severe or recurrent infections with encapsulated.

Diagnosis

As mentioned previously, early diagnosis of PID is critical for preventing significant disease-associated morbidity, and even mortality. However, a national survey of PID in the United States found that more than 40% of patients with these disorders were not diagnosed until adulthood, despite the fact that many reported serious or chronic health conditions prior to diagnosis, such as sinusitis, bronchitis, and pneumonia. The importance of prompt recognition and management of PIDs is further highlighted by the rate of hospitalizations pre- and post diagnosis. Although 70% reported being hospitalized prior to diagnosis, nearly half (48%) reported no hospitalization since diagnosis.

A diagnosis of PID should be suspected in both children and adults who have recurrent pneumonias and/or ear, sinus and cutaneous infections. Although this Table does not provide a comprehensive list of all signs and symptoms of PID, patients meeting any of these criteria should be referred immediately to a clinical immunologist for further evaluation. The immunologist will perform a comprehensive immune evaluation that often begins with a complete blood count (CBC) and blood smear. These tests are used to evaluate for the presence of lymphopenia, abnormal or unusual lymphocytes or phagocytic cells, and any associated gross hematologic abnormalities that may be indicative of PIDs. Significant lymphopenia, for example, may be the first indication of T-cell (cellular) immunodeficiency. Other important diagnostic tools include lymphocyte proliferation assays and flow cytometry which allow for the enumeration of B-cells, T-cells, and NK, and the evaluation of lymphocyte markers, T-cell variability, and adhesion receptors that may be associated with specific immune defects. Standard flow cytometry analysis is abnormal in most cases of SCID and in many cases of CID.

The initial evaluation of patients with suspected B-cell (antibody-deficiency) disorders involves the measurement of serum IgG, IgA, IgM, and IgE levels (note that the measurement of IgD is not useful for the diagnosis of PIDs). Serum levels that are

clearly below age-appropriate reference values may be indicative of B-cell immunodeficiencies. However, some patients with these disorders have normal or only modestly reduced immunoglobulin levels; therefore, the best approach for confirming a diagnosis of an antibody-deficiency disorder is the measurement of serum specific antibody titers (usually IgG) in response to vaccine antigens. This approach involves immunizing a patient with protein antigens (e.g., tetanus toxoid) and polysaccharide antigens (e.g., pneumococcus) and assessing pre- and post-immunization antibody levels. In many PIDs, antibody responses to these antigens are diminished or even absent.

Neutrophil function assays (e.g., dihydrorhodamine response [DHR]) and stimulation assays for cytokine responses are helpful for confirming a diagnosis of innate disorders. For example, abnormal neutrophil oxidase function is usually indicative of CGD. Complement studies, which examine the level and/or function of specific complement proteins, are essential for the diagnosis of complement deficiency disorders. These studies should be performed by accredited laboratories that have demonstrated competence in these assays and experience in performing investigations into PID.

In some cases, more advanced testing involving specialized molecular methods may be required to confirm a diagnosis of PID. Once the diagnosis is established, it is important that therapy be initiated as soon as possible, since delays can lead to permanent organ damage or even death from overwhelming infection.

Treatment

The treatment of PIDs is complex and generally involves both supportive and definitive strategies. As such, therapy should be coordinated by an immunologist with expertise in the management of these disorders.

SCID/CID

Initial therapy for patients with SCID or other CIDs is supportive and involves aggressive management of the established infection, immunoglobulin (Ig) replacement therapy (discussed in more detail in the next section), and antibiotic and antifungal prophylaxis to reduce the frequency and severity of infections. There is currently no standardized approach to the use of prophylactic antibiotics in patients with established PIDs since randomized, controlled studies in this area are lacking. Commonly used regimens are derived from studies focusing on the prevention of otitis media in children and include: sulfisoxazole**,** amoxicillin, trimethoprim-sulfamethoxazole (TMP-SMX) and azithromycin. Patients with SCID should also be protected from exposure to infectious agents. In the hospital setting, protective isolation in positive pressure rooms is recommended. Furthermore, live attenuated vaccines (e.g., such measles/mumps/rubella/varicella, bacillus Calmette-Guerin, infant rotavirus, and oral polio virus) are contraindicated in patients with SCID as they can lead to severe, disseminated and fatal infections. There is no risk of disseminated infections from killed or inactivated vaccines and, therefore, these may be administered according to routine indications and schedules in patients with PIDs.

Since SCID is fatal unless the underlying defect is corrected, definitive therapy with BMT or hematopoietic stem cell transplantation **(**HSCT**)** should be initiated as quickly as possible. When performed from a human leukocyte antigen (HLA)-identical sibling, these procedures lead to excellent long-term survival and long-lasting immune reconstitution. Good results have also been obtained with HLA-mismatched related donors when the procedures are performed within the first 3.5 months of life; however, less satisfactory outcomes have been noted in older patients. Gene therapy, which involves introducing a functional copy of the patient's defective gene into appropriate cells, has also been shown to lead to immune reconstitution and improved survival in patients with certain SCIDs, such as adenosine deaminase (ADA) deficiency and SCID-X1 (an X-linked inherited SCID characterized by an early block in T-cell differentiation). Enzyme replacement therapy with weekly intramuscular injections of pegylated bovine ADA is also available for the management of patients with ADA deficiency.

B-cell immunodeficiencies

The mainstay of therapy for most B-cell (antibody-deficiency) disorders is intravenous (IV) or subcutaneous Ig replacement therapy; in fact, many patients will require this treatment indefinitely. There are currently five Ig replacement products approved for the treatment of PID in Canada. IV and subcutaneous formulations are considered equally effective in reducing the frequency and severity of infections, and there is insufficient evidence to suggest that one product is superior to another. When deciding on a specific product, patient preference should be taken into consideration. Some patients may prefer a subcutaneous formulation since therapy can be administered at home. Note that intramuscular Ig replacement therapy is not considered to be as effective as IV or subcutaneous therapy and, therefore, is not recommended for the treatment of PID.

The recommended starting dose of Ig replacement therapy is 400–600 mg/kg/4 weeks for the IV formulation and 100–150 mg/kg/week for the subcutaneous formulation. The most common adverse events associated with this therapy are headache, flushing, chills, myalgia, wheezing, tachycardia, lower back pain, nausea, and hypotension. In patients experiencing multiple adverse reactions to one product, consideration may be given to switching to another product or route of administration.

For patients with recurrent infections, prophylactic antibiotic therapy (particularly with agents that provide coverage of *Streptococcus pneumoniae* and *Haemophilus influenzae*) may also be needed in addition to Ig replacement therapy. Depending on the etiology of the specific B-cell disorder, prophylactic antifungal therapy may also be required. Since B-cell immunodeficiencies are often associated with sensorineural hearing loss and pulmonary complications, regular hearing assessments and monitoring of pulmonary status and function is recommended. As with primary T-cell defects, vigilance for malignancies and autoimmune disorders is also important in patients with B-cell disorders.

At present, there are no definitive management strategies that can be routinely recommended for patients with B-cell disorders. However, gene therapy is currently being investigated for some antibody deficiencies and may represent a future treatment option for these patients.

Innate disorders

The management of innate disorders depends on the type of defect. For phagocyte disorders, therapy is primarily supportive and includes both antibiotic and antifungal prophylaxis. Cytokine replacement (e.g., interferon-gamma) and BMT have also been shown to be effective in patients with CGD. Gene therapy may also be a potential definitive treatment option in the future.

There is no specific definitive therapy for complement deficiencies. Treatment of these disorders focuses on antibiotic prophylaxis for the prevention of recurrent infections. Since some patients with complement disorders are at increased risk of meningococcal infections with *Neisseria meningitidis*, multivalent meningococcal vaccinations should also be considered. Pneumococcal and*Haemophilus influenzae* vaccines may also be needed in patients with frequent infections caused by encapsulated organisms.

Prognosis

The prognosis of patients with PIDs varies depending on the etiology of the disorder. However, patient outcomes and long-term survival have improved significantly since the 1970s given our improved management of infections and early access to antibiotics, advances in BMT and HSCT techniques, and enhanced intensive care services. Furthermore, routine vaccinations provide herd immunity to those at risk, decreasing the circulation of infectious disease. Further progress in the diagnosis and management of PIDs is expected as research on the genes responsible for immunodeficiencies and the use of definitive treatments such as gene therapy continues.

Conclusions

PID refers to a heterogeneous group of disorders that result from defects in immune system development and/or function. Although the signs and symptoms of PIDs are highly variable, most disorders involve increased susceptibility to infection, with many leading to significant disease-associated morbidity and mortality. Given the complexity of these disorders, referral to an immunologist is required for appropriate diagnosis and management. Severe disorders such as SCID requires definitive therapy for immune reconstitution (e.g., BMT, HSCT) as soon as possible. B-cell or antibody-deficiency disorders are the most common types of PIDs. The mainstay of treatment for patients with these disorders is Ig replacement therapy. Patients with innate immunodeficiency disorders often present with unusual or difficult to eradicate infections. Treatment varies depending on the type of defect (e.g., phagocyte disorder or complement deficiency), but may involve antifungal and antibiotic prophylaxis, cytokine replacement, vaccinations and BMT.

Key take-home messages

► With the exception of IgA deficiency (prevalence = 1 in 300-500), PIDs are more frequent than previously believed, with an estimated prevalence of 1 in 1200.

► Clinical presentation is highly variable, but most disorders involve increased susceptibility to infection.

► PIDs should be suspected in patients with: recurrent sinus or ear infections or pneumonias within a 1 year period; failure to thrive; poor response to prolonged use of antibiotics; persistent thrush or skin abscesses; or a family history of PID.

► Consultation with a clinical immunologist is required to confirm the diagnosis of PID and to establish an appropriate treatment plan.

► SCID is fatal unless the underlying defect is corrected and, therefore, definitive therapy with BMT or HSCT should be initiated as quickly as possible.

► Ig replacement therapy is the mainstay of therapy for antibody-deficiency disorders, and is also an important supportive treatment for many patients with other forms of PID including SCID/CID.

► Antibiotic and antifungal prophylaxis are also recommended for many PIDs to prevent the frequency and severity of infections.

SECONDARY IMMUNODEFICIENCY DISEASES

KEY POINTS

- A deficit in the immune system can lead to unusually severe or uncommon recurrent infections.
- Secondary immune deficiencies or acquired deficiencies, more frequent than primary immune deficiencies, are problems of the immune system that are notgenetic and which are caused by external factors.
- Secondary immunodeficiency disorders can occur in, for example, malnutrition, aging, many types of cancer (such as leukemia, lymphoma, multiple myeloma), and certain chronic infections such as acquired immunodeficiency syndrome (AIDS).
- Immunosuppression is one form of secondary immunodeficiency performed to prevent the body from rejecting an organ transplant, treating graft-versus-host disease after a bone marrow transplant, or for the treatment of auto-immune diseases, such as rheumatoid arthritis or Crohn's disease.
- A person who is undergoing immunosuppression or whose immune system is weak for other reasons (for example, chemotherapy, HIV, and Lupus), is said to be immunocompromised.

TERMS

- immunodeficiency

A depletion in the body's natural immune system, or in some component of it.

- immunosuppressive

Having the capability to suppress the immune system, capable of immunosuppression.

- immunocompromised

Having an immune system that has been impaired by disease or treatment.

- secondary infection

any infection that arises subsequent to a pre-existing infection; but especially a nosocomial infection

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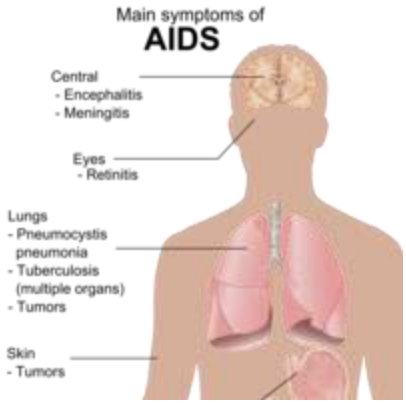
Immunodeficiency (or immune deficiency) is a state in which the immune system's ability to fight infectious disease is compromised or entirely absent. Immunodeficiency may also decrease cancer immunosurveillance. Most cases of immunodeficiency are acquired ("secondary") but some people are born with defects in their immune system, or primary immunodeficiency. Transplant patients take medications to suppress their immune system as an anti-rejection measure, as do some patients suffering from an over-active immune system. A person who has an immunodeficiency of any kind is said to be immunocompromised. An immunocompromised person may be particularly vulnerable to opportunistic infections, in addition to normal infections that could affect everyone. Distinction between primary versus secondary immunodeficiencies are based

on, respectively, whether the cause originates in the immune system itself or is, in turn, due to insufficiency of a supporting component of it or an external decreasing factor of it.

Secondary Immunodeficiencies

Secondary immunodeficiencies, also known as acquired immunodeficiencies, can result from various immunosuppressive agents, for example, malnutrition, aging and particular medications (e.g., chemotherapy, disease-modifying antirheumatic drugs, immunosuppressive drugs after organ transplants, glucocorticoids). For medications, the term immunosuppression generally refers to both beneficial and potential adverse effects of decreasing the function of the immune system, while the term immunodeficiency generally refers solely to the adverse effect of increased risk for infection. Many specific diseases directly or indirectly cause immunosuppression. This includes many types of cancer, particularly those of the bone marrow and blood cells (leukemia, lymphoma, multiple myeloma), and certain chronic infections. Immunodeficiency is also the hallmark of acquired immunodeficiency syndrome (AIDS) , caused by the human immunodeficiencyvirus (HIV). HIV directly infects a small number of T helper cells and also impairs other immune system responses indirectly.



Main Symptoms of AIDS

Acquired immunodeficiency syndrome (AIDS) is defined in terms of either a CD4+ T cell count below 200 cells per μL or the occurrence of specific diseases in association with an HIV infection. In the absence of specific treatment, around half the people infected with HIV develop AIDS within 10 years. The most common initial conditions that alert to the presence of AIDS are pneumocystis pneumonia and cachexia.

Immunodeficiency is the failure of the immune system to protect against disease or malignancy. Primary Immunodeficiency is caused by genetic or developmental defects in the immune system. These defects are present at birth but may show up later on in life. Secondary or acquired immunodeficiency is the loss of immune function as a result of exposure to disease agents, environmental factors, immunosuppression, or aging.

TYPES OF SECONDARY (ACQUIRED) IMMUNODEFICIENCIES

Immunodeficiencies associated with infections

Bacterial, viral, protozoan, helminthic and fungal infections may lead to B cell, T cell, PMN and macrophage deficiencies. Most prominent among these is acquired immunodeficiency syndrome (AIDS). Secondary immunodeficiencies are also seen in malignancies.

Immunologic abnormalities in the AIDS

All acquired immunodeficiencies have been outdone by AIDS that is caused by Human Immunodeficiency Virus (HIV)-1. This virus was first discovered in 1981 and the patients exhibited fungal infections with opportunistic organisms such as Pneumocystis carinii and in other cases, with a skin tumor known as Kaposi's sarcoma. There are two major types of HIV: HIV-1 and 2, the former being the strain frequently found in North America. HIV is spread through sexual intercourse, infected blood and body fluids as well as from mother to offspring. HIV, which was discovered in 1983, is a retrovirus with RNA that is reverse transcribed to DNA by reverse transciptase (RT) following entry into the cell. The DNA is integrated into the cell genome as a provirus that is replicated along with the cell. HIV-1 does not replicate in most other animals but infects chimpanzees although it does not induce AIDS in them. Severe combined immunodeficient mice (SCID) reconstituted with human lymphocytes can be infected with HIV-1. The HIV-1 virion consists of a viral envelope made up of the outer lipid bilayer of the host cell in which are embedded glycoproteins composed of the transmembrane gp41 along with the associated gp120. The gp120 binds the CD4 expressed on host cells. Within the viral envelope is the viral core or nucleocapsid consisting of a layer of matrix protein composed of p17 and an inner capsid made up of p24. The viral genome consists of two single stranded RNA molecules associated with two RT molecules as well as other enzymes including a protease and an integrase.

Replication cycle and targets of therapy

The virus attaches to the CD4 molecule on Th cells, monocytes and dendritic cells through the gp120 of HIV. For HIV infection, a co-receptor is required. The co-receptor is a chemokine receptor such as CXCR4 or CCR5. CCR5, expressed predominantly on macrophages, and CXCR4 on CD4+ T cells serve as coreceptors for HIV infection. After the fusion of HIV envelope and the host membrane, the nucleocapsid enters the cell. The RT synthesizes viral DNA which is transported to the nucleus where it integrates with the cell DNA in the form of a provirus. The provirus can remain latent till the cell is activated when the provirus also undergoes transcription. Virions, consisting of the transcribed viral RNA and proteins, are produced. These bud out of the host cell membrane from where they acquire the envelope. Thus, therapeutic agents have been developed that target viral entry and fusion, as well as serve as RT, protease and integrase inhibitors. Highly active anti-retroviral therapy is a cocktail of 3 or more such agents.

Immunological Changes

The virus replicates rapidly and within about two weeks the patient may develop fever. The viral load in the blood increases significantly and peaks in two months, after which there is a sudden decline because of the latent virus found in germinal centers of the lymph nodes. CTL develop very early whereas antibodies can be detected between 3 - 8 weeks. The CTL killing of Th cells around 4 - 8 weeks leads to a decrease in CD4+ T cells. When the CD4+ T cell count decreases below 200 per cubic mm, full blown AIDS develops.

Immunotherapy

There are several barriers to development of an effective HIV vaccine.

- Attenuated vaccine may induce the disease
- CD4+ T cells may be destroyed by the vaccine
- Antigenic variation of HIV
- Low immunogenicity of the virus by downregulation of MHC molecules
- Lack of animal models
- Lack of in vitro tests

The following reagents have been considered in developing vaccines

- Immunization with deletion mutants to reduce pathogenicity
- Vaccination with recombinant proteins
- Gene encoding proteins introduced into virus vectors may be used for vaccination
- Chemokines that compete for the co-receptors
- IL-2 to boost the Th cells.

Immunodeficiencies associated with aging

These include a progressive decrease in thymic cortex, hypo-cellularity of and reduction in the size of thymus, a decrease in suppressor cell function and hence an increase in auto-reactivity, a decrease in CD4 cells functions. By contrast B cells functions may be somewhat elevated.

Immunodeficiencies associated with malignancies and other diseases

B cell deficiencies have been noted in multiple myeloma, Waldenstrom's macroglobulinemia, chronic lymphocytic leukemia and well differentiated lymphomas. Hodgkin's disease and advanced solid tumors are associated with impaired T-cell functions. Most chemotherapeutic agents used for treatment of malignancies are also immunosuppressive.

Other conditions in which secondary immunodeficiencies occur are sickle cell anemia, diabetes mellitus, protein calorie malnutrition, burns, alcoholic cirrhosis, rheumatoid arthritis, renal malfunction, etc.

3. Final part Test-control
Initial knowledge level

1. An 8-month-old baby has a history of repeated gram-positive bacterial infections. The most probable cause for this condition is that
A) the mother did not confer sufficient immunity on the baby in utero.
B) the baby suffers from erythroblastosis fetalis (hemolytic disease of the newborn).
C) the baby has a defect in the alternative complement pathway.
D) the baby is allergic to the mother's milk.
E) None of the above.

2. A 50-year-old worker at an atomic plant who previously had a sample of his own bone marrow cryopreserved was accidentally exposed to a minimal lethal dose of radiation. He was subsequently transplanted with his own bone marrow. This individual can expect
A) to have recurrent bacterial infections.
B) to have serious fungal infections due to deficiency in cell-mediated immunity.
C) to make antibody responses to thymus-independent antigens only.
D) All of the above.
E) None of the above.

3. Which of the following immune deficiency disorders is associated exclusively with an abnormality of the humoral immune response?
A) X-linked agammaglobulinemia (Bruton's agammaglobulinemia)
B) DiGeorge syndrome
C) Wiskott-Aldrich syndrome
D) chronic mucocutaneous candidiasis
E) ataxia telangiectasia

4. A sharp increase in levels of IgG, with a spike in the IgG region seen in the electrophoretic pattern of serum proteins is an indication of
A) IgA or IgM deficiency.
B) multiple myeloma.
C) macroglobulinemia.
D) hypogammaglobulinemia.
E) severe fungal infections.

5. Patients with DiGeorge syndrome may fail to produce IgG in response to immunization with T-dependent antigens because
A) they have a decreased number of B cells that produce IgG.
B) they have increased numbers of suppressor T cells.
C) they have a decreased number of helper T cells.
D) they have abnormal antigen-presenting cells.
E) they cannot produce IgM during primary responses.

6. A 2-year-old child has had three episodes of pneumonia and two episodes of otitis media. All the infections were demonstrated to be pneumococcal. Which of the following disorders is most likely to be the cause?
A) an isolated transient T-cell deficiency
B) a combined T- and B-cell deficiency
C) a B-cell deficiency
D) transient anemia
E) The child has AIDS.

7. A healthy woman gave birth to a baby. The newborn infant was found to be HIV-seropositive. This finding is most likely the result of
A) the virus being transferred across the placenta to the baby.
B) the baby is making anti-HIV antibodies.
C) the baby's erythrocyte antigens cross-reacting with the virus.
D) the mother's erythrocyte antigens cross-reacting with the virus.
E) maternal HIV-specific IgG being transferred across the placenta to the baby.

8. Immunodeficiency disease can result from
A) a developmental defect of T lymphocytes.
B) a developmental defect of bone marrow stem cells.
C) a defect in phagocyte function.
D) a defect in complement function.
E) All of the above.

9. A 9-month-old baby was vaccinated against smallpox with attenuated smallpox virus. He developed a progressive necrotic lesion of the skin, muscles, and subcutaneous tissue at the site of inoculation. The vaccination reaction probably resulted from
A) B-lymphocyte deficiency.
B) reaction to the adjuvant.
C) complement deficiency.
D) T-cell deficiency.
E) B- and T-lymphocyte deficiency

10. The most common clinical consequence(s) of C3 deficiency is (are)
A) increased incidence of tumors.
B) increased susceptibility to viral infections.
C) increased susceptibility to fungal infections.
D) increased susceptibility to bacterial infections.
E) All of the above.

11. Which of the following statements regarding the functional properties of cytokines in patients with secondary immunodeficiencies is *false*?
A) They typically have pleiotropic properties.
B) They often exhibit functional redundancy.

C) They often display antigen specificity.
D) They exhibit synergistic or antagonistic properties.
E) They assist in the regulation and development of immune effector cells.

12. What happens to naive antigen-nonspecific T cells in the vicinity when IL-2 is secreted by antigen-specific T cells activated due to presentation of antigen by APCs?
A) They proliferate due to their exposure to IL-
B) They often undergo apoptosis.
C) They begin to express IL-2 receptors.
D) They secrete cytokines associated with their T_H phenotype.
E) Nothing happens.

13. Which of the following cytokines have receptors that exhibit structural similarity that helps to account for their functional redundancy?
A) IL-3, IL-15, and GM—CSF
B) IL-1, IL-2, and M—CSF
C) IL-2, IL-3, and IL-8
D) IL-3, TNF-b, and RANTES
E) IL-3, IL-4, and IFN-g

14. What type of immune response is *not* mediated by the T_H subset in patients with secondary imuunodeficiency?
A) responses to viral infections
B) delayed-type hypersensitivity
C) activation of cytotoxic T cells
D) activation of IgE synthesis
E) responses to intracellular pathogens

15. IL-1, IL-6, and TNF-a are proinflammatory cytokines that are known to
A) cause increased vascular permeability.
B) act in concert with chemokines to promote migration of inflammatory cells to sites of infection.
C) initiate acute-phase responses.
D) have endogenous pyrogen properties.
E) All of the above.

Final knowledge level

1. Case Study A 4-year-old child who just moved to a new town with his mother is brought to a pediatrician's office with a complaint of the child's failure to thrive, including loss of appetite, weight loss, and persistent cough. On physical examination the child appears pale and sickly, has a low-grade fever, but no other physical signs. His past history is unremarkable except for a fractured leg at 1 year of age that required a blood transfusion during surgery, but it healed without complication. A battery of tests are performed to arrive at a diagnosis. X-ray examination showed some bilateral pulmonary infiltrates. Blood count is normal, with slightly reduced white cell numbers. An ELISA to detect serum immunoglobulins showed elevated IgG, IgM, and IgA. Skin tests performed with mumps, tetanus, and candida antigens were negative. Antibiotic therapy was started, but on the next visit the child was sicker and now had enlarged lymph nodes, spleen, and liver. What diagnosis should the physician be thinking of at this point and how could it be confirmed?

2. A 7-year-old boy with an infected wound on his leg is admitted to the emergency department. His mother states that a high fever with diarrhea occurred during the last 12 hours. Within the last 2 hours he had become very lethargic, was unable to stand, and was very disoriented. The attending physician observes that his blood pressure is dangerously low and suspects that the boy is suffering from bacterial septic shock caused by the wound infection. Discuss the etiology of bacterial septic shock as well as the role of cytokines in the pathogenesis of this disease. Speculate on future therapeutic strategies that might be employed by using monoclonal antibodies or other biologic agents to treat this disease.

3. In what region can the largest number of people living with HIV currently be found?

a. Asia and the Pacific
b. Sub-Saharan Africa
c. Latin America and the Caribbean
d. North America
e. Central and Eastern Europe

4. What does the acronym HIV stand for?

a. Hemo-insufficiency virus
b. Human immunodeficiency virus
c. Human immobilization virus
d. all of the above
e. correct answer - absent

5. What does the acronym AIDS stand for?

a. active immunological disease syndrome
b. acquired immune deficiency syndrome
c. acquired immunological derivative syndrome

d. acquired immunodeficiency syndrome
e. all of the above

6. What is the main means of HIV transmission worldwide?
a. unprotected heterosexual sex
b. homosexual sex
c. intravenous drug use
d. mother-to-child transmission
e. all the above

7. Spread of HIV by sexual transmission can be prevented by:
a. abstinence
b. practising mutual monogamy with an uninfected partner
c. correct use of condoms
d. all of the above
e. coorect answer - absent

8. Women are most likely to contract HIV through:
a. unprotected heterosexual sex
b. injecting drug use
c. contaminated blood
d. saliva
e. correct answer- absent

9. HIV can be contracted from:
a. condoms
b. kissing
c. mosquito bites
d. drinking from the same glass as an infected person
e. none of the above

10. Immunodeficiency should be suspected when recurrent infections are the following:
a. Severe
b. Complicated
c. In multiple locations
d. Resistant to treatment
e. All of the above

11. Age when recurrent infections began is important.
a. Onset before age 6 mo suggests a T-cell defect because maternal antibodies are usually protective for the first 6 to 9 mo.
b. Onset between the age of 6 and 12 mo may suggest combined B- and T-cell defects or a B-cell defect, which becomes evident when maternal antibodies are disappearing (at about age 6 mo).

c. Onset much later than 12 mo usually suggests a B-cell defect or secondary immunodeficiency.
d. All of the above
e. Correct answer – absent

12. Characteristic clinical findings in patients with DiGeorge syndrome:
a. hypocalcemic tetany
b. a congenital heart disorder
c. unusual facies with low-set ears
d. developmental delay
e. all of the above

13. Characteristic clinical findings in patients with Chdiak-Higashi syndrome:
a. hypocalcemic tetany
b. a congenital heart disorder
c. unusual facies with low-set ears
d. developmental delay
e. none of the above

14. Characteristic clinical findings in patients with Hyper-IgE syndrome:
a. chronic gingivitis
b. recurrent aphthous ulcers
c. skin infections
d. severe neutropenia
e. none of the above

15. Characteristic clinical findings in patients with leukocyte adhesion deficiency:
a. delayed umbilical cord detachment
b. leukocytosis
c. periodontitis
d.poor wound healing
e. all of the above

CORRECT ANSWERS:

Initial knowledge level:

1. E 2. E 3. A 4. B 5. C 6. C 7. E 8. E 9. D 10. D 11. C 1**2. E** 1**3.A** 1**4. D** 1**5. E**

Final knowledge level:

Answer to Case Study

1. This is a possible case of childhood AIDS, presumably attributable to the earlier transfusion with blood obtained from a blood bank. Although present-day tests to screen blood from donors are highly reliable, there are still isolated cases of HIV transmission by blood transfusion. Following a long incubation period the child presented with a slightly lowered white cell count, somewhat elevated immunoglobulin levels, but a seriously compromised T-cell function. The latter was determined by the absence of skin reactions to mumps, tetanus toxoid, and candida antigens, all of which elicit T-cell-mediated delayed-type hypersensitivity reactions in normal individuals. A follow-up test of the levels of circulating $CD4^+$ and $CD8^+$ cells revealed a ratio of 0.4, indicating that helper $CD4^+$ cells, the target of the HIV virus, have declined markedly. Further study of the lung infiltrate, which failed to respond to antibiotic therapy, would be done by bronchoscopy and lavage. Microscopic examination of the washings would probably show an opportunistic organism such as *Pneumocystis carinii*, a common cause of death in patients with AIDS. Final confirmatory evidence would come from an examination of the child's serum for the presence of antibody to HIV antigens, a clear indication of an infection with HIV, and from the use of an HIV-specific PCR to ascertain the presence the viral genes.

2. Bacterial septic shock is a condition that can develop within a few hours following infection by certain gram-negative bacteria, including *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Enterobacter aerogenes,* and *Neisseria meningitidis*. The symptoms are often fatal and include a drop in blood pressure, fever, diarrhea, and widespread blood clotting in various organs. It develops when bacterial cell wall endotoxins stimulate macrophages to overproduce IL-1 and TNF-a. Therapeutic strategies using monoclonal antibodies capable of neutralizing the effects of IL-1 and TNF-α.

3.B; 4.B; 5.B; 6.E; 7.D; 8.A; 9.E; 10.E; 11.D; 12.E; 13.E; 14.E; 15.E.

Structure of class.

Plan and organizational structure of the lesson:

Preparatory stage: organization of lesson and test control of incoming level of knowledge (5 academic hours or 225 minutes)

№	Content	Level of learning	Methods of the learning control	Materials of the methodological support	Time, min.
1.	The preparatory phase Control input level of knowledge, skills.	I	1. Front rapid survey	Tests. Scheme.	25
2.	The main stage - The history of primary and secondary immunodeficiencies, HIV/AIDS; - Epidemiologiacal peculiarities these immunodeficiency onditions; - Immune response in patients with primary and secondary immunodeficiencies, HIV/AIDS; - Classification of primary immunodeficiency; Clinical symptoms and diagnosis primary and secondary immunodeficiencies, HIV/AIDS; - Approach to the treatment of primary and secondary immunodeficiencies, HIV/AIDS	II II II I-II II II I-II II I-II II-III	2. Individual oral examination. 3. Test control.	1.Tables. 2. Folio-grams. 3. Tests I-III 1. 2. Slideshow.	180
3.	The final stage Monitoring and adjustment of professional knowledge, skills and abilities: basic principles-purpose of immunotropic therapy in patients with primary and secondary immunodeficiencies, HIV/AIDS;	III III III	1. Testing. 2. Solving custom applications. 6. Oral examination.	1. Tests III-IV levels. 2. Situational problems.	15
4.	To sum up the lessons. Homework for the next topic.				2 3

Control questions:

1. Immune Deficiency Syndromes
a. Primary Immunodeficiency Syndromes
b. Secondary Immunodeficiency Syndromes/infections
2. Acquired Immunodeficiency Syndrome (AIDS)
a. Initial Description and Epidemiology
b. Human Immunodeficiency Virus
c. Clinical Course
d. Prevention, Control, and Therapy of HIV Infection
3. Neoplasms of the Immune System
a. B-Cell Neoplasms
b. T-Cell Neoplasms
c. Hodgkin's Disease
4. Approach to the patient with suspected immunodeficiency
5. Vaccines and avoidance of exposure to infection in patients with primary immunodeficiency.
6. Antibiotics and sometimes surgery with primary immunodeficiency.
7. Replacement of missing immune components with primary immunodeficiency.

Practical skills:

1. To be able to collect anamnesis and diagnose of different types of immunodeficiencies.
2. To know instrumental and laboratory methods for diagnosis of HIV/AIDS.
3. To formulate the modern principles of diagnosis and treatment of different types of immunodeficiencies.

The conclusions.

1. Possessed modern knowledge about the mechanisms of immunological reactions of biostructures damage, genetic and environmental bases of primary immunodeficiency.
2. Formed basic principles of clinical and laboratory and instrumental diagnostics of secondary immunodeficiency.
3. Determined the basic methods of laboratory diagnosis of HIV/AIDS.
4. Determined the basic approaches to the treatment of immunodeficiency conditions.

References:

1. Stephen T. Holgate, Martin K. Church, MPharm, David H. Broide, Fernando D Martinez. Allergy, 4th Edition. – Saunders Ltd. (2012). – 432 pages
2. Abul K. Abbas, Andrew H. H. Lichtman, Shiv Pillai Cellular and Molecular Immunology. - Saunders; 7 edition (2011). – 560 pages

3. Roitt's Essential Immunology, Includes Desktop Edition. Peter J. Delves, Seamus J. Martin, Dennis R. Burton, Ivan M. Roitt. Wiley-Blackwell; 12 edition (2011). – 560 pages

4. How the Immune System Works, Includes Desktop Edition. Lauren M. Sompayrac. Wiley-Blackwell; 4 edition (2012). – 152 pages

5. J. Wyatt, R. N. Illingworth, C. A. Graham, K. Hogg. Oxford Handbook of Emergency Medicine. Oxford University Press (2012). – 768 pages

6. H. Chapel, M. Haeney, S. Misbah, N.Snowden.Essentials of Clinical Immunology, 6th Edition. Wiley-Blackwell (2014). – 376 pages

7. A.Brian Baldo, N.H. Pham. Drug Allergy. Clinical Aspects, Diagnosis, Mechanisms, Structure-Activity Relationships. Springer New York (2013). – 447 pages

METHODICAL INSTRUCTION

Practical class №6

1. **THEME: ORGAN-SPECIFIC AND SYSTEMIC AUTOIMMUNE DISEASE: DIAGNOSIS AND TREATMENT** (5 academic hours).

2. **Actuality of the topic:**

1. Subject: autoimmune aggression and immunological tolerance. Organic non-specific/systemic and specific autoimmune disease.

2. Theme relevance: today the students' understanding of the mechanisms of immune system function in patients with autoimmune diseases, which will determine the selection of the proper modern treatment, is particularly important.

3. The purpose of class:
   - **study** – students must know the phenomenon of immunological tolerance, the basic theory of pathogenesis of autoimmune diseases
   - **professionally oriented** – students must be able to based on clinical and laboratory data to diagnose organic non-specific and specific organic autoimmune disease, determine the correct tactics of treatment
   - **educational** – students should know the factors that influence the development of autoimmune diseases, have a sense of responsibility for the timeliness and correctness of professional action.

4. Materials: Equipment to run powerpoint presentation

Main books. Short information due to the topic.

5. Integrative links theme:
   This practical topic is connected with such topics as "The structure and principles of the immune system functioning" and "Clinical and laboratory assessment of human immune status ".

Interdisciplinary integration:

Subject	To know	To be able to
1. Therapy	Diagnostic Criteria autoimmune Diseases	Differentiated by clinical and laboratory signs of various rheumatic diseases
2. Endocrinology	Clinical and laboratory features of thyroiditis, diabetes first type, Addison's disease	To diagnose this disease, prescribe treatment
3. Hematology	Clinical and laboratory signs of hemolytic anemia,	To diagnose this disease, prescribe treatment

	thrombocytopenia, agranulocytosis immune	
4. Ophthalmology	Clinical and laboratory signs of sympathetic ophthalmopathy, phacogenic uveitis	To diagnose this disease, prescribe treatment
5. Obstetrics and Gynecology	The main autoimmune diseases in obstetric practice	To diagnose this disease, prescribe treatment

6. The content of the theme class.

The content of the topic

6. Student has to know:

1. Understand the classification of autoimmunity
2. Know the diseases associated with autoantibodies production
3. Understand the mechanisms of damage in patients with autoimmune diseases
4. Definition of autoimmune diseases
5. Classification of autoimmune diseases
6. Basic principles of autoimmune diseases

7. Study questions.

1. Reasons for the development, triggers and genetic basis of systemic vasculitis
2. Immunological mechanisms and types of injury biostructures
3. Basic principles of diagnosis of systemic autoimmune diseases
4. Principles of treatment of autoimmune diseases
5. Prevention of autoimmune diseases

Main part

Autoimmune disease encompasses an array of 80 to 100 disorders affecting different body systems/organs. It has been difficult to determine the overall burden of autoimmune diseases, primarily because epidemiologic studies have not focused on them as a single entity and also because many of them are rare. However, research on individual autoimmune diseases, along with some studies on the diseases collectively, indicates that the burden is substantial in terms of the number of people affected, morbidity, mortality, and financial cost.The most recent estimate of the overall prevalence of 29 autoimmune diseases is approximately 9%, and studies have suggested that the prevalence is rising. Autoimmune diseases are chronic illnesses, with most having no available cure. As a result, lifelong treatment is needed for diseases that cause substantial morbidity, disability, mortality, and costs. It has been estimated that nearly half of all cases of autoimmune diseases remain undiagnosed

because of challenges in diagnosis. More than 45% of individuals with an autoimmune disease reported that they had been labeled as a chronic complainer in the early stages of their disease because no cause for their symptoms could be determined.

Evidence-based guidelines for diagnosis, management, and/or follow-up are available for some autoimmune diseases, but diagnosis frequently remains a challenge, because symptoms are often overlapping and definitive diagnostic testing is lacking for most diseases.

Autoimmune disease encompasses a broad array of disorders, and they vary according to the body systems/organs they affect and their associated morbidity. Researchers have identified direct evidence (the ability to transfer autoimmune disease) for 15 autoimmune diseases, and there is indirect evidence (the ability to reproduce the autoimmune disease in animal models) and circumstantial evidence (the association of autoantibodies with disease in appropriate clinical settings) for an autoimmunity component in more than 80 additional diseases.

The autoimmune diseases with the highest reported prevalence rates are Graves' disease, rheumatoid arthritis, and Hashimoto's thyroiditis; prevalence rates are lower for such diseases as celiac disease and autoimmune hepatitis. Among the other more commonly occurring autoimmune diseases are systemic lupus, Sjogren's syndrome, multiple sclerosis, myasthenia gravis, inflammatory bowel diseases (ulcerative colitis and Crohn's disease), pernicious anemia, scleroderma, primary biliary cirrhosis, Addison disease, and thrombocytopenic purpura. Estimates of the prevalence of fibromyalgia have been similar to that of common autoimmune diseases.

The prevalence of autoimmune diseases differs according to gender, age, and race/ethnicity. Most autoimmune diseases occur far more frequently in female individuals than in male individuals, and although these diseases can occur at any age, many occur during the middle adult years, which represents the childbearing years for women. Some diseases, such as type 1 diabetes, have an onset primarily in childhood and adolescence, and others, such as rheumatoid arthritis, occur primarily among older adults. Differences in the prevalence of autoimmune diseases according to race/ethnicity are only beginning to emerge, and the variations have been studied only within the context of individual diseases. Many autoimmune diseases follow a progressive course, even with appropriate management, and serious or life-threatening complications may develop. Functional limitations, disability, and poor quality of life are substantial concerns.

Much is still unknown about how autoimmune diseases or fibromyalgia develop, but investigators have explored host, genetic, and environmental factors and continue to evaluate potential pathways.

PATHOGENESIS

The immune system is designed to work with a balance of responding to a wide variety of foreign threats, such as harmful bacteria, viruses, or cancer cells, while maintaining self-tolerance (i.e., being nonresponsive to self-antigens). However, in a small proportion of individuals, this balance is disrupted, and there is unregulated activation of the immune system and loss of self-tolerance. The resulting autoimmunity can lead to autoimmune diseases, a heterogeneous group of disorders that involve damage to organs, tissues, or cells. Virtually every body system can be affected, and the target of

the immune system can be a specific organ, as in thyroiditis, or multiple organs, as in systemic lupus.

In organ-specific diseases, such as thyroiditis, type 1 diabetes, inflammatory bowel disease, or multiple sclerosis, a normal immune response is misdirected against a self-antigen or organ, and inflammation and production of autoantibodies are usually confined to antigens specific to the target organ. Multiple organs are targets in systemic autoimmune diseases, such as systemic lupus, Sj?gren's syndrome, or systemic sclerosis. In these types of autoimmune diseases, autoantibodies are directed to different autoantigens, typically resulting in chronic activation of innate and adaptive immune cells and an array of clinical manifestations. Some autoimmune diseases are characterized by an organ-specific immune process but are systemic because they also involve autoantibodies to autoantigens outside of a specific organ. For example, rheumatoid arthritis is primarily a joint-selective disease, but other autoantibodies can cause extra-articular manifestations.

Organ-specific autoimmune diseases differ according to whether disease is mediated primarily though autoantibodies, autoreactive T cells, or a combination of the two. Systemic autoimmune diseases can be categorized as being associated with either cell-mediated immunity or autoantibodies, or immune complexes. T-cell or B-cell activation can cause tissue damage directly, by binding to cell-surface autoantigens, or indirectly by forming antibody-antigen complexes that become deposited in tissues. The autoimmunity process is cyclic, as tissue damage leads to the release of cytokines, activated T cells, and additional self-antigens, further stimulating the immune response.

The detection of an autoantibody does not necessarily indicate the presence of an autoimmune disease, as some autoantibodies, such as rheumatoid factor and antinuclear antibodies, are found in individuals without evidence of an autoimmune disease. In addition, autoantibodies can be detected years before a related autoimmune disease develops. Some level of autoimmunity is, in fact, present in all individuals, which means that other factors must be involved in the development of an autoimmune disease.

RISK FACTORS

Genetic Factors

Genetics have been found to play a major role in rendering a person susceptible to an autoimmune disease. In general, autoimmune diseases occur concurrently within affected individuals and their families at higher than expected rates, but there are differences in the diseases that cluster within families. The mode of inheritance of an autoimmune disease is complex, and research indicates that the genes involved in autoimmune disorders are pleiotropic (meaning they affect more than one trait) rather than disease-specific. This research suggests that common alleles may have the potential for alternate clinical phenotypes under different sets of genetic and environmental factors, and data support the premise that clinically distinct autoimmune diseases may have common susceptibility genes. Currently, at least 68 genetic risk variants have been associated with various autoimmune diseases, and several loci have been identified as being associated with more than one autoimmune disease. Studies with monozygotic twins have been done to determine the genetic basis for many

autoimmune diseases. Reported concordance rates include 12% to 30% for rheumatoid arthritis, 25% to 57% for systemic lupus, 30% for multiple sclerosis, 30% to 50% for type 1 diabetes, 70% to 75% for celiac disease, and up to 80% for Graves' disease. The concordance rate does not reach 100% for any autoimmune disease, which means that factors other than genetics must have a role in the pathogenesis.

Environmental Factors

The role of environmental factors on the development of autoimmune disease has been studied, but exact triggers and how their interaction with genetic predisposition bears on pathogenesis have not yet been defined. Among the environmental factors that have been found to have influence are infectious agents, stress, sex hormones (estrogens and androgens), and cigarette smoking.

Infectious Agents

Animal models have provided the best evidence of infectious agents inducing an autoimmune disease by immune-mediated mechanisms. On the basis of studies with these models, researchers have theorized that the immune response is triggered by antigens of a micro-organism that closely resembles self-antigens, a mechanism that has been termed molecular mimicry. Another theory is that autoimmunity is induced by a mechanism known as the bystander effect: the invading micro-organism directly damages tissue during active infection, thereby exposing self-antigens to the immune system. The diseases most often associated with infection as an etiological factor are multiple sclerosis, type 1 diabetes, rheumatoid arthritis, systemic lupus, fibromyalgia, myasthenia gravis, and Guillain-Barre syndrome. The micro-organisms most often implicated are viral, including Epstein-Barr virus, hepatitis C virus, parvovirus, and cytomegalovirus.

Stress

Several studies in animals and humans have demonstrated that physical and psychologic stress affects the immune system, most probably the result of downstream neuroendocrine alterations that modulate immune function. As a result, researchers have hypothesized that stress may be involved in the development of autoimmune diseases. Inflammatory autoimmune diseases, such as rheumatoid arthritis and systemic lupus, are the most likely diseases to be influenced by stress. Psychologic stress as a trigger for autoimmune diseases and fibromyalgia is further suggested by studies in which as many as 80% of individuals reported emotional stress or major life events before the onset of symptoms, primarily in cases of rheumatoid arthritis, fibromyalgia, and Graves' disease. However, most studies have been retrospective and have lacked the statistical power to determine significance.

Sex Hormones

Sex hormones and their metabolites and receptors are involved in immunoregulation and the development of autoreactivity through their roles in lymphocyte maturation, activation, and synthesis of antibodies and cytokines. Studies have shown that sex hormones are a factor in the pathogenesis of autoimmunity and that the expression of sex hormones is altered in individuals with autoimmune diseases. Systemic lupus offers the strongest evidence for sex hormones as a development factor because of its incidence trend (i.e., high after puberty and low after menopause) and the fluctuations in disease severity according to menstrual cycles and pregnancy. More research is

needed to better understand the role of sex hormones in autoimmunity and in specific autoimmune diseases.

Cigarette Smoking

Cigarette smoking has also been found to be a potential trigger for autoimmune diseases, most notably rheumatic diseases (rheumatoid arthritis and systemic lupus) and, to a lesser degree, thyroiditis. The exact mechanisms behind the influence of cigarette smoke on the pathogenesis of autoimmune diseases are uncertain.

GENERAL CHARACTERISTICS

Although each autoimmune disease is a distinct entity with its own constellation of signs, symptoms, and clinical manifestations, many autoimmune diseases share some common characteristics, including female preponderance, similar symptom profiles, difficulty in diagnosis, importance of history and physical examination in diagnosis, and similarity in the approach to disease management.

Female Preponderance

Autoimmune diseases have a definite gender bias, with women accounting for nearly 80% of cases overall. The female-to-male ratio varies according to disease, from Hashimoto's thyroiditis, which has a female preponderance of 95%, to vitiligo, which has a female preponderance of 52%. Fibromyalgia is also more prevalent in women (3.4% vs. 0.5%)http://www.netce.com/coursecontent.php?courseid=753&works=true - bibl.workscited.58. However, a few diseases have been reported to occur more often in men than women, including type 1 diabetes, ulcerative colitis, Guillain-Barr? syndrome, and psoriasis.

FEMALE PREDOMINANCE OF AUTOIMMUNE DISEASES AND FIBROMYALGIA

Disease	Percentage of Sufferers Who Are Female
Hashimoto's thyroiditis	95%
Sjogren's syndrome	94%
Addison disease	93%
Scleroderma	92%
Fibromyalgia	80% to 90%
Systemic lupus erythematosus	89%
Graves' disease	88%
Rheumatoid arthritis	75%
Myasthenia gravis	73%
Multiple sclerosis	64%
Vitiligo	52%

Similar Symptom Profiles

The symptom profiles associated with autoimmune diseases and fibromyalgia are another shared characteristic. Extreme fatigue is common, and other shared symptoms

include low-grade fever, dizziness, and general malaise. In addition, vague, nonspecific symptoms tend to wax and wane over the long-term, causing periods of remission with intermittent disease flare-ups. Clinical presentations with overlapping symptom profiles, along with a high rate of co-occurring autoimmune diseases, make it difficult to confirm the diagnosis of an autoimmune disease or fibromyalgia.

Difficulty in Diagnosis

Evidence of the difficulty in diagnosing autoimmune diseases or fibromyalgia is demonstrated in the results of surveys that have shown that individuals consult an average of four (and as many as 13) healthcare providers, typically over 2 to 4 years, before a diagnosis is made. There are several reasons for the challenge. First, the initial symptoms are often subtle, nonspecific, and intermittent until the disease enters the acute stage. Symptoms can also affect many body organs, making it difficult for specialists in one area to recognize a disease within another specialty area. In addition, because most individual autoimmune diseases are rare, a primary care clinician may be unfamiliar with the clinical manifestations of each disease. Lastly, the diseases lack a single distinguishing feature, creating the need for clinicians to rely on varying combinations of information gathered from the history, physical examination, and laboratory and imaging studies. Diagnostic criteria have been developed to aid in the diagnosis of some autoimmune diseases.

Importance of History and Physical Examination in Diagnosis

A carefully taken history and comprehensive physical examination are particularly vital for the diagnosis of autoimmune diseases and fibromyalgia. Clinicians should prompt patients about symptoms that the patient may not consider important enough to report. Clinicians should also ask about any family and personal history of autoimmune diseases.

Studies of autoimmune diseases within families have shown significantly higher frequencies of autoimmune disease in general and of specific autoimmune diseases among first-degree relatives compared with controls. Studies have also demonstrated that an individual with a diagnosed autoimmune disease is often at increased risk for the co-occurrence of another autoimmune disease. These studies have focused primarily on individuals with an index disease of multiple sclerosis, rheumatoid arthritis, autoimmune thyroiditis (hypothyroidism), type 1 diabetes, inflammatory bowel disease, and vitiligo. Among the most significant findings are a 90-fold and 68-fold higher prevalence of Hashimoto's thyroiditis and Graves' disease, respectively, among individuals with systemic lupus. Other studies have indicated an increased risk of type 1 diabetes and ulcerative colitis among persons with multiple sclerosis, and an increased risk of rheumatoid arthritis, multiple sclerosis, and a combined category of six other diseases (Addison disease, hemolytic anemia, primary biliary cirrhosis, immune thrombocytopenia purpura, Sj?gren's syndrome, and systemic sclerosis) among persons with inflammatory bowel disease. In approximately 60% of individuals with Sj?gren's syndrome, the syndrome is secondary to another autoimmune disease, most commonly rheumatoid arthritis, systemic lupus, or systemic sclerosis. Celiac disease has been associated with the co-occurrence of several autoimmune diseases, most notably Sjogren's syndrome and type 1 diabetes. Autoimmune diseases of connective tissue have generally been associated with higher rates of co-occurrence of

other autoimmune diseases. Higher-than-expected rates of fibromyalgia have also been found in individuals with autoimmune diseases, most notably systemic lupus, rheumatoid arthritis, thyroiditis, and Sjogren's syndrome. Obtaining an accurate history necessitates effective patient-physician communication, which is challenging given the high number of people of various racial/ethnic minorities or with inadequate language proficiency or health literacy.

Approach to Disease Management

The specific treatment of autoimmune diseases depends on the particular systems or organs affected, but the overall goals of treatment are similar. These goals are primarily to relieve symptoms, preserve organ function, and control the autoimmune process, often with immunomodulatory/immunosuppressant drugs. Challenges in treatment are related to the complexity of symptoms, the need to manage long-term medications for preserving organ function, and the long-term adverse effects of immunosuppressant drugs. As with diagnostic criteria, practice guidelines for the treatment of autoimmune diseases are available but limited. The long-term management of individuals with autoimmune diseases requires a multidisciplinary approach, with potential referral to specialists, such as rheumatologists, endocrinologists, gastroenterologists, neurologists, nutritionists, physical/occupational therapists, and counselors. This multidisciplinary care is best coordinated by the primary care provider, with clear articulation of specific roles. Because of the influence of stress on the immune system-coupled with the stress of a chronic disease-the management of autoimmune diseases should include stress reduction interventions.

The management of autoimmune diseases is often complicated by patients' responses to the diagnosis and their coping with the disease. Adherence to the treatment plan is often difficult because of denial about the diagnosis, work and life demands, and frustration with the lack of symptom response to treatment. Unresolved symptoms lead to a high rate of use of complementary and/or alternative methods among individuals with autoimmune diseases or fibromyalgia. The chronic nature of the conditions and the need for adherence to long-term management with frequent follow-up visits is essential for optimal outcomes but is also challenging, especially for individuals in racial/ethnic minority populations who may have different perceptions of health and the disease. A strong, supportive patient-clinician relationship is integral to ensuring adherence and effective management.

Patient-Clinician Relationship in Disease Management

To enhance the patient-provider relationship, healthcare professionals are advised to gain an understanding of the patient's perspective of his or her illness or disease and to ensure that the patient's primary concerns have been addressed. Patient trust in healthcare providers has been rated higher for clinicians who seek the patient's perspective of his or her illness. In turn, the healthcare professional's comprehensive knowledge of the patient and higher levels of patient trust have been reported to be substantial influences on adherence to medical advice, patient satisfaction, and improved health status. Effective communication is a cornerstone of the patient-provider relationship. Some communication behaviors that have been found to be positively associated with health outcomes include empathy, reassurance and support, explanations, positive reinforcement, humor, discussion of psychosocial issues, health

education and information sharing, courtesy, and summarization and clarification. Other factors essential for effective communication and a successful relationship are knowledge of the patient's language preference; an understanding of and respect for the patient's personal cultural values, beliefs, and practices (referred to as cultural competency); and an awareness of the patient's health literacy level.

ORGAN-SPECIFIC AUTOIMMUNE DISEASES

THYROIDITIS

Thyroiditis is the most common autoimmune disease. Autoimmune thyroiditis encompasses both Hashimoto's thyroiditis, also known as chronic lymphocytic thyroiditis, and Graves' disease. Hashimoto's disease and Graves' disease are the leading causes of hypothyroidism and hyperthyroidism, respectively. Hashimoto's disease is more common than Graves' disease.

In Hashimoto's disease, antithyroid antibodies destroy thyroid cells, resulting in the decreased production of thyroid hormones. Hashimoto's disease can be associated with either subclinical or overt hypothyroidism, and subclinical disease is the more commonly encountered of the two in the primary care setting. Left untreated, hypothyroidism can cause fatigue, weight gain, mental slowing, heart failure, and elevated lipid levels.

In Graves' disease, circulating thyroid antibodies target the TSH receptor, which stimulates the thyroid gland, causing enlargement of the thyroid gland and increased production of thyroid hormone. As with Hashimoto's disease, thyroid dysfunction with Graves' disease may be subclinical or overt. Mild ophthalmopathy is present in as many as half of individuals with Graves' disease, and severe ophthalmopathy occurs in 3% to 5%. This ophthalmopathy is the result of edema and lymphocytic infiltration of orbital fat, connective tissue, and eye muscles, and exophthalmos is the characteristic sign of Graves' disease. If not treated, overt hyperthyroidism can result in atrial fibrillation, congestive heart failure, osteoporosis, and neuropsychiatric problems.

POTENTIAL ENVIRONMENTAL RISK FACTORS

In individuals with genetic susceptibility, iodine deficiency, infection, smoking, and stress have been identified as environmental triggers for both types of autoimmune thyroiditis. Recent childbirth may be an additional trigger for Graves' disease.

ASSOCIATION WITH OTHER AUTOIMMUNE DISEASES

A coexisting autoimmune disorder is present in approximately 14% of individuals with Hashimoto's disease and nearly 10% of individuals with Graves' disease. In a British study involving more than 3,000 individuals with autoimmune thyroiditis, rheumatoid arthritis was the most common coexisting autoimmune disorder, appearing in approximately 4% of individuals with Hashimoto's disease and 3% of those with Graves' disease. Among the other autoimmune disorders that have been found to be associated with Hashimoto's thyroiditis are pernicious anemia, systemic lupus, Addison disease, celiac disease, Sj?gren's syndrome, systemic sclerosis (scleroderma), type 1 diabetes, and vitiligo.

Genetic studies have indicated a close relationship between type 1 diabetes and autoimmune thyroid disease, and a fourfold risk of thyroiditis has been found among individuals with type 1 diabetes. In a small study (254 participants), nonthyroid autoimmune diseases were found in approximately 9% of individuals with Graves'

disease, and the specific nonthyroid diseases varied according to the presence or absence of ophthalmopathy. Type 1 diabetes was the most prevalent disease among individuals who did not have ophthalmopathy (approximately 7%), and vitiligo was the most prevalent autoimmune disease among those who had ophthalmopathy (4%).
Another small study has suggested that Hashimoto's disease and/or subclinical hypothyroidism may be a predisposition to fibromyalgia; signs and symptoms of fibromyalgia were found in nearly one-third of individuals.

CLINICAL MANIFESTATIONS

Both Hashimoto's disease and Graves' disease may be present with no symptoms or with subtle, nonspecific symptoms, especially with early or subclinical disease. With Hashimoto's disease, approximately 20% of individuals have symptoms at the time of diagnosis, although symptoms may not develop until years after thyroid dysfunction. Symptoms of Graves' disease are usually present for at least 2 to 3 months before diagnosis.
The symptoms associated with Hashimoto's disease are the same regardless of whether hypothyroidism is present. In addition to nonspecific symptoms, such as fatigue, weakness, lethargy, and muscle aches, hypothyroidism can also affect a variety of body systems.

SIGNS AND SYMPTOMS OF AUTOIMMUNE THYROID DISEASE

Body System	Hashimoto's Disease	Graves' Disease
General	Fatigue Weakness Lethargy Hypothyroid speech Forgetfulness Increased sensitivity to medications	Fatigue Weakness Sleep disturbances
Psychiatric	Depression	Emotional instability Nervousness, anxiety
Metabolic	Weight gain Cold intolerance	Weight loss Heat intolerance
Skin	Pale, dry, cold skin (may appear jaundiced) Coarse skin Thick, brittle nails Dry, coarse, brittle hair or hair loss	Warm, moist skin Pretibial myxedema Hair loss
Cardiovascular	Slow pulse Bradycardia Diastolic hypertension Peripheral edema	Rapid pulse (≥90 beats/minute) Tachycardia, palpitations, atrial fibrillation Elevated systolic and diastolic blood pressure Edema Dyspnea on exertion
Pulmonary	Slow, shallow respirations	Shortness of breath Increased respiratory rate and depth

Neurologic	Delayed ankle reflexes	Fine finger/hand tremor
Musculoskeletal	Sore muscles Pain and/or stiffness in joints	Proximal muscle weakness or wasting Back pain History of fractures
Digestive	Constipation	Increased appetite Diarrhea Vomiting Abdominal pain
Hematologic	Easy bruising Macrocytic anemia Normocytic normochromic anemia	Easy bruising
Renal	--	Polyuria Polydipsia
Reproductive	Menorrhagia Irregular periods Decreased libido Increased rate of miscarriage, still birth, and fetal death	Amenorrhea Irregular periods Decreased fertility Increased risk for miscarriage
Ophthalmologic	--	Tearing Gritty sensation Eye discomfort/pain Diplopia Exophthalmos

Fatigue and weakness are also among the most common symptoms associated with Graves' disease, and as with Hashimoto's disease, symptoms can be related to many body systems, with the overactivity of the thyroid having the opposite effect. For example, hypothyroidism is typically associated with bradycardia, while hyperthyroidism is usually associated with a rapid, bounding pulse and/or palpitations.

DIAGNOSTIC EVALUATION

Because of the frequency of nonspecific symptoms and the wide array of other symptoms, healthcare professionals should elicit a detailed history, with emphasis on questions related to:

- Appetite, recent unexplained weight loss, or weight gain
- Tightness, fullness, or pain in the neck
- Eye pain or discomfort, changes in visual acuity
- Nervousness and/or anxiety
- Emotional status
- Abdominal pain
- Constipation or diarrhea
- Exertional dyspnea
- Increased perspiration
- Heat or cold intolerance

- Regularity of menstrual cycles
- Sleep disturbances
- Hair loss

The comprehensive physical examination should begin with assessment of blood pressure, weight, pulse, and other vital signs. A slow pulse is a clinically significant finding of hypothyroidism, and a rapid pulse (i.e., 90 beats per minute or more) is a clinically significant finding of hyperthyroidism. Among individuals with hyperthyroidism, tachycardia occurs less often among older individuals than younger ones.

Palpation and auscultation of the thyroid should be done to determine if the gland is enlarged and if nodules are present. In individuals with hypothyroidism, the thyroid gland may not be palpable or a goiter may be present. An enlarged thyroid gland is a significant sign of hyperthyroidism, occurring in 70% to more than 90% of individuals with the disorder. The goiter associated with hyperthyroidism is typically diffuse and symmetric, which distinguishes Graves' disease from toxic nodular goiter, in which palpation of the goiter usually reveals nodes.

Evaluation of the skin is also important. Skin that is both cool and dry is a clinically significant finding for hypothyroidism; the skin may also feel coarse or appear pale or yellowish [106]. Skin that is both warm and moist is a significant finding for hyperthyroidism. Hair loss is common with both types of thyroiditis. An eye examination is integral to the diagnosis of Graves' disease, as exophthalmos is a hallmark characteristic and is often the first sign of this disease. Eyelid retraction is the most clinically significant finding of hyperthyroidism, followed by eyelid lag; other ophthalmologic signs of Graves' disease are periorbital edema and limited eye movements. Hypothyroid speech-a low-pitched, hyponasal (as if speaking with a cold) voice, spoken at a slow pace-is found in about one-third of individuals with hypothyroidism. This speech is the finding with the most clinical significance for diagnosis of hypothyroidism. A neurologic evaluation is also useful in the diagnosis. Delayed ankle reflexes are a clinically significant finding of hypothyroidism, and fine finger tremor is a clinically significant finding of hyperthyroidism. Tremor is less likely to occur in older than younger individuals with hyperthyroidism. No single clinical finding, when absent, is significant for ruling out hypothyroidism. The lack of thyroid enlargement, a pulse of less than 90 beats per minute, and the absence of finger tremor are findings with the most significance in ruling out hyperthyroidism. Among the differential diagnoses that should be considered when evaluating an individual with suspected Hashimoto's thyroiditis are chronic thyroiditis, thyroid nodules, euthyroid sick syndrome, and lymphoma of the thyroid. The differential diagnosis for Graves' disease includes toxic nodular goiter, subacute thyroiditis, and papillary carcinoma of the thyroid.

Laboratory Testing

Thyroid function tests can confirm a diagnosis of Hashimoto's thyroiditis or Graves' disease. The single best screening test for either disease is the sensitive TSH assay (also known as thyrotropin level), and the free thyroxine (T4) level and the total

triiodothyronine (T3) level also help confirm the diagnosis. An elevated TSH level with low levels of T3 and free T4 indicates Hashimoto's hypothyroidism. Subclinical hypothyroidism is indicated by a repeatedly high TSH level with normal free T4 and T3 levels. In contrast, a low TSH level with increased T3 and T4 levels indicates hyperthyroidism. The patient's history is important to remember when interpreting the results of laboratory testing, as a low TSH level can also be caused by glucocorticoids, severe illness, or pituitary dysfunction. Thyroid autoantibodies (i.e., thyroid peroxidase and thyroglobulin antibodies) may be helpful in the diagnosis.

Other Testing

A radioiodine-uptake scan is not useful in diagnosing hypothyroidism, but it can help distinguish hyperthyroidism from subacute thyroiditis, which is associated with low uptake values, and from multinodular toxic goiter. If a radioiodine-uptake scan is not possible, ultrasonography of the thyroid gland may be done instead, and increased blood flow by Doppler correlates with an increased uptake. Ultrasonography is also useful for detecting nodules and evaluating suspicious structural abnormalities. A fine-needle biopsy should be done to exclude malignancy when a dominant nodule is present.

TREATMENT OPTIONS

The American Thyroid Association have developed guidelines for the treatment of hypothyroidism and hyperthyroidism. Treatment of either thyroid dysfunction must be tailored to the individual patient, and the patient should have a clear understanding of the indications and implications of all forms of therapy, including risks, benefits, and side effects. Clinicians should also encourage the patient to be an active participant in the decision-making process regarding the type of therapy. The goal of treatment for either condition is to achieve a euthyroid state.

Hashimoto's Disease

Most primary care clinicians can diagnose and treat hypothyroidism, but the organization recommends consultation with an endocrinologist for patients with:

Age 18 years or younger

Pregnancy

Cardiac disease

Disease that is unresponsive to treatment

Another endocrine disease

A goiter, nodule, or other structural change in the thyroid gland

Overt hypothyroidism involves lifelong thyroid replacement medication, typically levothyroxine. Levothyroxine is prescribed as a daily, oral dose, and treatment begins with a low dose and is gradually titrated up according to the results of TSH testing. An initial daily dose of 25 to 50 mcg has been recommended; lower doses may be more appropriate for older individuals or those with cardiovascular disease. Clinical evaluation of the patient and TSH testing should be done every 4 to 6 weeks after a change in dose. When titrating the dose of levothyroxine, healthcare professionals must consider the effects of other drugs the patient takes. Many drugs, including cholestyramine, ferrous sulfate, sucralfate, calcium, and some antacids containing aluminum hydroxide, can interfere with levothyroxine absorption. Also, rifampin and

sertraline may accelerate levothyroxine metabolism, calling for a higher replacement dose.

Hashimoto's Disease Without Hypothyroidism

Recommendations have also been made for individuals who have Hashimoto's disease without hypothyroidism (i.e., who have a goiter but normal TSH levels). Treatment is not required for individuals who are asymptomatic and have a small goiter. However, many endocrinologists prescribe levothyroxine for patients with a goiter, even if the level of TSH is normal, with a goal of decreasing the size of the goiter.

Subclinical Hypothyroidism

The appropriate approach to subclinical hypothyroidism has been debated. Proponents of treatment note that although subclinical hypothyroidism is usually asymptomatic, treatment has been shown to offer benefit in reducing the risks of several adverse events, including cardiovascular events, hyperlipidemia, and neuropsychiatric effects. In addition, subclinical hypothyroidism can progress to overt hypothyroidism, with a wide range in risk of progression (3% to 20%).

Graves' Disease

As noted, the goal of treatment of Graves' disease is to make the thyroid function normally or to disable the gland completely and treat the resultant hypothyroidism. The three primary treatment options are radioactive iodine (usually 131I), antithyroid drugs, or thyroidectomy. In addition, treatment with a beta blocker is recommended to provide relief of symptoms (such as tremor, palpitations, and sweating) until a euthyroid state is reached.

Treatment with Radioactive Iodine

Treatment with 131I is considered to be the treatment of choice for most people; however, pregnancy and breastfeeding are absolute contraindications. A pregnancy test should be obtained before treatment with 131I for all women of childbearing age who are sexually active.

The isotope is given orally (as a capsule or in water), and there is no consensus on the optimal dose. The dose is usually determined with a dose-calculation algorithm, and the typical dose range is 5 to 15 mCi of 131I Randomized trials have shown no significant differences in outcome between the use of calculated doses and fixed doses, and fixed doses are now used in many institutions.

Treatment with antithyroid drugs may be indicated in some individuals, particularly older individuals or those with cardiac disease, before administration of 131I. Antithyroid drugs should be stopped 1 week before treatment with radioactive iodine is begun and should not resume until approximately 6 weeks after treatment.

The American Thyroid Association recommend that individuals be followed up every 4 to 6 weeks for the first 3 months after treatment to monitor the transition to a euthyroid and/or hypothyroid state. Hypothyroidism can occur at any time after treatment, but most commonly occurs within 2 to 6 months. Treatment with partial replacement doses of levothyroxine can usually begin 2 months after treatment. The timing of thyroid-replacement treatment depends on the findings of laboratory testing and clinical evaluation.

The cure rate for treatment with radioactive iodine is more than 80%. Retrospective studies have shown that factors associated with a lack of response to 131I are a young

age, a large thyroid, severe thyrotoxicosis, previous exposure to antithyroid drugs, and a higher 131I uptake value. When necessary, a second dose should be given at least 6 to 12 months after the initial treatment, and antithyroid drugs should be stopped before and after a second treatment.

Treatment with 131I is safe, with the primary side effects being acute radiation thyroiditis and hypothyroidism; there is no adverse effect on fertility or on offspring conceived after treatment. The findings of some studies have suggested an increased risk for some types of cancer after treatment with 131I, but the results of other studies have demonstrated conflicting data, with no increases in the incidence of cancer.

Treatment with Antithyroid Drugs

Antithyroid drugs (thionamides) interfere with thyroid hormone synthesis by preventing iodine from combining with tyrosine residues in thyroglobulin. This approach is usually the treatment of choice for pregnant women, children and adolescents, and individuals who have severe Graves' ophthalmopathy. The goal of treatment is to achieve remission, defined as a biochemical euthyroid state for a minimum of 1 year after discontinuing treatment.

The most frequently prescribed antithyroid drugs are methimazole and propylthiouracil. Methimazole has become the preferred drug in the United States, especially after a 2012 U.S. The FDA recommended that physicians should "carefully consider" the choice of drug for newly diagnosed Graves' disease and that propylthiouracil should not be used in children and adolescents unless the patient is allergic to or intolerant of methimazole and no other treatment options are available. The FDA now requires a boxed warning on the label of propylthiouracil to alert clinicians about the risk of liver damage.

Antithyroid drug therapy can be given in two ways: a titration regimen or a block-replace regimen. With a titration regimen, the initial dose is high and the dose is tapered over time. With a block-replace regimen, a high dose of an antithyroid drug is given, followed by levothyroxine once a euthyroid state has been reached. The starting dose depends on the severity of the hyperthyroidism, and the typical starting doses have been 10 to 40 mg/day for methimazole and 100 to 600 mg/day (in divided doses) for propylthiouracil. The starting dose is tapered according to the results of thyroid function testing, which should be done once a month until symptoms start to resolve and then every 2 to 3 months. The results of thyroid function testing are also considered when tapering the dose; testing should be done every month. Use of a block-replace regimen requires less frequent testing. Typical maintenance doses are 5 to 20 mg/day of methimazole or 100 to 200 mg/day of propylthiouracil.

Two starting doses of methimazole (15 mg/day and 30 mg/day) and propylthiouracil (300 mg/day) were compared in a small randomized study in Japan (240 participants). Overall, the 30 mg/day dose of methimazole normalized the serum free T4 level in significantly more individuals than the 15 mg/day dose or propylthiouracil at 12 weeks. The higher dose of methimazole was also significantly more effective in the subgroup with severe hyperthyroidism (free T4 level: 7 ng/dL or greater), but there was no difference among the three treatments in the subgroup with mild or moderate disease (free T4 level: less than 7 ng/dL).

With regard to duration of therapy, 1 year of treatment has been reported to offer better rates of remission than 6 months of treatment. However, there has been no significant difference in remission rates at 2 years between individuals treated for longer than 18 months compared with those treated for 18 months. A systematic review indicated that the optimal duration of a titration regimen was 12 to 18 months.

Hyperthyroidism will recur after antithyroid drug therapy in approximately 30% to 60% of individuals. Studies have suggested that recurrence after antithyroid drug therapy is associated with several factors, including:

- Severe hyperthyroidism
- Long duration of symptoms before initiation of treatment
- Age younger than 40 years
- Male gender
- Family history of autoimmune thyroid disease
- History of cigarette smoking
- Presence of clinical ophthalmopathy
- High serum T3 and T4 concentrations
- Large goiter at diagnosis and/or at end of therapy

However, the association between recurrence and any of these individual factors has not been strong enough to warrant use as a risk stratification factor. Another course of antithyroid drug therapy, treatment with radioactive iodine, or surgery can be used to treat recurrent hyperthyroidism. Side effects occur in approximately 5% of individuals receiving antithyroid drugs. The most common side effects are rash, arthralgia, gastrointestinal problems, and changes in taste/smell. The most serious side effect, occurring in about 0.1% to 0.3% of individuals, is agranulocytosis. The risk of agranulocytosis increases with higher drug doses and with age and can occur at any time during the course of treatment..

Thyroidectomy

Surgery was once frequently used to treat hyperthyroidism, but it is now the least-used treatment option. The AACE and the American Thyroid Association recommend that the specific indications for thyroidectomy are a large goiter, especially with compressive symptoms (which may be resistant to radioactive iodine treatment); severe ophthalmopathy (because of the risks associated with radioactive iodine); or an allergy or intolerance to antithyroid drugs. The primary advantage of thyroidectomy is that it provides definitive treatment of hyperthyroidism with none of the hazards associated with radioactive iodine, the other option with a good cure rate. In addition, surgery offers a rapid normalization of thyroid function. Thyroidectomy usually results in hypothyroidism, occurring in 12% to 80% of individuals during the first year and at a subsequent annual rate of 1% to 3%. Thyroidectomy is associated with a low rate of complications and a mortality rate of nearly zero. Total thyroidectomy is recommended over subtotal thyroidectomy because it has been associated with similar complication rates but better cure rates.

Treatment of Subclinical Hyperthyroidism

There is no consensus on whether subclinical hyperthyroidism should be treated. Treatment is generally unnecessary, but all individuals with subclinical hyperthyroidism should be followed up with clinical evaluation and laboratory testing at approximately 6-month intervals.

The Endocrine Society asserts that subtotal thyroidectomy may be indicated during pregnancy as therapy for maternal Graves' disease if 1) a patient has a severe adverse reaction to antithyroid drug therapy, 2) persistently high doses of antithyroid drug are required, or 3) a patient is nonadherent to antithyroid drug therapy and has uncontrolled hyperthyroidism. The optimal timing of surgery is in the second trimester.

Treatment of Ophthalmopathy

The primary problems caused by Graves' ophthalmopathy are dryness and edema. The AACE and the American Thyroid Association recommend many nonpharmacologic measures for symptoms related to mild Graves' ophthalmopathy, including artificial tears for lubrication, sunglasses to decrease photophobia, eye protectors during sleep, and elevation of the head of the bed to decrease periorbital edema. Other interventions include a diuretic at bedtime, application of cool compresses to the eyes, increased fluid intake, and avoidance of secondhand smoke, ceiling fans, and contact lenses. Clinicians should consult an ophthalmologist experienced with Graves' ophthalmopathy for patients with severe eye problems. Treatment may include glucocorticoids, retro-orbital radiation, or surgery.

Treatment of Thyroid Storm

A complication of Graves' disease is thyroid storm, a syndrome characterized by exaggerated signs and symptoms of hyperthyroidism accompanied by fever and altered mental status. Thyroid storm is most often precipitated by a concurrent illness or injury and may also occur following discontinuation of treatment with antithyroid drugs or with radioactive iodine. The diagnosis of thyroid storm relies on clinical evaluation, as laboratory testing cannot distinguish thyroid storm from uncomplicated hyperthyroidism. Thyroid storm is a complex, life-threatening syndrome, and an endocrinologist should be involved in the care. Individuals with thyroid storm should be treated in the intensive care unit, with treatment consisting of an antithyroid drug, a drug that inhibits release of thyroid hormone from the thyroid gland, and agents that decrease the peripheral effects of thyroid hormone.

FOLLOW-UP AND PROGNOSIS

The American Thyroid Association recommend annual follow-up visits for patients with either Hashimoto's or Graves' disease, after a stable TSH level has been achieved . Both organizations recommend that a TSH level be determined at least annually. This monitoring is important, as studies have shown that as many as 40% of individuals taking thyroid medication do not have a TSH level within the normal range. Clinicians should also ask direct questions about compliance with drug therapy. Routine follow-up visits provide healthcare professionals with the opportunity to evaluate patients for signs or symptoms of other autoimmune diseases, especially those that have been reported to be associated with thyroiditis, such as rheumatoid arthritis, systemic lupus, pernicious anemia, vitiligo, and fibromyalgia. In addition, because of the strong association between thyroiditis and type 1 diabetes, the patient should be evaluated closely for signs of this disease.

For patients with Hashimoto's disease, clinicians should carefully examine the thyroid during follow-up visits, as lymphoma of the thyroid is a serious, yet rare, complication. The FDA recommends that patients taking propylthiouracil for Graves' disease be closely monitored for signs and symptoms of liver injury, especially within the first 6 months after the start of treatment. Individuals with subclinical hypothyroidism should be followed up annually to determine if there are clinical or biochemical signs of loss of thyroid function, indicating progression to overt hypothyroidism.

The prognosis for individuals with autoimmune thyroid disease is good, and associated mortality for either autoimmune thyroid disease is low. Remission and mortality vary according to treatment, as discussed.

POINTS OF EMPHASIS IN PATIENT EDUCATION FOR AUTOIMMUNE THYROID DISEASE TREATMENTS

Treatment	**Education Points**
Hashimoto's Disease	
Levothyroxine	Take drug: At same time every day With full glass of water When stomach is empty Avoid the use of antacids.
Graves' Disease	
Radioactive iodine	Abstain from close personal contact for 1 week after treatment (2 weeks for children and pregnant women). Avoid pregnancy for 4 to 6 months after treatment.
Antithyroid drugs	Recognize signs and symptoms of agranulocytosis (fever, sore throat, mouth ulcers), and stop taking drug if they occur.

SYSTEMIC AUTOIMMUNE DISEASES

RHEUMATOID ARTHRITIS

Rheumatoid arthritis is a chronic disease characterized by inflammation of synovial tissue that can lead to long-term damage of the joint, resulting in chronic pain, loss of function, and disability. A cytokine network, which includes tumor-necrosis factor (TNF)-α, interleukin (IL)-1, and IL-6, has an integral role in the development of the inflammatory response. The disease is also associated with several extra-articular manifestations and comorbidities . The course and severity of the illness vary considerably, and the disease tends to progress over time, with the occurrence of intermittent disease flares. The burden of rheumatoid arthritis is significant. Arthritis (all types) was the second-leading chronic condition diagnosis for physician office visits in 2006, accounting for approximately 16% of all adult visits. According to a 2010 national survey, 19% of men and 24% of women reported disability related to "arthritis and rheumatism". The prevalence of arthritis-attributable work limitation has ranged from approximately 3% to 15% of working-age adults. In one survey, nearly 48% of respondents 65 years of age and older reported difficulty with functional activities, including limitations in activities of daily living and in working around the house or at a job. As such, rheumatoid arthritis ranks among the chronic diseases with the greatest effect on health-related quality of life and the most substantial socioeconomic impact. In addition, the mortality rate associated with rheumatoid arthritis is also high, representing the second highest death count among autoimmune diseases for women older than 65 years of age.

EPIDEMIOLOGY

Most of the statistics on rheumatoid arthritis have been gathered in surveys and studies that have reported on arthritis as a general category. Several of these studies point to a high-and increasing-prevalence of arthritis. Data from the 2003-2013 National Health Interview Survey (NHIS) showed a prevalence of diagnosed arthritis of nearly 22%. In a 2010 study, researchers estimated that nearly 1.5 million adults (18 years of age and older) have rheumatoid arthritis, which represents an increase from the nearly 1.3 million reported in 2008.

The prevalence of arthritis (all types) increases with age. According to data from the 2003-2013 NHIS, the prevalence is approximately 8% for individuals 18 to 44 years of age, compared with 29% for individuals 45 to 64 years of age and 50% for individuals 65 years of age and older. The overall prevalence of arthritis has also consistently been higher among women than men, and approximately 75% of all individuals with rheumatoid arthritis are female.

Data are limited on racial/ethnic differences in the prevalence of rheumatoid arthritis. The prevalence of arthritis (all types) has been found to be higher in the non-Hispanic white population (24%) compared with the non-Hispanic black (19%), Hispanic (11%), and Asian/Pacific Islander (8%) populations. In contrast, the prevalence has been higher in the American Indian/Alaska Native population (25%) and individuals of multiple races (21%) than in the white population [149]. The differences between the white population and the Hispanic, Asian/Pacific Islander, and American Indian/Alaska Native populations are all significant.

POTENTIAL ENVIRONMENTAL RISK FACTORS

Environmental factors that have been linked to rheumatoid arthritis include infection, smoking, and stress. Among the infectious micro-organisms thought to be associated with rheumatoid arthritis are Epstein-Barr virus, *Mycobacterium tuberculosis*, *Escherichia coli*,*Proteus mirabilis*, retroviruses, parvovirus B19, and hepatitis C virus. Approximately 8% to 15% of individuals have reported the onset of rheumatoid arthritis-related symptoms within a few days after an infectious illness.

Smoking has also been identified as a significant risk factor for the development of rheumatoid arthritis, and greater smoking intensity (number of cigarettes per day) and longer smoking history further increase the risk. The risk remains increased for at least 20 years after smoking cessation. Psychologic stress has been thought to play a role in the pathogenesis of rheumatoid arthritis by triggering the inflammatory process and exacerbating disease activity. In addition, because evidence of rheumatoid arthritis-associated antibodies has often been found many years before the onset of clinical symptoms, early environmental factors have been thought to be a contributor to the disease. High birth weight and early breastfeeding cessation are two such early factors.

ASSOCIATION WITH OTHER AUTOIMMUNE DISEASES

As noted, autoimmune diseases of connective tissue are more likely to be associated with other autoimmune diseases. Studies have shown that the coexistence of rheumatoid arthritis, thyroiditis, and type 1 diabetes is high. In addition, features of systemic lupus are common in individuals with rheumatoid arthritis; in one study, four or more lupus features were found in approximately 16% of individuals with rheumatoid arthritis within 25 years after diagnosis. This finding is significant because the co-occurrence of systemic lupus features and rheumatoid arthritis was associated with increased overall mortality.

As many as 25% of individuals with rheumatoid arthritis also have Sj?gren's syndrome, and the risk of rheumatoid arthritis appears to be higher in individuals who have inflammatory bowel disease. An inverse relationship between rheumatoid arthritis and multiple sclerosis has been reported. Fibromyalgia is also commonly found in association with rheumatoid arthritis, with reported rates ranging from 17% to 57%.

CLINICAL MANIFESTATIONS

Pain and stiffness in multiple joints are the primary characteristics of rheumatoid arthritis; approximately one-third of individuals with the disease initially have pain in only one joint. Other common symptoms of rheumatoid arthritis include fatigue, weakness, generalized muscular aches, and anorexia. Approximately 46% of individuals with rheumatoid arthritis have extra-articular manifestations, the most common of which is rheumatoid nodules, followed by pulmonary fibrosis, dry eye syndrome, and anemia of chronic disease. Rheumatoid nodules are soft, poorly delineated subcutaneous nodules, and they also occasionally affect internal organs such as the pleura, sclera, vocal cords, and vertebral bodies. Other frequently occurring extra-articular manifestations include pericarditis, pleuritis, vasculitis, cervical myelopathy, and neuropathy. No reliable predictors of extra-articular manifestations have been identified, but they have been reported to be associated with male gender, smoking, more severe joint disease, worse function, high levels of inflammatory markers, and a positive rheumatoid factor and antinuclear antibody (ANA) titer.

DIAGNOSTIC EVALUATION

When evaluating a patient for suspected rheumatoid arthritis, healthcare professionals should focus both the history and the physical examination on the joints. Questions about symptoms related to the joint should help determine which joints are involved, when joint pain occurs (e.g., during activity, at rest), how long pain and stiffness last, and how pain limits function. The most commonly involved joints are the wrist joints and the proximal interphalangeal and metacarpophalangeal joints; the distal interphalangeal joints and sacroiliac joints are typically not affected. Affected joints may become warm and tender after long periods of inactivity, and joint symptoms are usually bilateral. Small joints of the hands and feet are not usually painful at rest. Morning joint stiffness associated with rheumatoid arthritis usually lasts more than 1 hour, in contrast to osteoarthritis, in which morning stiffness usually resolves within 30 minutes after waking. For most individuals, symptoms develop over a long period of time (weeks to months); symptoms develop over days to weeks in approximately 15% of patients.

The findings on physical examination are usually normal, except for an occasional low-grade fever. The involved joint(s) may feel warm and boggy and may be tender to the touch, but there is usually no accompanying erythema. Affected joints have limitations in the range of motion, and the strength of muscles near affected joints is usually decreased. The patient may keep an affected joint in flexion to avoid pain related to extension. Lymph nodes in the epitrochlear, axillary, and cervical regions may be enlarged. Rheumatoid nodules are often found in pressure areas (e.g., the elbows and finger joints) and the extensor surface of the forearm.

Diagnostic Criteria

In 1988, the American Rheumatism Association (now known as the American College of Rheumatology [ACR]) published its Criteria for the Classification of Rheumatoid Arthritis, and these criteria remained the standard for several years. However, the criteria were criticized for a lack of sensitivity to early disease. In 2010, the ACR and the European League Against Rheumatism (EULAR) collaborated on a new classification system that focuses on features of earlier stages of rheumatoid arthritis that are associated with persistent and/or erosive disease, rather than defining the disease by its late-stage features. The impetus for this change in focus was the need for earlier diagnosis in order to begin disease-modifying drugs as soon as possible.

The new classification criteria apply only to newly presenting individuals, and two requirements must first be met: there must be evidence of currently active clinical synovitis (i.e., swelling) in at least one joint as determined by an expert assessor, and the synovitis must not be better explained by another diagnosis. The ACR/EULAR note that all joints may be assessed, except for the distal interphalangeal joints, the first metatarsophalangeal joint, and the first carpometacarpal joint, as these are most often involved in osteoarthritis. Individuals who are eligible according to the first two criteria are then evaluated by four additional criteria related to joint involvement, serologic testing, acute-phase reactants, and duration of symptoms (***Table***) . The classification system includes a scoring system, with a possible total of 10 points; a score of 6 or more indicates "definite" rheumatoid arthritis. Although a person with a score of less

than 6 does not have definite rheumatoid arthritis, the score may increase on subsequent testing.

2010 ACR/EULAR CLASSIFICATION CRITERIA FOR RHEUMATOID ARTHRITIS*

Category	Criteria	Score
Joint involvement	1 large joint (shoulder, elbow, hip, knee, ankle)	0
	2 to 10 large joints	1
	1 to 3 small joints (MCP, PIP, second to fifth MTP, thumb IP joints), with or without involvement of large joints 2	2
	4 to 10 small joints, with or without involvement of large joints	3
	>10 joints (at least 1 small joint and any combination of any other joints)	5
Serology (at least 1 test result is needed for classification)	Negative RF and ACPA	0
	Low-positive RF or low-positive ACPA	2
	High-positive RF or high-positive ACPA	3
Acute-phase reactants (at least 1 test result is needed for classification)	Normal CRP and normal ESR	0
	Abnormal CRP or abnormal ESR	1
Duration of symptoms	<6 weeks	0
	≥6 weeks	1

*See text for initial criteria and descriptions of criteria. MCP = metacarpophalangeal; PIP = proximal interphalangeal; MTP = metatarsophalangeal; IP = interphalangeal; RF = rheumatoid factor; ACPA = anti-citrullinated protein antibody; CRP = C-reactive protein; ESR = erythrocyte sedimentation rate.

2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Ann Rheum Dis. 2010;69:1580-1588.

The ACR/EULAR recommended serologic testing involves a rheumatoid factor and an anti-citrullinated protein antibody (ACPA). A positive rheumatoid factor has long been known as an indicator of rheumatoid arthritis, and studies have shown that this test is positive in approximately 69% to 90% of people with the disease. However, the test may be positive in healthy individuals as well as in individuals with other rheumatic diseases (e.g., Sjogren's syndrome, systemic sclerosis, systemic lupus), with chronic infections, or with pulmonary disease. The false-positive rate of rheumatoid factor for rheumatoid arthritis has been reported to be 15%. As a result, the sensitivity and specificity of the test are 69% and 85%, respectively. Testing for ACPA began in the late 1990s, and although the sensitivity of the test (67%) is similar to that of the rheumatoid factor, its false-positive rate is lower, yielding a specificity of 95%. According to the ACR/EULAR classification scoring system, the highest score is given if the results of either the rheumatoid factor or the ACPA test is highly positive, and no points are given if both tests are negative. An ANA titer has a reported sensitivity of

about 40% among individuals with rheumatoid arthritis, and false-positive results are common. The ANA titer is not part of either the 1988 or 2010 diagnostic criteria.
Other recommended baseline laboratory testing includes a complete blood cell count (CBC) with differential, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP). However, the ESR and CRP results should be interpreted with caution, as the tests are normal in about 40% of people with rheumatoid arthritis. Baseline renal and hepatic functioning should also be determined, not because these tests are sensitive or specific for rheumatoid arthritis but because they are important in guiding the choice of medications .
Radiographic evaluation has been recommended as part of the diagnostic work-up for rheumatoid arthritis, but the findings on conventional radiographs of involved joints are often normal, especially in early-stage disease. The findings on imaging studies are not part of the 2010 classification criteria for rheumatoid arthritis. However, imaging studies may be helpful in the differential diagnosis and in establishing baseline images for comparison during follow-up. An analysis of 11 studies of magnetic resonance imaging (MRI) as a diagnostic tool showed a wide range in sensitivity and specificity, with the authors concluding that the data are inadequate to justify widespread use of MRI in the diagnosis of rheumatoid arthritis.

Differential Diagnosis

A wide range of medical conditions should be considered in the differential diagnosis of rheumatoid arthritis, including:

- Connective tissue diseases (e.g., systemic lupus, systemic sclerosis)
- Psoriatic arthritis, gout, and other forms of arthritis
- Fibromyalgia
- Polymyalgia rheumatica
- Thyroid disease
- Sarcoidosis
- Hemochromatosis
- Still's disease
- Viral arthritis
- Paraneoplastic syndrome (when onset is after 55 years of age)

Overlapping signs and symptoms can make it challenging to distinguish rheumatoid arthritis from many of these conditions, especially connective tissue diseases and other forms of arthritis. A positive ANA titer may help distinguish systemic lupus from rheumatoid arthritis, and determination of a TSH level can aid in a diagnosis of hypothyroidism. Early in the course of rheumatoid arthritis, self-limited viral syndromes should be considered, especially hepatitis B and C, parvovirus, rubella (infection or vaccination), and Epstein-Barr virus.

TREATMENT OPTIONS

The primary goal of treatment for rheumatoid arthritis was once to alleviate symptoms, but the advent of disease-modifying drugs as a standard of care has shifted the focus to remission or the prevention of further joint damage. The ACR published guidelines for

the treatment of rheumatoid arthritis in 2012 and was in the process of updating them in 2011. In 2008, the ACR published guidelines on the use of disease-modifying antirheumatic drugs. EULAR has developed guidelines for the management of early rheumatoid arthritis and for the use of disease-modifying drugs.

Disease-Modifying Antirheumatic Drugs

Early treatment is essential to achieving optimal outcomes with disease-modifying drugs, and the ACR recommends that patients with suspected rheumatoid arthritis be referred within 3 months after the initial diagnosis so appropriate treatment can begin as soon as possible. Similarly, EULAR recommends that treatment with disease-modifying drugs begin at the time of diagnosis.

Disease-modifying antirheumatic drugs, or DMARDs, are antimetabolite/cytotoxic agents, and several nonbiologic and biologic disease-modifying drugs are now available, allowing clinicians and patients to select a specific drug after considering several factors. In its recommendations for the use of disease-modifying drugs, the ACR discussed the use of 10 drugs (five nonbiologic and five biologic agents) and noted that other drugs were not included because they were used infrequently, were associated with a high incidence of adverse events, or were not recommended for other reasons. For example, anakinra, an interleukin-1 antagonist, has been found to be less effective than the other biologic agents and so was omitted from the review of the literature informing the guidelines. Since the publication of the guidelines, two additional biologic agents and one nonbiologic agent have received FDA approval for the treatment of rheumatoid arthritis.

DISEASE-MODIFYING ANTIRHEUMATIC DRUGS APPROVED BY THE U.S. FOOD AND DRUG ADMINISTRATION FOR THE TREATMENT OF RHEUMATOID ARTHRITIS

Agent	Indication*	Dose and Administration	Time to Efficacy	Most Common Adverse Effects
Nonbiologic Agents				
Methotrexate	Any disease duration, any degree of disease activity, with or without poor prognosis features	12-25 mg PO, IM, or SC weekly	1 to 2 months	Nausea, diarrhea, fatigue, mouth ulcers, rash, alopecia
Leflunomide	Any disease duration, any degree of disease activity, with or without poor prognosis features	100 mg PO daily for 3 days, then 10-20 mg PO daily	1 to 3 months	Nausea, diarrhea, rash, alopecia; highly teratogenic, even after use is discontinued
Hydroxychloroquine	Short or intermediate disease duration, low disease activity, no poor prognosis features	200-400 mg PO daily	2 to 6 months	Nausea, headache, possible retinopathy
Minocycline	Short disease duration, low disease activity, no poor prognosis features	100 mg PO daily	1 to 3 months	Dizziness, skin pigmentation

Sulfasalazine	Any disease duration, any degree of disease activity, no poor prognosis features	2-3 g PO daily (in divided doses)	1 to 3 months	Nausea, diarrhea, headache, mouth ulcers, rash, alopecia, oligospermia (reversible)
Tofacitinib**	Moderately to severely active disease despite treatment with methotrexate or intolerance of methotrexate	5 mg daily	1 to 2 months	Infections, headache, diarrhea
Biologic Agents				
Anti-tumor necrosis factor-α agents (adalimumab, etanercept, infliximab)	In combination with methotrexate: Disease duration of less than 3 months, high disease activity, features of poor prognosis, and no previous treatment with disease-modifying drugs Alone: Inadequate response to methotrexate monotherapy AND disease duration >3 months, moderate disease activity, and poor prognosis features OR disease duration >3 months, high disease activity, with or without poor prognosis features	Adalimumab: 40 mg SC every 2 weeks	Few days to 3 months	Infusion reactions, increased risk of infection (especially fungal)
		Etanercept: 25 mg SC twice weekly or 50 mg SC weekly	Few days to 3 weeks	
		Infliximab: 3 mg/kg IV at weeks 0, 2, and 6, then every 8 weeks	Few days to 3 months	
Golimumab (anti-tumor necrosis factor-α)**	In combination with methotrexate: moderate-to-severe disease	50 mg SC monthly	1 to 5 months	Serious infections, upper respiratory infection, nasopharyngitis
Abatacept	Inadequate response to methotrexate-based combination or sequential administration of other nonbiologic agents, moderate-to-high disease activity, and features of poor prognosis	500-1,000 mg (depending on body weight) IV at weeks 0, 2, and 4, then every 4 weeks	2 weeks to 3 months	Headache, nasopharyngitis, dizziness, urinary tract infection, bronchitis

Rituximab	In combination with methotrexate: Inadequate response to methotrexate-based combination or sequential administration of other nonbiologic agents, high disease activity, and features of poor prognosis	1,000 mg IV at week. 0 and 2, then every 24 weeks	By 2 months	Upper respiratory infection, bronchitis, nasopharyngitis, urinary tract infection
Tocilizumab**	Alone or in combination with methotrexate: Moderate-to-severe disease refractory to 1 or more anti-tumor necrosis factor-α agents	4-8 mg/kg IV monthly	2 weeks to 5 months	Serious infection, upper respiratory infection, nasopharyngitis, headache, hypertension
*Disease duration defined as short (less than 6 months), intermediate (6 to 24 months), or long (more than 24 months). Degree of disease activity is defined according to scores on one of several validated disease activity instruments; presence of poor prognosis features is defined as functional limitation, extra-articular disease, positive rheumatoid factor and/or positive anti-citrullinated protein antibody test, and/or osseous erosions on radiograph. **Not included in American College of Rheumatology guidelines, as the guidelines predated FDA approval.				

Among the recommended nonbiologic agents are methotrexate, generally considered to be the standard first-line treatment; the tetracycline minocycline; the antimalarial drug hydroxychloroquine; the Janus kinase inhibitor tofacitinib; and drugs developed specifically for rheumatoid arthritis, sulfasalazine and leflunomide. The biologic agents include three anti-TNF-α agents (adalimumab, etanercept, and infliximab); abatacept, a selective costimulation modulator; and rituximab, an anti-CD20 monoclonal antibody that depletes B lymphocytes. A nonbiologic agent-usually methotrexate-is typically recommended as first-line treatment, and it can be given alone or in combination with one or two other nonbiologic agents. The ACR does not recommend the use of biologic agents for individuals with early rheumatoid arthritis (i.e., 6 months or less) and a low-to-moderate degree of disease activity and does not recommend the use of biologic agents in combination

In people with newly diagnosed active rheumatoid arthritis, the National Collaborating Centre for Chronic Conditions recommends offering a combination of DMARDs (including methotrexate and at least one other DMARD, plus short-term glucocorticoids) as first-line treatment as soon as possible, ideally within 3 months of the onset of persistent symptoms.

Anti-Inflammatory Medications

Anti-inflammatory medications are used to reduce joint pain and swelling associated with rheumatoid arthritis. Because these drugs do not change the course of disease, they must be used in conjunction with a disease-modifying drug. Treatment typically begins with a nonselective nonsteroidal anti-inflammatory drug (NSAID); a cyclooxygenase-2 (COX-2)-selective inhibitor and/or glucocorticoids may also be

used. A gastroprotective agent (proton-pump inhibitor) should be prescribed with an NSAID for individuals at high risk for gastrointestinal complications [176].

There is good evidence that nonselective NSAIDs and COX-2 inhibitors have comparable efficacy and that COX-2 inhibitors are comparable to each other. Although COX-2 inhibitors have better tolerability in general compared with NSAIDs, there is considerable variability across individual drugs in terms of protection against serious gastrointestinal events. A large, double-blind, randomized trial involving nearly 4,500 individuals with rheumatoid arthritis or osteoarthritis demonstrated that the COX-2 inhibitor celecoxib was associated with lower risks of adverse gastrointestinal events than a nonselective NSAID plus a proton-pump inhibitor (diclofenac plus omeprazole)..

The adverse event profiles of both nonselective NSAIDs and COX-2 inhibitors should be considered when selecting a specific drug for an individual patient. All individuals treated with NSAIDs should be monitored for long-term complications such as gastrointestinal bleeding, cardiovascular events (e.g., myocardial infarction, stroke), and gastric ulcers and bleeding. The increased risk of cardiovascular events associated with some COX-2 inhibitors has been well publicized, and special care should be taken when prescribing these drugs.

In addition to their anti-inflammatory properties, glucocorticoids may substantially reduce the rate of further joint erosion and should be considered as a temporary adjunct to treatment with disease-modifying drugs. However, because of the substantial risk of adverse effects, glucocorticoids should be given for the shortest time and at the smallest dose possible, and treatment should be discontinued gradually-over at least 1 month-to avoid rebound effects. Administration of a glucocorticoid as an intra-articular injection may reduce swelling and inflammation in a single joint, but the clinical benefit is short term.

Complementary/Alternative Medicine

Many individuals with rheumatoid arthritis turn to complementary and alternative medicine to alleviate symptoms. The use of complementary and alternative medicine among individuals with rheumatoid arthritis has ranged from 28% to 90%, and the rates of use among all individuals varies across racial/ethnic populations. Most herbal supplements used by individuals with rheumatoid arthritis are safe, but the evidence of their benefit has been weak to moderate. Despite the wide use of complementary and alternative medicine, most individuals (63% to 72%) do not report the use to their healthcare providers. Because of this, clinicians should ask direct questions about the use of complementary and alternative medicine approaches and initiate discussions about their use.

Nonpharmacologic Therapy

Physical therapy and/or occupational therapy can help individuals improve their ability to carry out activities of daily living at home, at work, and socially. In addition, physical therapists can provide instruction in a program of range-of-motion and strengthening exercises, in joint protection, and in ways to conserve energy. Evidence of benefit from nonpharmacologic approaches is lacking, however. An overview of systematic reviews found that there was unclear benefit (low quality of evidence) for most nonpharmacologic therapies, including balneotherapy, electrical stimulation,

transcutaneous electrical nerve stimulation, assistive devices, and splints. The exceptions were comprehensive occupational therapy and joint protection, which were shown to improve function (with no difference in pain) according to high-quality evidence, and low-level laser therapy, which was shown to reduce pain and improve function according to evidence of moderate quality.

Regular participation in activities such as walking or aerobic exercises is recommended, as they can help improve joint mobility, muscle strength, and aerobic fitness; decrease fatigue; and maintain psychologic well-being. Also, because emotional stress can exacerbate disease activity, stress management interventions should be encouraged. Several randomized controlled trials have indicated that significant improvements in pain management and function have resulted from cognitive-behavioral therapy that has focused on therapist-guided training in coping strategies (e.g., relaxation, goal setting, imagery, and cognitive restructuring of negative thoughts related to pain). **Surgical Procedures**

Surgical procedures to treat rheumatoid arthritis are reserved for individuals who have structural joint damage that causes high pain levels, loss of range of motion, or severely limited function (severe disability and/or inability to work) despite pharmacologic and nonpharmacologic therapy. The goals of surgical interventions are to restore function and quality of life, prevent further deterioration of the joint, relieve pain, and correct deformity. The challenge with surgical treatment is that many joints are often involved; priority should be given to the joint that causes the greatest disability and pain. Among the options for surgical treatment are synovectomy, carpal tunnel release, resection of the metatarsal heads, specialized hand surgery, arthrodesis, and joint replacement. The preoperative functional status is an important factor in the postoperative outcome, making early referral for surgery important.

Treatment of Extra-Articular Manifestations

The overall treatment of individuals with rheumatoid arthritis also includes treatment targeted at extra-articular manifestations. Because extra-articular manifestations are associated with poor prognosis, they should be identified and managed promptly. Treatment is primarily limited to glucocorticoids.

FOLLOW-UP AND PROGNOSIS

Close follow-up is needed for individuals with rheumatoid arthritis to evaluate response to treatment, ensure control of symptoms, and monitor for treatment side effects and disease-related comorbidities.

Response to Treatment

Both the ACR and the EULAR recommend that evidence of disease activity be evaluated, through subjective and objective measures, at each follow-up visit. The follow-up assessment may include:

Self-reports of degree of joint pain, duration of morning stiffness, limitation of function, and duration of fatigue

Tender and swollen joints on physical examination

Evidence of disease progression on physical examination (e.g., loss of motion, instability, malalignment, and/or deformity)

Elevated ESR or CRP level

Progression of radiographic damage of involved joints (with use of radiographic assessment scales)
Global assessment of disease activity (by the physician and the patient)
Standardized questionnaires to assess functional status and/or quality of life
The recommended follow-up interval is every 1 to 3 months until remission is achieved, and adjustments to the doses and/or choices of monotherapy or combination therapy with disease-modifying drugs should be made if the response is inadequate. Treatment with disease-modifying drugs can lead to some level of remission in approximately 30% to 40% of individuals, but complete remission is rare, and most individuals will have persistent disease.

Monitoring and Treatment of Drug Side Effects

A systematic approach to long-term drug monitoring is necessary because of the potential for serious adverse events associated with the long-term use of disease-modifying antirheumatic drugs and glucocorticoids.

Among the side effects of long-term use of disease-modifying drugs are infection; bone marrow suppression; gastrointestinal, hepatic, renal/genitourinary, cardiovascular, and neurologic effects; pulmonary toxicity; and skin reaction/rash . Infusion site reactions are also commonly associated with anti-TNF-α agents. The ACR recommends that individuals treated with leflunomide, methotrexate, or sulfasalazine have a CBC, liver function studies, and a serum creatinine every 2 to 4 weeks for the first 3 months after the beginning of treatment; every 8 to 12 weeks during the 3- to 6-month period, and every 12 weeks subsequently. Individuals taking rituximab should have a CBC and platelet count done every 2 to 4 months, and individuals treated with tocilizumab should have a CBC, platelet count, and liver function studies every 4 to 8 weeks.

Individuals receiving hydroxychloroquine are at risk for severe retinopathy, and ophthalmologic follow-up is important for early detection and minimization of toxicity. The reported incidence of retinopathy associated with hydroxychloroquine is low, especially within the first 5 years of use at a low dose (less than 6.5 mg/kg/day), but the potential severity calls for ophthalmologic follow-up.

All drugs used to treat rheumatoid arthritis are associated with a high risk of conventional and opportunistic infections, and measures to prevent infection should be taken. The ACR recommends that in addition to receiving an influenza vaccination before beginning treatment with disease-modifying antirheumatic drugs, individuals should receive the vaccination annually thereafter. In addition, a pneumococcal vaccination should be done every 5 years. Live vaccines should be avoided in individuals receiving a biologic disease-modifying drug. Targeted prophylaxis for individuals at high risk for infection may also be appropriate.

The use of glucocorticoids, especially over the long term, is associated with a wide range of potential adverse events, including osteopenia/osteoporosis, hypertension, cataracts, glaucoma, dyspepsia, weight gain, avascular necrosis of bone, Cushingoid changes, and adverse psychologic effects. The ACR published guidelines in 2010 for the prevention and treatment of glucocorticoid-induced osteoporosis. The ACR recommends daily calcium intake (dietary plus supplement) of 1,200 to 1,500 mg and supplemental vitamin D (400 to 800 IU) to prevent osteoporosis in all individuals taking glucocorticoids. The ACR guidelines also include recommendations for the use

of bisphosphonates according to an individual's risk, noting that risk is best assessed with the Fracture Risk Assessment (FRAX) tool, which provides a better overall clinical risk profile than bone mineral density alone. In addition, baseline dual x-ray absorptiometry, height, prevalent fragility fractures, and serum 25-hydroxyvitamin D level should be obtained before the start of treatment with glucocorticoids and should be monitored throughout the course of treatment. Better adherence to the ACR guidelines are needed, as one study showed that a baseline bone scan was done in only 39% of patients and appropriate treatment was also prescribed for only 39%.

COMORBIDITIES ASSOCIATED WITH RHEUMATOID ARTHRITIS

Comorbidity	**Prevalence**	
	Lifetime	**Current**
Any gastrointestinal problem	50%	15%
Hypertension	47%	32%
Any psychiatric problem	36%	16%
Depression	34%	15%
Any endocrine problem	30%	20%
Any genitourinary problem	30%	4%
Cataract	27%	10%
Any lung problem	25%	12%
Any cardiovascular problem	22%	9%

Prognosis

Of all the autoimmune diseases, rheumatoid arthritis is a leading cause of mortality, especially among women older than 65 years of age. Studies have consistently shown higher rates of mortality for individuals with rheumatoid arthritis than for the general population. Furthermore, the increasing survival rates documented for the population at large since the 1950s and 1960s have not been found for individuals with rheumatoid arthritis . The increased mortality has been linked to several factors, including extra-articular manifestations, markers of disease severity, and diminished function within the first year. By far, cardiovascular disease has been thought to confer the greatest risk for increased mortality.

A meta-analysis of observational studies demonstrated that mortality related to cardiovascular disease is increased by about 50% in individuals who have rheumatoid arthritis (compared with individuals who do not have the disease). The increased risk cannot be explained by an increased incidence of traditional cardiovascular disease risk factors. The underlying inflammatory mechanism is thought to have a role, and the increased use of disease-modifying drugs is expected to help improve survival in addition to function. To date, only methotrexate has been shown to be associated with a reduced risk of cardiovascular disease among individuals with rheumatoid arthritis. The increased risk of cardiovascular disease highlights the need for clinicians to assess traditional and nontraditional cardiovascular risk factors, such as hypertension, obesity, smoking, hyperlipidemia, inflammation, insulin resistance, and family history of

cardiovascular disease, and provide counseling, preventive measures, and treatment as appropriate.[.

PATIENT EDUCATION

Education and self-management are valuable components of an overall treatment plan for a chronic illness such as rheumatoid arthritis. Studies have demonstrated that patient education improves function, patients' global assessment, adherence to the treatment plan, and psychologic status.

Clinicians should emphasize the importance of noting new symptoms that may be related to adverse effects of treatment drugs and the need for strategies to minimize these effects[. For example, clinicians should counsel patients treated with glucocorticoids and/or immunosuppressant agents about ways to prevent osteoporosis and reduce the risk of infection and should emphasize to all patients the importance of modifying lifestyle factors that increase the risk for cardiovascular disease.

MOST COMMON SIGNS AND SYMPTOMS OF SYSTEMIC LUPUS ERYTHEMATOSUS

Organ/Body System	Symptoms
General	Fatigue Low-grade, unexplained, episodic fever Weight loss Generalized adenopathy
Cutaneous	Butterfly-shaped rash on face Photosensitivity Alopecia Oral mucosal sores, ulcers Raynaud phenomenon
Musculoskeletal	Arthralgia, arthritis Myalgia, muscle tenderness
Cardiovascular	Pericarditis Pericardial effusion Myocarditis
Respiratory	Pleuritic pain Pleurisy (with coughing and dyspnea)
Renal	Glomerulitis, glomerulonephritis
Neurologic	Cognitive dysfunction Headache Seizures Cranial or peripheral neuropathy
Gastrointestinal	Abdominal pain Nausea/vomiting
Ocular	Dry eye syndrome, uveitis, scleritis

The clinical manifestations of systemic lupus often differ among older individuals. Malar and discoid rash and glomerulonephritis are less common in the older population compared with the younger population, whereas arthritis, fever, serositis, dry eye

syndrome, Raynaud phenomenon, lung disease, and neuropsychiatric symptoms are more common in the older population.

DIAGNOSTIC EVALUATION

The diagnosis of systemic lupus is challenged by the waxing and waning of symptoms over time and variations in the degree of disease severity and in the organ systems involved. Because of the lower prevalence and differences in clinical manifestations among older individuals, diagnosis is especially challenging for that population.

The malar rash associated with systemic lupus can be easily misdiagnosed as rosacea or seborrheic dermatitis, but it is usually asymptomatic, lacking symptoms such as burning, itching, and tingling, that accompany other facial rashes. The differential diagnosis of systemic lupus includes several other autoimmune disorders, such as early rheumatoid arthritis, undifferentiated connective tissue disease, fibromyalgia, vasculitis, and idiopathic thrombocytopenia purpura.

Diagnostic Criteria

Criteria for the classification and diagnosis of systemic lupus have been established. The criteria include 11 clinical and immunologic manifestations, and at least four of the 11 criteria are needed for a definitive diagnosis of systemic lupus . Because the criteria are based on the presence of signs and symptoms at any time during the course of the illness, an individual with early or atypical disease may not meet the criteria for definitive diagnosis. It is not uncommon for people with systemic lupus to meet only two of the clinical criteria, with the diagnosis subsequently confirmed by laboratory testing.

CLASSIFICATION CRITERIA OF THE AMERICAN COLLEGE OF RHEUMATOLOGY FOR THE DIAGNOSIS OF SYSTEMIC LUPUS ERYTHEMATOSUS

Major Criteria	Definition of Criteria
Malar rash	Fixed erythema (flat or raised) over malar eminences, usually sparing the nasolabial folds
Discoid rash	Erythematous raised patches with adherent keratotic scaling and follicular plugging; atrophic scarring may occur in older lesions
Photosensitivity	Skin rash resulting from exposure to sun (by patient history or physician observation)
Oral ulcers	Ulceration in mouth or nasopharyngeal passage, usually painless (observed by physician)
Nonerosive arthritis	Tenderness, swelling, or effusion in 2 or more peripheral joints
Pleuritis or pericarditis	Evidence of pleuritic pain documented by convincing history of pleuritic pain, by pleural friction rub heard by physician, or by demonstration of effusion OR Evidence of pericarditis documented by electrocardiogram, by pericardial friction rub heard by physician, or by demonstration of pericardial effusion

Renal disorder	Persistent proteinuria OR Cellular casts (red cell, hemoglobin, granular, tubular, or mixed)
Neurologic disorder*	Seizures OR Psychosis
Hematologic disorder	Hemolytic anemia with reticulocytosis OR Leukopenia (<4,000/mm3 on 2 or more occasions) OR Lymphopenia (<1,500 mm3 on 2 or more occasions) OR Thrombocytopenia (<100,000/mm3) (in the absence of offending drugs)
Immunologic disorder	Abnormal anti-DNA titer OR Abnormal anti-Sm titer OR Evidence of antiphospholipid antibodies
Positive antinuclear antibody (ANA) titer	By immunofluorescence or an equivalent assay at any point in time and in the absence of drugs
*In the absence of offending drugs or known metabolic derangements (such as uremia, ketoacidosis, or electrolyte imbalance).	

Physical examination can identify nearly half of the diagnostic criteria, including malar and discoid rash, oral ulcers, arthritis, and pleuritis or pericarditis. The absence of these signs may not necessarily exclude systemic lupus as a potential diagnosis, however, because of the waxing and waning of symptoms.

Seizures and psychosis are the only two neurologic criteria for the classification of systemic lupus, but many other neuropsychiatric disorders occur in conjunction with the disease. Neuropsychiatric disorders have been reported in up to 63% of patients with systemic lupus, and approximately 30% to 47% of these disorders are directly attributable to the disease. The disorders may be evident before, at the time of, or after the diagnosis of systemic lupus. In a population-based study of 68 patients with systemic lupus, the most common neuropsychiatric disorders were headache (87%), cognitive dysfunction (46%), and mood disorders (26%).

Laboratory testing can help to identify the remaining clinical criteria: renal, hematologic, and neurologic disorders. The work-up should include a CBC with differential, platelet count, chemistry profile (especially kidney and liver function studies), and urinalysis. Evidence of renal involvement may include proteinuria or red blood cell casts and leukocytes in the urine . Hematologic testing may indicate anemia (in about 40%), thrombocytopenia (in about 25% to 35%), and leukopenia (about 15% to 20%). Metabolic abnormalities (e.g., uremia, electrolyte imbalance, or ketoacidosis) may be signs of neurologic disorders; seizures or psychosis are other signs.

Other recommended laboratory testing includes an ANA titer, as well as anti-double-stranded DNA, antibody to Sm nuclear antigen (anti-Sm), antiphospholipid antibodies, anti-Ro/SSA, and anti-La/SSB antibodies. The ANA titer is highly sensitive for systemic lupus, with a positive result in approximately 93% to 100% of individuals with the disease. However, the specificity is low, and a positive titer will also be found in 60% to 80% of people with systemic sclerosis and 40% to 70% of people with Sjogren's syndrome, as well as in a substantial number of healthy individuals. Given the low specificity, in combination with the low prevalence of systemic lupus in the primary care setting, the College of American Pathologists recommends an ANA titer when there is a "reasonable clinical suspicion" of systemic lupus on the basis of the history, physical examination, and other laboratory tests. A negative ANA titer (less than 1:160 on standard substrate) essentially rules out a diagnosis of systemic lupus . The ANA titer is best determined with fluorescent testing because it has better sensitivity and specificity than testing with enzyme-linked immunosorbent assay and can also demonstrate an ANA pattern.

ANTIBODY TESTING FOR SYSTEMIC LUPUS

Diagnostic Test	Prevalence*	Comments
Antinuclear antibody titer	93% to 100%	Positive titer also found in systemic sclerosis (up to 80%) and Sjogren's syndrome (up to 70%), as well as many healthy individuals
Anti-double-stranded DNA	70% to 80%	Positive test highly specific for systemic lupus Associated with greater risk of skin disease and lupus nephritis
Anti-Ro	30% to 40%	Also associated with Sjogren's syndrome (up to 70%) Associated with greater risk of skin disease, lupus nephritis, and fetal heart problems
Antiphospholipid antibodies	20% to 30%	Associated with greater risk of thrombosis and pregnancy loss
Anti-Sm	10% to 30%	Positive test highly specific for systemic lupus Associated with greater risk of lupus nephritis
Anti-La	15% to 20%	Associated with Sjogren's syndrome (up to 50%) Associated with fetal heart problems
*Among people with systemic lupus.		

Anti-double-stranded DNA and anti-Sm tests can help confirm a diagnosis of systemic lupus, as they have greater specificity than the ANA titer; however, they are not as sensitive as the ANA tier. The prevalence of positive anti-Ro/SSA and anti-LA/SSB titers is also low, and these titers are more often positive among older people. Serum complement levels may also be useful, as decreased levels indicate active or impending exacerbation of disease. The prevalence of positive anti-double-stranded DNA titers and of decreased complement levels is lower among older individuals than among younger ones.

When systemic lupus is suspected, the Finnish Medical Society Duodecim recommends the following basic laboratory investigations be completed:

- Blood count
- Platelets
- Erythrocyte sedimentation rate
- Antinuclear antibody titer

REFERRAL

The ACR recommends that primary care providers refer patients with suspected lupus to a rheumatologist to confirm the diagnosis and to evaluate the activity and severity of disease. The rheumatologist will establish a treatment plan, and when the disease is mild-to-moderate, the primary care provider can monitor the clinical course of the disease and drug-related toxicities. Because of the range in systems/organs that may be affected, a variety of other specialists may be needed during the course of disease. In addition, referral to physical and occupational therapies, social workers, and psychologists may also be appropriate.

TREATMENT OPTIONS

Data from large, randomized, controlled trials in the treatment of systemic lupus are lacking, creating a weak evidence base for recommendations. The ACR published guidelines for the management of systemic lupus in 1999, before the advent of many of the drugs currently used. EULAR published guidelines in 2013, acknowledging the lack of strong evidence. Very few drugs have FDA approval for use in systemic lupus, and researchers have been evaluating the efficacy and safety of drugs approved for other conditions, most notably rheumatoid arthritis; however, as of 2011, none have shown enough benefit for approval. Several drugs have been used in clinical practice, with their use depending on the severity of disease.

TREATMENT OPTIONS FOR SYSTEMIC LUPUS

Agent	Typical Dose*	Indication	Side Effects
Nonsteroidal anti-inflammatory drugs (NSAIDs)	At or near the upper limit of the dose range	Mild-to-moderate arthritis, fever, mild serositis	Gastrointestinal bleeding, renal and hepatic toxicity
Immunosuppressants/cytotoxic agents	Dose varies	Usually used in conjunction with a low-dose glucocorticoid	Infection, leukopenia, anemia, thrombocytopenia, myelosuppression, lymphoma, gastrointestinal effects, alopecia
Antimalarial Agents			
Hydroxychloroquine	200 mg PO twice daily	Preferred first-line treatment; effective for arthritis and rash and for preventing disease flares	Dizziness, nausea and diarrhea (usually resolves over time), macular damage
Glucocorticoids			

Prednisone (low dose)	≤10 mg PO daily	Usually used in conjunction with hydroxychloroquine	Osteopenia/osteoporosis, infection, hypertension, avascular necrosis of bone, weight gain, glaucoma, cataracts, psychologic effects
Prednisone (moderate dose)	≤20 mg PO daily	Moderate disease (without organ involvement) with inadequate response to first-line treatment	
Methylprednisolone (high dose)	40-60 mg PO daily or 1 g IV daily X3	Lupus nephritis, cerebritis, thrombocytopenia	
Topical	Low or intermediate dose	Facial lesions	Skin atrophy, infection, contact dermatitis
	Intermediate dose	Lesions on trunk or extremities	
	High dose	Lesions on palms or soles	
Azathioprine	25-150 mg PO daily	Nonarthritic disease refractory to antimalarial agent and/or glucocorticoids; maintenance therapy for lupus nephritis, neuropsychiatric lupus	Hepatitis, pancreatitis
Methotrexate	7.5-20 mg PO weekly	Mild-to-moderate disease refractory to first-line treatment; lupus nephritis, neurologic complications	Hepatic fibrosis, cirrhosis, pulmonary infiltrates, stomatitis, mucositis; teratogenic
Cyclophosphamide	IV, dose varies	Digital vasculitis; disease with organ involvement (lupus nephritis, cerebritis)	Irreversible ovarian or testicular failure (with long-term use); nausea, alopecia, herpes zoster; teratogenic
Mycophenolate mofetil	1.5-3 g PO daily	Mild-to-moderate lupus nephritis (induction and maintenance therapy); refractory thrombocytopenia; cutaneous manifestations; uncontrolled disease	Diarrhea, nausea; teratogenic

Leflunomide	10-20 mg PO daily	Mild-to-moderate disease refractory to first-line treatment	Diarrhea, alopecia, rash; teratogenic
Topical Calcineurin Inhibitors			
Tacrolimus or pimecrolimus	0.1%	Severe cutaneous lesions resistant to other agents	Peeling and burning sensation
Monoclonal Antibody			
Belimumab	10 mg/kg IV every 2 weeks for 6 weeks, then every 4 weeks	Adjunctive therapy for autoantibody-positive, mild-to-moderate systemic lupus	Nausea, fever, diarrhea, nasopharyngitis, insomnia; possibly teratogenic
*For most drugs, the typical dose may vary, as no recommended dose has been established because of the lack of FDA approval.			

The goal of treatment of systemic lupus is to adequately control disease and alleviate symptoms while minimizing the risk of treatment side effects. The approach to the treatment of older individuals with newly diagnosed systemic lupus is the same as that for younger individuals, but treatment is complicated in older people because of a greater likelihood of comorbidities and an increased risk of treatment-related toxicity.

Treatment During Pregnancy

Pregnancy in women with systemic lupus is associated with risks for both the mother and the fetus, and pregnant women should be managed as high-risk obstetric patients. Pregnancy may cause disease flares, especially in the third trimester and postnatal period, but flares are usually mild and can be controlled without excessive risk to either the mother or the fetus. Many treatment agents may be used during pregnancy, including hydroxychloroquine, prednisone, and azathioprine; evidence suggests that mycophenolate mofetil, cyclophosphamide, and methotrexate should be avoided. Systemic lupus increases the risk for fetal loss, especially in women who have antiphospholipid antibodies. A history of lupus nephritis, antiphospholipid antibodies, and anti-Ro and/or anti-La antibodies are associated with increased risk for pre-eclampsia, miscarriage, stillbirth, premature delivery, intrauterine growth restriction, and fetal congenital heart block. Heparin and aspirin are usually given throughout pregnancy to reduce the risk of miscarriage and thrombotic events.

Disease Activity/Response to Treatment

Disease activity should be assessed by a validated instrument, and the most widely used tools are the Systemic Lupus Activity Measure (SLAM), Systemic Lupus Erythematosus Disease Activity Index (SLEDAI), Lupus Activity Index (LAI), British Isles Lupus Assessment Group (BILAG) index, and the European Consensus Lupus Activity Measure (ECLAM). EULAR also recommends evaluation of quality of life through patient history and/or a patient global assessment at each visit and annual assessment of organ damage.

Laboratory testing every 6 to 12 months should include urinalysis, CBC, ESR, CRP, albumin, and creatinine levels. Anti-double-stranded DNA titer and serum complement levels should also be obtained, as an increase in the anti-double-stranded DNA titer and decreases in the serum complement levels often signal a disease flare. As defined by an international panel of experts, a flare is "a measurable increase in disease activity in one or more organ systems involving new or worse clinical signs and symptoms and/or laboratory measurements. It must be considered clinically significant by the assessor and usually there would be at least consideration of a change or an increase in treatment". Early treatment with a glucocorticoid may reduce the total dose needed to suppress the flare.

Because of the risk for lupus nephritis, patients should be followed up closely for signs of progression of disease to the kidneys. For patients who have persistently abnormal urinalysis results or elevated serum creatinine level, a urine protein/creatinine ratio (or 24-hour urine for protein), urine sediment, and ultrasound of the kidney should be done, and referral for a biopsy should be considered. When evidence of renal disease is found, CBC, serum creatinine level, urinalysis with microscopic evaluation, and quantitative testing of urinary protein should be done at 3-month intervals.

Approximately 50% to 60% of neuropsychiatric manifestations occur within the first year after diagnosis, and patients should be evaluated carefully for relevant signs and symptoms. A focused history can be used to elicit information about such symptoms as seizures, paresthesias, numbness, weakness, headache, epilepsy, and depression. Clinicians should also assess patients for cognitive impairment by asking questions about problems with multitasking, household tasks, or memory. If cognitive impairment is suspected, the patient should be evaluated further.

Monitoring and Treatment of Drug Side Effects

Infection, osteopenia/osteoporosis, and bone marrow suppression are the major side effects of treatment for systemic lupus; gastrointestinal, hepatic, renal/genitourinary, cardiovascular, and neurologic effects may also occur. Recommended testing for individuals receiving methotrexate, mycophenolate mofetil, or azathioprine is a CBC and platelet count every 3 months. Individuals treated with methotrexate should also have liver function studies done every 3 months. A serum glucose level should be obtained yearly for patients treated with glucocorticoids. Monitoring during treatment with cyclophosphamide should be done monthly, with a CBC, platelet count, and urinalysis. No laboratory testing is recommended to monitor treatment with hydroxychloroquine.

Prevention of Infection

Infection has been estimated to be responsible for 30% to 50% of morbidity and mortality among individuals with systemic lupus and is a leading cause of mortality. Approximately 80% of infections are caused by bacterial micro-organisms, with the skin, respiratory tract, and urinary tract accounting for more than two-thirds of affected sites.

Viral infections occur less commonly, and parvovirus B19 and cytomegalovirus are the most common viral micro-organisms. Symptoms related to viral infections often mimic disease flares. Women with systemic lupus are at increased risk for infection with the

human papillomavirus (HPV)-16 virus and thus are at risk for premalignant cervical lesions. Several factors have been proposed as risk factors for infection, including:

- Active disease
- Neutropenia/lymphopenia
- Low serum complement levels
- Involvement of major organ systems (e.g., kidney, lung, central nervous system)
- Treatment with immunosuppressive agents
- Treatment with antimalarial drugs has been shown to have a significant protective effect against infection, further confirming that treatment with antimalarial agents should be the standard of care unless contraindicated.

Prevention of Osteoporosis (in mostly aytoimmune diseases)

As noted, long-term use of glucocorticoids is associated with a wide range of potential adverse events, including osteopenia/osteoporosis, hypertension, cataracts, glaucoma, dyspepsia, weight gain, avascular necrosis of bone, Cushingoid changes, and adverse psychologic effects. Of these side effects, osteoporosis is of particular concern, with a prevalence of 4% to 24% among patients with systemic lupus. According to the 2010 ACR guidelines, the following are recommended for the prevention and treatment of glucocorticoid-induced osteoporosis:

Daily calcium intake (dietary plus supplement) of 1,200 to 1,500 mg and supplemental vitamin D (400 to 800 IU) to prevent osteoporosis in all individuals taking glucocorticoids

Use of bisphosphonates according to an individual's risk (noting that risk is best assessed with the FRAX tool, which provides a better overall clinical risk profile than bone mineral density alone)

Dual x-ray absorptiometry, height, prevalent fragility fractures, and serum 25-hydroxyvitamin D level at baseline (before treatment starts) and at intervals throughout the course of treatment

Prevention of Treatment-Related Eye Disease

As discussed, hydroxychloroquine increases the risk for retinopathy, although this toxicity is rare at doses of less than 6.5 mg/kg/day for fewer than 5 years. Still, ophthalmologic follow-up is important for early detection and minimization of this potentially serious side effect. Routine examination of the eyes should be done for patients treated with glucocorticoids who are at high risk for cataracts and glaucoma.

Prevention of Comorbidities

EULAR guidelines recommend a high index of suspicion and prompt evaluation for comorbidities commonly associated with systemic lupus, such as atherosclerosis, hypertension, dyslipidemia, and non-Hodgkin's lymphoma. Among patients with systemic lupus, the prevalence of hypertension or dyslipidemia has been reported to range from approximately 11% to 75% . Racial disparities exist, with cardiovascular events occurring at a younger age in black women and men. Although the increased risk of cardiovascular disease in the systemic lupus population cannot be fully explained by traditional cardiovascular risk factors, experts agree that such risk factors

should be evaluated at least annually and that modifiable risk factors should be treated according to established guidelines.

Hypertension and cardiovascular problems are among the most common comorbidities in individuals with systemic lupus. Hypertension is the leading current comorbidity, and any gastrointestinal problem is the leading lifetime comorbidity. Psychiatric problems and depression are the second and third leading current and lifetime comorbidities. Follow-up care should include patient assessment and preventive strategies for these comorbidities, as well as treatment as appropriate.

The risk of cancer is slightly increased for individuals with rheumatic diseases in general and for systemic lupus specifically. Although several types of cancer have been reported to occur more frequently, the risk is greater for hematologic cancers, especially non-Hodgkin's lymphoma. The underlying link between cancer and systemic lupus is unknown, but both the disease itself and medication exposure are thought to be factors. Cervical cancer has also been linked to systemic lupus, but a meta-analysis published in 2010 demonstrated that the risk for cervical cancer was not increased; rather, the risk for HPV infection and cervical dysplasia were increased. Clinicians should assess patients for signs and symptoms of cancer and should ensure that routine cancer screening is carried out. Shorter intervals for gynecologic evaluation are reasonable for women with systemic lupus.

Because of the high percentage of thyroiditis and the potential for polyautoimmunity among people with systemic lupus, clinicians should carefully consider the possibility of these diseases during follow-up, especially among those at highest risk. The highest risk for polyautoimmunity has been associated with female gender, articular involvement, familial autoimmunity, and positive anti-Ro titer.

Systemic lupus often has a substantial impact, with disease-related symptoms interfering with quality of life and ability to work. In a small study of women with systemic lupus between 21 and 75 years of age, the most common concerns were prognosis and course of disease; body image; effects of treatment; emotional difficulties; inability to plan due to disease unpredictability; fatigue; pain; career prospects and loss of income; memory loss/concentration problems; reliance on others to assist with everyday tasks; and pregnancy issues. Healthcare professionals should ask patients about their ability to cope with the disease and should suggest support groups or counseling as appropriate.

Prognosis

Systemic lupus is one of the leading causes of death among autoimmune disorders, and its associated mortality is higher than that expected for the general population. Mortality among women is consistent across all age-groups. Survival has improved substantially over the years, from a 4-year survival of 50% in the 1950s to a 5-year survival rate of 95% today. Ten-year and 15-year survival rates have been reported to be approximately 90% and 80%, respectively. Improved survival is thought to be the result of earlier diagnosis, recognition of mild disease, increased use of ANA testing, and better treatment options. Lower survival rates are associated with a younger age at the time of diagnosis, and mortality rates are twofold to threefold higher in the black population than in the white population.

Corticosteroids in rheumatology

Corticosteroids (such as prednisone; methylprenisolone, Medrol®) have both anti-inflammatory and immunoregulatory activity. They can be given orally, intravenously, intramuscularly or can be injected directly into the joint. Corticosteroids are useful in early disease as temporary adjunctive therapy while waiting for DMARDs to exert their antiinflammatory effects. Corticosteroids are also useful as chronic adjunctive therapy in patients with severe disease that is not well controlled on NSAIDs and DMARDs. The usual dose of predinisone is 5 to 10mg daily. Although prednisone can be started at higher doses (15 to 20mg daily), attempts should be made to taper the dose over a few weeks to less than 10mg daily. Once started, corticosteroid therapy may be difficult to discontinue and even at low doses. Some patients are very sensitive to the tapering of prednisone which may be done slowly over a few weeks.

Weight gain and a cushingoid appearance (increased fat deposition around the face, redness of the cheeks, development of a "buffalo hump" over the neck) is a frequent problem and source of patient complaints. Other side effects of prednisone include weight gain, increased blood pressure, increased blood sugar, increased risk of cataracts, and avascular necrosis of bones.

Steroid medications are also associated with accelerated osteoporosis even with relatively low dose prednisone at doses of 10 mg daily. Patients with and without osteoporosis risk factors on low dose prednisone should undergo bone densitometry (DEXA Scan) to assess fracture risk. Bisphosphonates such as alendronate (Fosamax®), risedronate (Actonel®), ibandronate (Boniva®) are recommended to prevent and/or treat osteoporosis in addition to adequate calcium and vitamin D supplementation.

Higher doses of prednisone are rarely necessary unless there is a life-threatening complication of RA and, if used for prolonged periods, may lead to serious steroid toxicity. Although a few patients can tolerate every other day dosing of corticosteroids which may reduce side effects, most require corticosteroids daily to avoid symptoms. Once a day dosing of prednisone is associated with fewer side effects than the equivalent dose given twice or three times daily. Generally steroids are given in the morning upon wakening to mimic the body's own steroid surge. Repetitive short courses of high-dose corticosteroids, intermittent intramuscular injections, adrenocorticotropic hormone injections, and the use of corticosteroids as the sole therapeutic agent are all to be avoided.

Intra-articular corticosteroids (e.g., triamcinolone or methylprednisolone and others) are effective for controlling a local flare in a joint without changing the overall drug regimen.

Disease Modifying Anti-rheumatic Drugs (DMARDS)

Although both NSAIDs and DMARD agents improve symptoms of active rheumatoid arthritis, only DMARD agents have been shown to alter the disease course and improve radiographic outcomes. DMARDs have an effect upon rheumatoid arthritis that is different and may be slower. In most cases, when the diagnosis of rheumatoid arthritis is confirmed, DMARD agents should be started. The presence of erosions or joint space narrowing on x-rays of the involved joints is a clear indication for DMARD therapy, however one should not wait for x-ray changes to occur. The currently available drugs include:

- Methotrexate (Rheumatrex®, Trexall®)
- Hydroxychloroquine (Plaquenil ®)
- Sulfasalazine (Azulfidine®)
- Leflunomide (Arava®)
- Tumor Necrosis Factor Inhibitors– etanercept (Enbrel®, adalimumab (Humira ®), and infliximab (Remicade®), certolizumab pegol (Cimzia®), golimumab (Simponi®)
- T-cell Costimulatory Blocking Agents—abatacept (Orencia®)
- B cell Depleting Agents—rituximab (Rituxan®)
- Interleukin-6 (IL-6) Inhibitors– tocilizumab (Actemra®)
- Interleukin-1 (IL-1) Receptor Antagonist Therapy—anakinra (Kineret®)
- Intramuscular Gold
- Other Immunomodulatory and Cytotoxic agents— azathioprine (Imuran®) and cyclosporine A(Neoral®, Sandimmune®)

Methotrexate

Methotrexate is now considered the first-line DMARD agent for most patients with RA. It has a relatively rapid onset of action at therapeutic doses (6-8 weeks), good efficacy, favorable toxicity profile, ease of administration, and relatively low cost. When looking at groups of patients on different DMARDS, the majority of patients continue to take Methotrexate after 5 years, far more than other therapies reflecting both its efficacy and tolerability. Methotrexate is effective in reducing the signs and symptoms of RA, as well as slowing or halting radiographic damage. It was as effective as leflunomide and sulfasalazine in one study, and its effectiveness given early and in higher doses approached the efficacy of etanercept and adalimumab as single therapies in terms of signs and symptom improvement. Methotrexate is also effective in many other forms of inflammatory arthritis including psoriatic arthritis and other spondyloarthopathies, and is used in many other autoimmune diseases.

Mechanism:

The anti-inflammatory effects of methotrexate in rheumatoid arthritis appear to be related at least in part to interruption of adenosine and possible effects on other inflammatory and immunoregulatory pathways. The immunosuppressive and toxic

effects of methotrexate are due to the inhibition of an enzyme involved in the metabolism of folic acid, dihydrofolate reductase.

Dosage:

Dosing typically begins at 12.5-15 mg once per week. A dose escalation to 20 mg within the first three months is now fairly well accepted in clinical practice. Maximal dose is usually 25 mg per week but is sometimes increased further to 30 mg. Methotrexate can be given orally or by subcutaneous injection. The latter route of administration can be advantageous for patients who have methotrexate-associated nausea. Patients starting methotrexate should be carefully evaluated for renal insufficiency, acute or chronic liver disease, significant alcohol intake or alcohol abuse, leukopenia (low white blood cell counts), thrombocytopenia (low platelet counts), or untreated folate deficiency. Obesity, diabetes and history of hepatitis B or C are factors that have been suggested but not confirmed to increase methotrexate hepatotoxicity (liver injury). Salicylates (and other NSAIDs) and the antibiotic trimethoprim (Bactrim®, Septra®) block the renal excretion of methotrexate and increase serum levels with an increased risk of toxicity. If alternatives exist, concomitant use of methotrexate and trimethoprim is to be avoided. The coadministration of NSAIDS with methotrexate is routine in patients with rheumatoid arthritis and is considered safe by rheumatologists as long as liver function tests and blood counts are closely monitored.

Usual Time to Effect:

The onset of action is seen in as early as 4 to 6 weeks. However the dose required to achieve a response is variable in individual patients and may require 4-6 weeks after a dose increase to determine if the drug is working. A trial of 3 to 6 months at an increased dose (e.g. 20 mg/wk) is suggested. In patients with partial responses to methotrexate, additional medications are usually added to rather than substituted for methotrexate to achieve combination therapies.

Side Effects:

Fortunately the most serious complications of methotrexate therapy: hepatic cirrhosis, interstitial pneumonitis, and severe myelosuppression are quite rare, especially with proper monitoring. Stomatitis and oral ulcers, mild alopecia and hair thinning, and GI upset may occur and are related to folic acid antagonism. These side effects can be improved with folic acid supplementation. Folic acid given at a dose of 1mg daily does not diminish the efficacy of methotrexate and is routinely given with methotrexate to decrease these side effects. Some patients complain of headache, fatigue, and feeling "wiped out" (also called methotrexate "fog"). These side effects can often be overcome by increasing folic acid or using an activated form of folic acid known as folinic acid (leukovorin®) given as a 5mg dose 12 hours and sometimes 24 hours after methotrexate is given. Some patients complain of GI upset (nausea or diarrhea) with oral methotrexate. This may be lessened when methotrexate is taken at night. In most

cases this is completely eliminated when methotrexate is given by subcutaneous administration.

Before starting methotrexate, baseline studies should include complete blood count, liver chemistries, serum creatinine, hepatitis B and C serologies, and chest X-ray. Routine toxicity monitoring should include a CBC, liver profile, serum albumin and serum creatinine every 4-8 weeks.

Methotrexate can be combined safely with nearly every other FDA-approved DMARDs for RA, including sulfasalazine, hydroxychloroquine, TNF inhibitors, abatacept, rituximab, tocilizumab, anakinra, and leflunomide. In all clinical trials combining methotrexate with one of these DMARDs, no unexpected toxicities or synergistic toxicities were observed with the exception of higher liver toxicity with leflunomide which is also metabolized by the liver.

Hepatotoxicity (liver injury) has not been significant if patients with pre-existing liver disease, alcohol abuse, or hepatic dysfunction are excluded from treatment with methotrexate. Patients are instructed to limit alcohol containing beverages to no more than one-two per week. Baseline or surveillance liver biopsies are <u>not</u> indicated unless pre-existing liver disease is suspected. Elevated liver enzymes do not directly correlate with toxicity but therapy should be stopped and doses of methotrexate reduced if transaminases are elevated to 2 times the upper limit of normal. Liver biopsy should be done if elevated liver enzymes persist or if methotrexate therapy is to be continued.

Interstitial pneumonitis is a rare complication of methotrexate (<2%), but the clinician should be alert to symptoms of cough or shortness of breath that may herald the onset of this severe complication. Methotrexate pneumonitis may occur at any time during therapy and is not dose related. A baseline chest x-ray is useful for comparison. Patients with poor pulmonary reserve from other causes may be excluded from therapy over concerns of increased morbidity if methotrexate pneumonitis occurs. A more chronic form of interstitial lung disease and fibrosis is also seen in patients with rheumatoid arthritis. This may be increased with methotrexate.

Myelosuppression (lowering of blood counts) is also rare at the low doses of methotrexate utilized for rheumatoid arthritis. Patients at particular risk include those with renal insufficiency from other causes or use of trimethoprim (Bactrim®, Septra®) which increases levels of methotrexate. In the absence of leukopenia (lowered white blood cell counts), there has not been conclusive information to link methotrexate use in rheumatoid arthritis with increased risk of infection. The exception is a slight increased risk of localized herpes zoster infection (shingles).

Cancer risk with methotrexate. Although there are case reports of**lymphoma** associated with methotrexate therapy including cases where the lymphoma resolved after cessation of therapy, increased occurrence of malignancy has not been found in large population-based studies. It is important to recognize that patient with rheumatoid arthritis have an increased risk of developing lymphoma as a

consequence of their autoimmune disease, independently from any potential medication effects.

Pregnancy and Conception with methotrexate. There have not been any notable effects on sperm production or ovarian function after the prolonged administration of methotrexate. However, **methotrexate is considered a teratogen**; therefore, women of childbearing potential or men with partners of childbearing potential must practice effective birth control. Women should discontinue methotrexate for at least one ovulatory cycle prior to attempting conception, while men should wait 3 months.

Hydroxychloroquine

Hydroxychloroquine is an antimalarial drug which is relatively safe and well-tolerated agent for the treatment of rheumatoid arthritis. Chloroquine is another antimalarial agent that is also sometimes used. Because these drugs have limited ability to prevent joint damage on their own, their use should probably be limited to patients with very mild, seronegative, and nonerosive disease. Hydroxychloroquine is sometimes combined with methotrexate for additive benefits for signs and symptoms or as part of a regimen of "triple therapy" with methotrexate and sulfasalazine.

Mechanism:

The mechanism of action of antimalarials in the treatment of patients with rheumatoid arthritis is unknown but is thought to involve changes in antigen presentation or effects on the innate immune system.
Dosage: Hydroxychloroquine (Plaquenil®) is the drug of choice among antimalarials. Chloroquine is not commonly used because of greater toxicity on the eye. The usual dose of Plaquenil is 400mg/day but 600mg/day is sometimes used as part of an induction regimen. It may be prescribed as a single daily dose or in divided doses twice per day.

Usual Time to Effect:

A period of 2 to 4 months is usual. Most agree that if a patient shows no response after 5-6 months that this should be considered a drug failure.

Side Effects:

The most important toxicities are on the eyes: corneal deposits, extraocular muscular weakness, loss of accommodation (and sensitivity to light), and a retinopathy that may progress to irreversible visual loss. Ocular toxicity is exceedingly rare, occurring in only 1 out of 40,000 patients treated at the doses recommended. Patients with underlying retinopathies or risks may not be good candidates for antimalarial drugs. Baseline ophthalmologic examination and a follow-up examination every 12 months are recommended during the period of treatment.

Sulfasalazine

Sulfasalazine **(Azulfidine®)** is an effective DMARD for the treatment of RA. Its effectiveness overall is somewhat less than that methotrexate, but it has been shown to reduce signs and symptoms and slow radiographic damage. It is also given in conjunction with methotrexate and hydroxychloroquine as part of a regimen of "triple therapy" which has been shown to provide benefits to patients who have had inadequate responses to methotrexate alone. Sulfasalazine is also used in the treatment of inflammatory bowel disease and spondyloarthropathies. Its mechanism of action in RA is unknown. Some of its effects may be due to folate depletion.

Dosage:

The usual dose is 2-3 grams per day in a twice daily dosing regimen. The dose may be initiated at 1 gram per day and increased as tolerated.

Usual Time to Effect:

It may take 6 weeks to 3 months to see the effects of sulfasalazine.

Side effects:

Sulfasalazine may cause hypersensitivity and allergic reactions in patients who have experienced reactions to sulfa medications. Mild gastrointestinal complaints are commonly seen and these can be decreased by using enteric coated formulations or administration of the medication with meals. Occasionally, mild cytopenias are seen. Patients may be screened before the use of sulfasalazine for a deficiency of the enzyme glucose-6-phosphate dehydrogenase (G6PD) which may predispose patients to red blood cell hemolysis and anemia. Blood monitoring is typically every 1-3 months depending on dose. Though sulfasalazine may cause increases in liver function tests, it is generally considered a preferable agent to methotrexate in patients with liver disease or in patients who have hepatitis B or C.

Leflunomide (Arava®)

Leflunomide is also an effective DMARD. Its efficacy is similar to methotrexate in terms of signs and symptoms, and is a viable alternative to patients who have failed or are intolerant to methotrexate. Leflunomide has been demonstrated to slow radiographic progression. Studies have demonstrated that it can also be carefully combined with methotrexate in patients with no preexisting liver disease, as long as the liver function tests are carefully monitored. Leflunomide has also been studied in psoriatic arthritis with some efficacy demonstrated.

Mechanism:

The mechanism of action of leflunomide is not fully understood but may be related to its ability to inhibit *de novo* pyrimidine biosynthesis through the inhibition of the enzyme dihydroorotate dehydrogenase. Laboratory studies have demonstrated that it also has effects on stimulated T cells.

Dosage:

The half-life of the active metabolite of leflunomide is very long. Leflunomide and its metabolites are extensively protein bound and undergo further metabolism before excretion. When initially approved, the medication was given using a loading dose of 100mg daily for three days then followed by 20 mg daily. Due to a significant incidence of GI side effects and diarrhea, most practitioners now use a shorter loading period with lower doses or initiate treatment at 10-20 mg/day with no loading dose,. The dose may be reduced to 10mg daily if not tolerated at the 20 mg dose.

Usual Time to Effect:

The onset of action is relatively rapid within 4-8 weeks. The onset of action of Arava may be seen earlier than methotrexate when using a loading dose.

Side Effects:

Leflunomide has been associated with liver transaminase elevations that reversed with cessation of the drug in clinical trials. Routine monitoring should include complete blood count and hepatic panel more frequently at the beginning of therapy then on a regular basis (at least every 2 months). Other toxicities that are common include mild diarrhea, GI upset and alopecia and hair thinning sometimes of sufficient severity to cause cessation of the drug.

Because **leflunomide and its metabolites are a teratogen**, extreme care must be taken for treatment of women of child bearing potential. Women must be warned about the possible risk to the fetus and cautioned to use adequate birth control. Women wishing to become pregnant must take cholestyramine 8gm 3 times daily for 11 days and then have two leflunomide metabolite levels drawn 14 days apart to document serum concentration less than 0.02mg/L. Leflunomide treatment does not appear to be associated with an increased risk for infection.

Tumor necrosis factor (TNF) inhibitors

Tumor necrosis factor alpha (TNF) is a pro-inflammatory cytokine produced by macrophages and lymphocytes. It is found in large quantities in the rheumatoid joint and is produced locally in the joint by synovial macrophages and lymphocytes infiltrating the joint synovium. TNF is one of the critical cytokines that mediate joint damage and destruction due to its activities on many cells in the joint as well as effects on other organs and body systems. TNF antagonists were the first of the biological DMARDS to be approved for the treatment of RA. These drugs began to enter the

market for rheumatoid arthritis in 1999 and are now considered a part the ACR recommendations for treatment of RA. There are currently five TNF inhibitors FDA approved for the treatment of RA (listed in order of their approval for RA); **etanercept (Enbrel®), infliximab (Remicade®),adalimumab (Humira®), certolizumab pegol (Cimzia®), and golimumab (Simponi®)**. Etanercept if a soluble TNF receptor-Fc immunoglobulin fusion construct; infliximab, adalimumab, and golimumab are monoclonal antibodies; and certolizumab pegol is an anti-TNF antigen binding domain-polyethylene glycol construct. While differing in structure, the efficacy and safety of the drugs is similar across the class in reducing the signs and symptoms of RA, as well as in slowing or halting radiographic damage, when used either as monotherapy or in combination with methotrexate.

Usual Time to Effect: TNF inhibitors have a rapid onset of action sometimes with improvements seen within 2 to 4 weeks. However, additional improvements can be seen over 3-6 months.

Side Effects: With all TNF antagonists, there is an increased risk of infection both mild and severe. The most common are upper respiratory infections, pneumonia, urinary tract infections, and skin infections. Studies are currently ongoing regarding the practice of temporarily holding the administration of any biologic DMARD in the presence of infection and use of antibiotics. However, many rheumatology practices are following that practice.

In addition to routine infections, opportunistic infections have been seen. Disseminated tuberculosis due to reactivation of latent disease has been seen with all TNF inhibitors; therefore, screening for latent TB is prudent before treatment with any TNF inhibitor. Invasive fungal infections, including histoplasmosis, coccidioidomycosis, candidiasis, aspergillosis, blastomycosis, and pneumocystosis have all been seen in patients receiving TNF inhibitors. Patients with histoplasmosis or other invasive fungal infections may present with disseminated, rather than localized, disease. Antigen and antibody testing for histoplasmosis may be negative in some patients with active infection. Empiric anti-fungal therapy should be considered in patients at risk for invasive fungal infections who develop severe systemic illness.[13] Because reactivation of Hepatitis B has been seen with TNF use, patients should be screened before beginning TNF therapy.

In some clinical trials of TNF antagonists, lymphomas were more commonly observed in patients treated with TNF inhibitors compared to placebo controls but the incidence rates do not appear, at this time, to exceed those reported in the RA population prior to the availability of TNF inhibitors. It is important to note that RA itself is a risk factor for Non-Hodgkins lymphomas. Other malignancies have been seen in patients taking TNF inhibitors. There does appear to be an increase in nonmelanoma skin cancer (basal and squamous cell) in patients receiving these agents. Regular dermatologic assessment is recommended with any suspicious lesions promptly evaluated. The administration of TNF inhibitors in patients with a prior malignancy should be

discussed with the patient and their oncologist to assess potential risk and benefit. TNF inhibitors are not recommended in patients with demyelinating disease or with congestive heart failure. Transient neutropenia (lowering of white blood cell counts) or other blood dyscrasias have been reported with TNF inhibitors. Some patients develop positive antinuclear antibodies (ANA), and cases of clinical lupus are reported but rare. The new onset of psoriasis has also been seen.

T-cell Costimulatory blockade

Abatacept (Orencia®)

Abatacept is the first of a class of agents known as T-cell costimulatory blockers. These agent interfere with the interactions between antigen-presenting cells and T lymphocytes and affect early stages in the pathogenic cascade of events in rheumatoid arthritis. T lymphocytes become activated due to an unknown stimulus but likely involving the interaction between antigen presented in the context of the Class II Major Histocompatability Complex molecule on the surface of antigen presenting cells. T cells recognize antigens as foreign and if they receive a second stimulus, will become active, proliferate, traffic to inflamed sites, and secrete proinflammatory cytokines including TNF. One of the important second signals for T cell activation is mediated by the molecules CD80 and CD86 found on antigen presenting cells and the CD28 molecule on the T cell surface.

Mechanism of action:

Abatacept is a fusion protein that combines the extracellular domain of the molecule CTLA4 (CD154) with the Fc portion of a human immunoglobulin molecule. CTLA4 has very high affinity for CD28. When abatacept binds to CD28 on the T cell surface, it prevents the second signal from being delivered, thus turning down the T cell response. Additional effects are decreasing the production of T cell derived cytokines including TNF.

Dosing:

Abatacept is administered either via IV or subcutaneously. When given by intravenous infusion it is used once per month after initial doses at baseline, 2 weeks, and 4 weeks. The IV dose is based on body weight, with patients <60 kg receiving 500 mg, 60-100 kg receiving 750 mg, and >100 kg receiving 1000 mg. The medication is administered over a period of approximately 30 minutes to one hour. The subcutaneous version, a fixed dose of 125 mg regardless of weight, is administered once weekly with or without an intravenous loading dose based on body weight as above.

Time to Effect:

Responses are typically seen within 3 months. In clinical trials, patients with initial responses continued to show improvements through the first year.

Adverse effects:

As with other biological DMARDS infections are increased in patients receiving abatacept. These have ranged from mild to severe. Respiratory infections including pneumonia were more common in clinical trials in patients with underlying COPD, thus extreme caution is suggested in this population. Opportunistic infections have been seen, though only a few cases of TB have been seen to date. TB screening is recommended. Malignancies have been seen in clinical trials but the rates appear to be similar for those expected in patients with rheumatoid arthritis. Infusion reactions have been seen in clinical trials that are typically mild.

B-Cell Depletion

Rituximab (Rituxan®)

B cells are an important inflammatory cell with multiple functions in the immune response. They serve as antigen presenting cells, directly interact with T-cells and others, can secrete cytokines, and differentiate into antibody-forming plasma cells. The depletion of B cells has been shown to be effective in reducing signs and symptoms of RA and in slowing radiographic progression. One B cell depleting agent, Rituximab, is currently available for the treatment of rheumatoid arthritis. Rituximab (Rituxan®) was originally developed to treat non-Hodgkin's lymphoma. Ritxuimab causes a rapid and sustained depletion of circulating B cells in the circulation with clinical improvements in many patients. Clinical trials have demonstrated that Rituximab is effective in decreasing signs and symptoms and in slowing radiographic progression in RA patients who have failed other DMARD therapies. The agent is currently approved in the US, however, only in patients who have failed TNF antagonists**.**

Mechanism:

Rituximab is a chimeric monoclonal antibody that binds to the CD20 molecule on the B cell surface leading to the removal of B cells from the circulation. A single course of ritximab (2 infusions of 1000 mg each given 2 weeks apart) leads to a rapid and sustained depletion of B lymphocytes in the peripheral blood. This effect is sustained for 6 months to 1 year or even longer. The levels of the autoantibody rheumatoid factor decrease, but the levels of other antibodies typically remain within the normal range after the first infusion, but may drop with subsequent courses.

Time to onset:

Effects from rituximab are not seen for up to 3 months after an infusion. Effects however may last 6 months and up to 2 years following a single infusion course.

Dosing:

The currently approved dose is 1000 mg administered intravenously over 3-4 hours with two doses given 2 weeks apart. Patients receive intravenous corticosteroids 30 minutes prior to each infusion. The optimal time for readministration is not yet clear. Some have advocated treatment every 6 months, while others wait for a return of symptoms to redoes. Doses of 500 mg have also been studied and appear to have similar clinical efficacy in patients who have failed to respond to DMARDS.

Adverse effects:

Infusion reactions are seen in patients who receive Rituximab infusions. These may include hives, itching, swelling, difficualty breathing, fever, chills, and changes in blood pressure. These are usually mild and respond to slowing the infusion rate or additional medication (such as antihistamines) but may be severe. These are reactions were the most common with the first infusion.

As with other immunomodulatory therapies, **infections** may be increased in patients who are receiving rituximab. Rituximab may lead to the**reactivation of viral infections** that were dormant including hepatitis B. Cases of progressive multifocal leukoencephalopathy (PML), a severe and potentially fatal brain infection, have been seen in patients with autoimmune disease who receive rituximab though this condition has also been seen in patients with autoimmune diseases who are not administered rituximab.Immunizations should be completed before starting therapy with rituximab and live virus vaccinations avoided. Repeat administration of rituximab has been associated with decreases in levels of IgG and IgM antibodies with subsequent courese. Whether these decreases are clinically important is under study.

Interleukin-6 (IL-6)

Tocilizumab (Actemra®)

Tocilizumab is the first approved drug in a class of IL-6 inhibitors. Clinical studies have shown that tocilizumab is effective in decreasing signs and symptoms and in slowing radiographic progression in RA patients who have failed other DMARD therapies. The agent is currently approved in the US, however, only in patients who have failed TNF antagonists**.**

Mechanism of action: Tocilizumab binds specifically to both soluble and membrane-bound IL-6 receptors and has been shown to inhibit IL-6-mediated signaling through these receptors. IL-6 is a pleiotropic pro-inflammatory cytokine produced by a variety of cell types including T- and B-cells, lymphocytes, monocytes and fibroblasts. IL-6 has been shown to be involved in diverse physiological processes such as T-cell activation, induction of immunoglobulin secretion, initiation of hepatic acute phase protein synthesis, and stimulation of hematopoietic precursor cell proliferation and differentiation. IL-6 is also produced by synovial and endothelial cells leading to local production of IL-6 in joints affected by inflammatory processes such as rheumatoid arthritis

Dosage: When used in combination with DMARDs or as monotherapy the recommended starting dose is 4 mg/kg followed by an increase to 8 mg/kg based on clinical response

Usual time to effect: 4 to 8 weeks

Side effects: As with other biological DMARDs, an increase risk of infection and serious infection is present with tocilizumab. Because of a risk of GI perforation, patients with a history of diverticulitis should not receive tocilizumab. Tocilizumab has been associated with reduced platelet count, elevations in liver transaminases, increased lipid parameters (total cholesterol, triglycerides, LDL cholesterol, and/or HDL cholesterol) and neutropenia. Monitoring for any of these side effects should be considered every 4 to 8 weeks while on therapy.

Interleukin-1 (IL-1)

IL-1 is another proinflammatory cytokine implicated in the pathogenesis of RA. IL-1 receptor antagonist (IL1ra) is an endogenous blocker of the cytokine. Evidence supporting an anti-inflammatory role of IL-1ra in vivo is demonstrated by the observation that IL-1ra deficient mice spontaneously develop autoimmune diseases similar to rheumatoid arthritis as well as vasculitis. IL1 has effects on cartilage degradation leading to damage as well as inhibiting repair, and is a potent stimulus to osteoclasts leading to bone erosion. One IL1 antagonist, anakinra (Kineret®), is currenly approved for the treatment of RA. Other agents have been studied as well in RA.

Anakinra (Kineret™)

Anakinra

Anakinra, a human recombinant IL-1 receptor antagonist (hu rIL-1ra), is approved for the treatment of RA. Anakinra can be used alone or in combination with non-biologic DMARDs.
Mechanism: Anakinra is a recombinant human IL-1ra that differs from native IL-1ra by the addition of an N-terminal methionine. Anakinra blocks the biologic activity of IL-1 by binding to IL-1R type I with the same affinity as IL-1 beta.

Dosage: The recommended dose of anakinra is 100 mg/day administered daily by subcutaneous injection. The dose should be administered at approximately the same time each day. An autoinjection system is available for the medication.

Usual Time to Effect: 2 to 4 weeks.

Side Effects: The most commonly observed side effect in all of the clinical trials with anakinra is injection site reactions, occurring in approximately two-thirds of patients. These reactions are present as erythema, itching, and discomfort and typically resolve

over one to two months. In some patients these reactions can be severe leading to drug discontinuation.

A modest increase in the risk of serious infection was observed in RA patients in clinical trials treated with anakinra in combination with DMARDS other than TNF inhibitors, compared to placebo with DMARDs (2 % vs 1%). Opportunistic infections including tuberculosis are less common with anakinra than with TNF antagonists. Mild to moderate decreases in absolute neutrophil counts were seen more commonly in anakinra treated patients in clinical trials, some severe. The rate of malignancies reported for anakinra was not increased relative to expected rates in the general population.

Biologic Treatment Schedule				
Etanercept	Prefilled syringeAutoinject pen	50 mg subcutaneous	Once per week	
Adalimumab	Prefilled syringeAutoinject pen	40 mg subcutaneous	Once every 2 weeks (may ↑ to weekly)	
Infliximab	IV infusion	3mg/kg -10 mg/kg	Day 1, 14, 42 then every 8 wks (interim can be as short as every 4 weeks)	2-3 hours
Abatacept	IV infusion	<60 kg/500 mg 60 to 100 kg/750 mg >100 kg /1 gram	Day 1, 14, 28 and every 4 weeks thereafter	30 minutes
	Prefilled Syringe	125 mg	With or without initial single IV infusion, then every month	
Rituximab	IV infusion	1000mg (500 mg also shown to be effective in DMARD inadequate responders)	Day 1 and 14	4 ? hours
Certolizumab Pegol	Prefilled syringe	200mg per syringe	Loading dose 400 mg at baseline, 2 weeks, 4 weeks then either 200 mg every other week or 400 mg every month	
Golimumab	Prefilled syringe Autoinject pen	50 mg	Every 4 weeks	
Tocilizumab	IV infusion	4 mg/kg or 8mg/kg	Every 4 weeks	1 hour

Patients receiving infused biological agents including may develop a clinical syndrome of fever, chills, body aches, and headache associated with the infusion of biologics. The symptoms can often be reduced or prevented by slowing the infusion rate, administration of diphenhydramine, acetaminophen, and sometimes corticosteroids before the infusion. Injection site reactions may be seen with injectable biologics. These are typically mild and generally do not result in drug discontinuation.

Other Immunomodulatory and Cytotoxic Agents

Some additional immunomodulatory drugs are used in RA including azathioprine (Imuran®), and cyclosporin A (Sandimmune®, Neoral®). Rarely cyclophosphamide (Cytoxan®) and d-Penicillamine are used. Because the potential of high toxicity, these agents are typically utilized for life-threatening extra-articular manifestations of RA such as systemic vasculitis or with severe articular disease that is refractory to other therapy.

Azathioprine (Imuran®) has some activity in rheumatoid arthritis but may take 8-12 weeks to see an effect. It is a purine analog that can cause bone marrow suppression and lowering of blood cell counts (white blood cells, red blood cells, and platelets) particularly in patients with renal insufficiency or when used concomitantly with allopurinol or ACE inhibitors. Increased risk of secondary malignancy due to azathioprine is controversial. Screening for levels of the enzyme thiopurine methyltransferase (TPMT) is recommended before initiating therapy with azathioprine. Certain individuals have deficiencies in this enzyme that metabolizes azathioprine with a concomitantly increased risk of toxicitiy for the medication. Side effects include nausea, and alopecia. Blood tests to monitor blood counts and liver function tests are necessary for patients on azathioprine.

Cyclosporine (Sandimmune®, Neoral®) *has some activity as a disease modifying therapy in rhematoid arthritis.* Studies have demonstrated that cyclosporine can be combined with methotrexate in RA patients to capture clinical responses. *It* is an immunosuppressive agent approved for use in preventing renal and liver transplant rejection and also has activity in psoriasis and other autoimmune diseases. Cyclosporine inhibits T cell function by inhibiting transcription of interleukin-2. Main toxicities include infection and renal insufficiency. Increase in blood pressure is common and may require treatment. Careful monitoring of renal function and blood pressure is needed for the entire time a patient is taking cyclosporine. Numerous medication interactions may affect blood levels of cyclosporine and lead to more toxicity. The package insert contains important information concerning these medication interactions. Cyclosporine increases risks of infection and may also increase the risk of malignancies including lymphoma.

Cyclophosphamide (Cytoxan®) is a potent immunosuppressive agent that is reserved for severe cases of refractory rheumatoid arthritis and those with manifestations such as vasculitis. It is used in the treatment of other autoimmune conditions including lupus and vasculitis. Cyclophosphamide is an alkylating agent with serious toxicities including bone marrow suppression, hemorrhagic cystitis, premature ovarian failure, infection and secondary malignancy particularly an increased risk of bladder cancer. Blood counts must be carefully monitored with this medication.

d-Penicillamine (Cuprimine®, Depen®) historically has some activity as a treatment for rheumatoid arthritis. It is prescribed primarily for patients with persistent

aggressive disease who have failed other available DMARDS. Like gold it is a relatively toxic drug that has limited utility due to issues of tolerability and efficacy that is not as robust as other currently available agents. Major side effects include severe rash and effects on renal function. Careful monitoring of kidney function is required with this drug. Patients may develop a lupus like illness or other autoimmune diseases when taking d-Penicillamine.

Intramuscular Gold

Gold is effective in the treatment of rheumatoid arthritis when it is given intramuscularly. Intramuscular gold salts were, until the 1990′s, the most often used DMARD agents but have been replaced by Methotrexate and other DMARDS as the preferred agents to treat RA. Two injectable compounds are available, **(Myochrysine® and Solganal®)**. Gold compounds are rarely used now due to their numerous side effects and monitoring requirments, their limited efficacy, and very slow onset of action. An oral gold compound (Auranofin®) is also available but its efficacy is even more limited than injectable compounds.

Mechanism:

A number of mechanisms have been postulated, but how gold works in patients with rheumatoid arthritis remains unknown.

Dosage:

Myochrysine or Solganal therapy is started at 10 mg intramuscularly, 25mg is then given the second week, then 50mg is given weekly until a response has occurred or until a total of 1 g has been given. If there is a favorable response, therapy is tapered to 50mg every 2 weeks for 3 months, then every 3 weeks for 3 months and then finally to a maintenance monthly dose. No response after a total of 1g should be considered a treatment failure. Monthly gold should be continued indefinitely.

Usual Time to Effect:

Effects are achieved within 4 to 6 months or after administration of 1g of gold.

Side Effects:

Approximately 35% of patients on gold therapy experience side effects leading to discontinuation of the drug. Prior to each gold injection, patients should have a complete blood count and urine test for protein. The most common reaction is a rash, which can vary from a simple pruritic erythematous patch to a severe exfoliative dermatitis. Ulcerations and mucositis of the mouth, tongue, and pharynx can occur. If a mild mucocutaneous eruption occurs, therapy should be interrupted. If the eruption

abates, therapy can be restarted at a 10-15mg weekly, titrating upwards to 50mg weekly with careful monitoring for further rash.

Up to 10% of patients have mild proteinuria due to a gold-induced membranous glomerulonephropathy that can progress to the nephrotic range. Patients with a positive urine dipstick for protein should be evaluated with a 24-hour urine collection and gold therapy stopped if proteinuria exceeds 500mg/24 hours. Mild proteinuria generally resolves with the cessation of therapy. Occasionally patients will have isolated microscopic hematuria on gold therapy. If monitored closely gold therapy can be continued but other causes of hematuria must be excluded.

Immune thrombocytopenia, granulocytopenia, and aplastic anemia occur uncommonly but are absolute indications for cessation of gold therapy. Myochrysine, and less often Solganal, can produce a nitritoid reaction (flushing, dizziness, or fainting) occurring immediately after the gold injection. Rarely, there is a paradoxical increase in musculoskeletal pain that requires discontinuation of treatment.Long term use of gold may result in a bluish discoloration of the skin to occur that is typically irreversible.

Analgesic Drugs

Pain caused by inflammation is best treated with an anti-inflammatory drug (see above), although occasionally the addition of acetaminophen can be helpful. Chronic narcotic therapy is not used routinely due to side effects such as diminished mental status, hypersomnolence, constipation, and dependency. Furthermore, they have no anti-inflammatory activity. They may be needed for patients with severe joint destruction who are not surgical candidates.

Treatment During Pregnancy

Rheumatoid arthritis therapy during pregnancy is complicated by the fact that none of the drugs discussed above have been shown to be safe in pregnant women with adequate, controlled studies. Although joint symptoms frequently remit during pregnancy, this effect is not universal. Treatment decisions require careful consideration of the risks and benefits to the mother and fetus.

All DMARD therapy should be stopped in women planning to conceive and in pregnant and lactating women. Evidence of the risks of these agents to the fetus either exists or cannot be ruled out. Hyrdoxychloroquine (Plaquenil®) is probably the safest DMARD for use during pregnancy. Methotrexate, because of evidence of potential teratogencity should be stopped in men and women planning conception (see above). Leflunomide is teratogenic, and women who are considering conception should undergo a washout of this drug and have 2 separate demonstrations of blood levels of the metabolite of the drug are low. TNF antagonists are currently pregnancy category B though studies are ongoing to evaluate the outcomes of pregnancies in patients treated with these agents.

Although safety has not been proven in controlled trials, no evidence exists for risks to the fetus of low dose prednisone (less than 20mg daily) or of NSAIDs used in the first two trimesters. If necessary, joint symptoms are best managed with the lowest possible dose of prednisone. Potential prednisone complications include worsening of maternal gestational diabetes, hypertension and intrauterine growth retardation. NSAIDs should be avoided in the third trimester because of the potential for premature closure of the ductus arteriosus, prolonged labor and peripartum hemorrhage. Although both NSAIDs and prednisone are excreted in the breast milk, both are considered compatible with breast-feeding by the American Academy of Pediatrics.

Reduction of joint stress

Because obesity stresses the musculoskeletal system, ideal body weight should be achieved and maintained. Rest, in general, is an important feature of management. When the joints are actively inflamed, vigorous activity should be avoided because of the danger of intensifying joint inflammation or causing traumatic injury to structures weakened by inflammation. On the other hand, patients should be urged to maintain a modest level of activity to prevent joint laxity and muscular atrophy. Splinting of acutely inflamed joints, particularly at night and the use of walking aids (canes, walkers) are all effective means of reducing stress on specific joints. A consultation with a physical and an occupational therapist is recommended early in the course.

Surgical Approaches

Although rheumatoid arthritis is generally an inflammatory process of the synovium, structural or mechanical derangement is a frequent cause of pain or loss of joint function. Pain and joint mobility may be improved by a surgical approach. The primary physician, the rheumatologist, and the orthopedist all help the patient to understand the risks and benefits of the surgical procedure. The decision to have surgery is a complex one that must take into consideration the motivation and goals of the patient, their ability to undergo rehabilitation, and their general medical status.

Synovectomy is sometimes appropriate for patients with rheumatoid arthritis, though in many patients the relief is only transient. However, an exception is synovectomy of the wrist, which is recommended if intense synovitis is persistent despite medical treatment over 6 to 12 months. Persistent synovitis involving the dorsal compartments of the wrist can lead to extensor tendon sheath rupture resulting in severe disability of hand function.

Total joint arthroplasties, particularly of the knee, hip, wrist, and elbow, are highly successful. Arthroplasty of the metacarpophalangeal (knuckle) joints also can reduce pain and improve function. Other operations include release of nerve entrapments (e.g., carpal tunnel syndrome), arthroscopic procedures, and, occasionally, removal of a symptomatic rheumatoid nodule.

SLE treatment

Approach Considerations

Management of systemic lupus erythematosus (SLE) often depends on disease severity and disease manifestations, although hydroxychloroquine has a central role for long-term treatment in all SLE patients. The LUMINA (Lupus in Minorities: Nature versus Nurture) study and other trials have offered evidence of a decrease in flares and prolonged life in patients given hydroxychloroquine, making it the cornerstone of SLE management.

In general, cutaneous manifestations, musculoskeletal manifestations, and serositis represent milder disease, which may wax and wane with disease activity. These are often controlled with nonsteroidal anti-inflammatory drugs (NSAIDS) or low-potency immunosuppression medications beyond hydroxychloroquine and/or short courses of corticosteroids. More prolonged steroid use is generally reserved for patients with involvement of vital organs. For example, central nervous system involvement and diffuse proliferative renal disease must be recognized as more severe disease manifestations, and these are often treated with more aggressive immunosuppression. Evidence suggests a relative undertreatment of SLE patients with end-stage renal disease (ESRD), because the extent of lupus activity may be underestimated.

EULAR recommendations

In 2007, the European League Against Rheumatism (EULAR) released recommendations for the treatment of SLE. In patients with SLE without major organ manifestations, glucocorticoids and antimalarial agents may be beneficial. NSAIDs may be used for short periods in patients at low risk for complications from these drugs. Consider immunosuppressive agents (eg, azathioprine, mycophenolate mofetil, methotrexate) in refractory cases or when steroid doses cannot be reduced to levels for long-term use.

EULAR recommendations for the management of SLE with neuropsychiatric manifestations support the evaluation and treatment of these symptoms in the same way as they are evaluated and treated in patients without SLE; if symptoms persist, management of these symptoms as an extension of SLE should be considered. For example, in patients with neuropsychiatric manifestations that may have an inflammatory etiology, immunosuppressive agents may be considered.[53]

Other guidelines

In 2009, an American College of Rheumatology (ACR) Task Force generated a quality indicator set.In 2012, the ACR published " Guidelines for the Screening, Diagnosis, Treatment and Monitoring of Lupus Nephritis in Adults," as well as an evidence report for lupus nephritis. These and other guidelines are available at the ACR's Practice Management Web site.

Adjunctive therapies

No diet-based treatment of SLE has been proven effective. Patients with SLE should be reminded that activity may need to be modified as tolerated. Specifically, stress and

physical illness may precipitate SLE flares. Additionally, persons with SLE should wear sunscreen and protective clothing or avoid sun exposure to limit photosensitive rash or disease flares.

Consultations

The multisystemic nature of SLE often requires involvement of consultants, depending on the organ system involved. Consultation with any of the following specialists may be necessary:

- Rheumatologist
- Infectious disease specialist
- Neurologist
- Pulmonologist
- Cardiologist
- Gastroenterologist
- Nephrologist
- Dermatologist
- Hematologist
- High-risk obstetrician

Biologic DMARD Therapy

Belimumab

The monoclonal antibody belimumab (Benlysta), a B-lymphocyte stimulator–specific inhibitor, has been found to reduce disease activity and possibly decrease the number of severe flares and steroid use in patients with SLE when used in combination with standard therapy.In March, 2011, the US Food and Drug Administration (FDA) approved the use of belimumab in combination with standard therapies (including steroids, nonbiologic DMARDS [eg, hydroxychloroquine, azathioprine, methotrexate]) to treat active autoantibody-positive SLE.Patients of African American or African descent did not show significant responses to belimumab in phase III post-hoc analysis, but they were not powered to assess for this effect; in a phase II trial, black individuals had a greater treatment response. These results indicate that the benefits of belimumab in SLE patients remain inconclusive and that further investigation is needed. Patients with severe active lupus nephritis or CNS lupus or patients previouslytreated with other biologics or cyclophosphamide have been excluded from participation in early trials.

The SLE Responder Index (SRI) is a tool that was developed following phase II trials and is composed of the following scores :

- SELENA-SLEDAI (Safety of Estrogens in Lupus Erythematosus: National Assessment– Systemic Lupus Erythematosus Disease Activity Index)
- BILAG (British Isles Lupus Assessment Group)
- PGA (physician global assessment)

SRI response is defined by the following :

- A 4-point or greater reduction in the SELENA-SLEDAI score
- No new BILAG A or no more than 1 new BILAG B domain score
- No deterioration from baseline in the PGA by 0.3 or more points.

A multinational phase III study (BLISS-52) that evaluated the efficacy and safety of belimumab in 867 patients with a minimum SELENA-SLEDAI score of 6, reported that patients given belimumab had significantly higher SRI scores at 52 weeks than did those given placebo. All groups had similar rates of adverse events.

Similarly, a phase III trial of 819 SLE patients who were positive for either antinuclear antibody or anti-double-stranded DNA at baseline screening found that belimumab at 10 mg/kg plus standard therapy resulted in a significantly greater SRI score (43.2%) than placebo (33.5%) at 1 year (those who received belimumab 1 mg/kg plus standard therapy had a 40.6% response rate). Overall, the addition of belimumab to standard therapy reduced SLE disease activity and severe flares, and the medication was well tolerated.

Rituximab

B-cell depletion with rituximab (Rituxan) has been used successfully for rheumatoid arthritis, but studies have shown mixed results for the treatment of SLE. An open study using rituximab showed excellent results as rescue therapy for patients with active SLE who were unresponsive to standard immunosuppressant therapy.There have also been case reports of patients with severe refractory SLE in which off-label use of rituximab showed benefits with tolerable safety profiles.

Pharmacologic agents targeting specific pathways such as cytokines and complement, as well as combinations of rituximab with costimulatory inhibition with anti-CD4OL or CTLA-41g, may prove to be more effective in treating SLE.

Emergency Department Management

Acute emergencies in patients with systemic lupus erythematosus (SLE) include the following:

- Severe neurologic involvement
- Systemic vasculitis
- Profound thrombocytopenia with a thrombotic thrombocytopenia (TTP)–like syndrome
- Rapidly progressive glomerulonephritis
- Diffuse alveolar hemorrhage

These conditions may be treated with high-dose intravenous steroids and cytotoxic therapy such as cyclophosphamide. Strokes, acute myocardial infarctions, and pulmonary emboli occurring as complications of SLE are managed in the same way as they are in patients without SLE. In patients who present with fever, it may be necessary to limit immunosuppression to steroids and to empirically treat for an infection until culture results have been received.

In rare cases, diffuse alveolar hemorrhage may require plasma exchange, or profound steroid-refractory thrombocytopenia may require therapy with intravenous immunoglobulin (IVIG). Catastrophic antiphospholipid antibody syndrome also requires aggressive acute management.

For more information, see the Medscape Reference article Antiphospholipid Syndrome.

Hospitalization

Fever in patients with systemic lupus erythematosus (SLE) is grounds for hospital admission because of the difficulty of distinguishing a disease flare from infection in these immunocompromised hosts. Patients with SLE are often complement deficient and functionally asplenic; therefore, they are at particular risk for infections with encapsulated organisms. For example, meningococcemia in young females with lupus may be catastrophic.

Although it is known that chronically low complement levels and functional asplenia may result in a low level of susceptibility to infection, it is not known to what degree. Overall, it is likely that the primary reason patients with SLE die of infections is immunosuppressive medications.Stress-dose steroid protocols should be used in patients who are receiving maintenance corticosteroids when they are admitted with infectious or perioperative stress.

Central nervous system lupus with depressed consciousness or alveolar hemorrhage may prompt transfer to an intensive care unit and consideration of protective intubation. Thrombotic thrombocytopenic purpura and catastrophic antiphospholipid antibody syndrome should prompt transfer to a center capable of offering plasma exchange therapy.

For more information, see the Medscape Reference articles Neurologic Manifestations of Systemic Lupus Erythematosus and Thrombotic Thrombocytopenic Purpura.

Lupus Nephritis

The 2012 American College of Rheumatology (ACR) guidelines for lupus nephritis recommend that treatment of this condition be largely based on classification by the International Society of Nephrology/Renal Pathology Society (ISN/RPS) histologic criteria (see Biopsies and Histologic Features).

Lupus nephritis is managed with a combination of glucocorticoids and immunosuppressive agents to slow the progression to end-stage renal disease (ESRD), along with maintaining normal blood pressure levels (ie, target of ≤130/80 mm Hg). In general, individuals with class I or II lupus nephritis do not need management with immunosuppression.

Patients with class III or IV disease, as well as those with a combination of class V and class III or IV disease, generally undergo aggressive therapy with glucocorticoid drugs

and immunosuppressants. Immunosuppressive therapy consists of induction and maintenance therapy. Induction therapy involves potent immunosuppressive drugs (eg, mycophenolate mofetil, cyclophosphamide) to achieve remission; these drugs are generally used for 3 months to 1 year, with an average of 6 months' treatment having been shown to be more efficacious and safer than long-term therapy.

A large randomized trial that compared induction therapy consisting of oral mycophenolate mofetil with cyclophosphamide therapy in patients with lupus nephritis showed that mycophenolate mofetil was not inferior to cyclophosphamide.[118] The investigators suggested that mycophenolate mofetil was associated with both a trend toward greater complete remissions and a greater safety profile. This study's findings were confirmed with the large, international Aspreva Lupus Management Study (ALMS) trial.

Once remission is achieved, start maintenance therapy with azathioprine or mycophenolate mofetil (ie, use less potent agents relative to long-term cyclophosphamide). The ALMS maintenance trial also found that mycophenolate mofetil was superior to azathioprine in the maintenance of the renal response to treatment and in the prevention of relapse in patients with lupus nephritis.[120] In the MAINTAIN trial, there was a trend toward fewer renal flares in patients receiving mycophenolate mofetil than in those receiving azathioprine; however, these results did not reach statistical significance.

When Griffiths et al compared the corticosteroid-sparing effect of cyclosporine with azathioprine in patients with severe SLE, they concluded that azathioprine may be considered first-line therapy, whereas cyclosporine requires close monitoring of blood pressure and serum creatinine. However, the investigators noted that in patients who are unable to tolerate azathioprine, cyclosporine may be considered.

Unfortunately, significant side effects are associated with cyclophosphamide-based regimens, which are the only ones with proven long-term efficacy. An alternative consideration is mycophenolate mofetil, which may be as effective as pulse cyclophosphamide but with less severe adverse effects. In refractory cases (lack of treatment response by 6 months), consider intensifying therapy with mycophenolate mofetil.

In patients with SLE and nephritis who progress to end-stage renal disease, dialysis and transplantation may be required; these treatments have rates of long-term patient and graft survival that are similar to those observed in patients without diabetes and SLE. However, transplantation is considered the treatment of choice because of improved survival rates.

For more information, see the Medscape Reference article Lupus Nephritis.

Adjunctive therapy

Unless contraindicated, hydroxychloroquine should be used as adjunctive therapy in lupus nephritis because of the potential for reduction in rates of disease flare; damage accrual, ,including renal damage; and risk of thrombotic events.

Administer angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs) to all patients with lupus nephritis, except pregnant women, who have proteinuria of 0.5 g or more per 24 hours (or equivalent by protein/creatinine ratios on spot urine tests). This treatment has been reported to not only reduce proteinuria by about 30% but also significantly delay the doubling of serum creatinine and the progression to ESRD (in patients with nondiabetic chronic renal disease).{{Rer139}

Statin therapy is recommended in patients with low-density lipoprotein cholesterol (LDL-C) levels greater than 100 mg/dL because both renal dysfunction alone and SLE alone are independent risk factors for accelerated atherosclerosis.

Final part Test-control
Initial knowledge level

1. Most autoimmune diseases are caused by a:

a. single genetic defect.
b. known infectious organism.
c. constellation of genetic and environmental events.
d. hormonaldysregulation.
e. B-cell defect.

2. Identifying an autoimmune disease in humans is often accomplished by

a. finding an antibody against self-components.
b. passively transferring specific T cells from a patient to a healthy individual.
c. showing that T cells or antibodies are the cause of the tissue damage.
d. circumstantial evidence, such as MHC association and clinical improvement, with immunosuppressive drugs.
e. finding the definitive agent or agents responsible for the disease.

3. The following is/are possible mechanism(s) for the recognition of self-components by the immune system in autoimmune diseases:

a. alteration of a self-antigen so it is recognized as foreign
b. leakage of sequestered self-antigen
c. loss of suppressor cells
d. infection with a microorganism that carries a cross-reactive antigen
e. any of the above.

4. Rheumatoid factor, found in synovial fluid of patients with rheumatoid arthritis, is most frequently found to be
   a. IgM reacting with L chains of IgG.
   b. IgM reacting with H-chain determinants of IgG.
   c. IgE reacting with bacterial antigens.

d. antibodytocollagen.
e. antibodyto DNA.

5. The pathology in autoimmune diseases due to antibody may be a result of
a. the formation of antigen —antibody complexes.
b. antibody blocking a cell receptor.
c. antibody-induced phagocytosis.
d. antibody-induced complement mediated lysis.
e. any of the above.

6. Autoimmune hemolytic anemia:
a. is usually due to warm agglutinins belonging to the IgM class directed against Rh antigens on red blood cells
b. may be due to the production of either cold agglutinins after viral infection or autoantibodies following drug treatment.
c. is the result of cytotoxic T cells lysing red blood cells
d. does not generally involve complement components in red blood cell lysis
e. can be characterized by a negative Coombs test

7. Systemic lupus erythematosus
a. is due to a mutation in double-stranded DNA.
b. is a classic example of a T-cell-mediated autoimmune disease.
c. has multiple symptoms and affects many organs.
d. results from antibodies specific to thyroid.
e. affectsonlyskinepithelialcells.

8. Diseases in which T_H cells and cytotoxic $CD8^+$ T cells probably play major roles in their pathology include all of the following *except*
a. myasthenia gravis.
b. Hashimoto's thyroiditis.
c. rheumatoid arthritis.
d. multiple sclerosis.
e. insulin-dependent diabetes mellitus.

9. A patient is found to have a form of diabetes in which his immune system is destroying his pancreatic islet cells. Which is the most likely explanation for this disease state?
a. The patient has an acquired immunodeficiency syndrome.
b. Immune complex formation and complement are the main contributors to insulitis.
c. In the islets of the pancreas, cells have upregulated MHC class II and Fas molecules, making them susceptible to cell death by immune cells.
d. There is an increase in suppressor cells.
e. $CD4^+$ T cells are being destroyed by pancreatic enzymes.

10. Hashimoto's thyroiditis
a. is due primarily to antibodies formed to thyroid-stimulating hormone receptors.
b. mimics an animal model in which the disease is induced by immunization with thyroglobulin.
c. can be transplacentally transmitted causing a neonatal form of the disease.
d. is an autoimmune disease which affects males and females equally.
e. is characterized by immune complex deposition in the thyroid.

11. The usual sequence of events in the development of an effective immune response to a viral infection is
a. interferon secretion, antibody synthesis, cellular immune response, NK cell ADCC (Antibody-Dependent Cell-Mediated Cytotoxicity).
b. antibody synthesis, interferon secretion, NK cell ADCC(Antibody-Dependent Cell-Mediated Cytotoxicity), cellular immune response.
c. NK cell ADCC (Antibody-Dependent Cell-Mediated Cytotoxicity), interferon secretion, antibody synthesis, cellular immune response
d. interferon secretion, cellular immune response, antibody synthesis, NK cell ADCC(Antibody-Dependent Cell-Mediated Cytotoxicity).
e. cellular immune response, interferon secretion, antibody synthesis, NK cell ADCC(Antibody-Dependent Cell-Mediated Cytotoxicity).

12. Differences between gram-positive and gram-negative bacteria include
a. staining with crystal violet.
b. ability of complement to lyse cells.
c. thickness of the peptidoglycan layer.
d. endotoxin in the cell walls of gram-negative bacteria.
e. all of the above.

13. Antigenic variation is a mechanism of immune evasion that results in
a. interference with attachment to host receptors.
b. induction of immune suppression.
c. alterations in important surface antigens such that escape variants arise as a result of immune selection.
d. mutations in surface antigens.
e. destruction of antigens by proteolytic enzymes

14. The best way to provide immunologic protection against tetanus neonatorum (of the newborn) is to
a. inject the infant with human tetanus antitoxin.
b. inject the newborn with tetanus toxoid.
c. inject the mother with toxoid within 72 hours of the birth of her child.
d. immunize the mother with tetanus toxoid before or early in pregnancy.
e. give the child antitoxin and toxoid for both passive and active immunization.

15.Active, durable immunization against poliomyelitis can be accomplished by oral administration of attenuated vaccine (Sabin) or by parenteral injection of inactivated (Salk) vaccine. These vaccines are equally effective in preventing disease because
a. both induce adequate IgA at the intestinal mucosa, the site of entry of the virus.
b. antibody in the serum protects against the viremia that leads to disease.
c. viral antigen attaches to the anterior horn cells in the spinal cord, preventing attachment of virulent virus.
d. both vaccines induce formation of interferon.
e. both vaccines establish a mild infection that can lead to formation of antibody.

Final knowledge level

1. A 60-year-old man who underwent a cardiac transplant 1 year ago returns for an annual heart evaluation. His medications include cyclosporine, metoprolol, aspirin, lisinopril, calcium, and atorvastatin. He has no allergies. His vital signs are stable. Lungs are clear. Heart is regular. Abdomen is benign. He has no pedal edema. His creatinine level is 3.4 mg/dL (baseline, 1.9 mg/dL). An electrocardiogram shows sinus tachycardia. He denies any fever, chills, shakes, nausea, vomiting, or fever. Which of the following is the appropriate management?
a. Check cyclosporine level
b. Obtain a renal ultrasound
c. Perform an echocardiogram
d. Place a Foley catheter
e. Schedule a renal biopsy

2. A recipient of a 2-haplotype MHC-matched kidney from a relative still needs immunosuppression to prevent graft rejection because
a. graft-versus-host disease is a problem.
b. major histocompatibility antigens will not be matched.
c. minor histocompatibility antigens will not be matched.
d. complement components will not be matched.
e. all of the above

3. Which major problem does a bone marrow transplantation in immunocompromised patients present?
a. potentially lethal graft-versus-host disease
b. high risk of T cell leukemia
c. inability to use a live donor
d. delayed hypersensitivity
e. all of the above

4. What is the role of class II MHC proteins on donor cells in graft rejection?
a. They are the receptors for interleukin-2, which is produced by macrophages when they attack the donor cells.

b. They are recognized by helper T cells, which then activate cytotoxic T cells to kill the donor cells.
c. They induce the production of blocking antibodies that protect the graft.
d. They induce IgE which mediates graft rejection.
e. all of the above

5. Grafts between genetically identical individuals (i.e., identical twins)
a. are rejected slowly as a result of minor histocompatibility antigens.
Bb. are subject to hyperacute rejection.
c. are not rejected, even without immunosuppression.
d. are not rejected if a kidney is grafted, but skin grafts are rejected.
e. all of the above

6. Chemically-induced tumors have tumor-associated transplantation antigens that
a. are always the same for a given carcinogen.
b. are different for two tumors of different histologic type even if induced by the same carcinogen.
c. are very strong antigens.
d. do not induce an immune response.
e. all of the above

7. Polyomavirus (a DNA virus) causes tumors in "nude mice" (nude mice do not have a thymus, because of a genetic defect) but not in normal mice. The BEST interpretation is that
a. macrophages are required to reject polyomavirus-induced tumors.
b. natural killer cells can reject polyomavirus-induced tumors without help from T lymphocytes.
c. T lymphocytes play an important role in the rejection of polyomavirus-induced tumors.
d. B lymphocytes play no role in rejection of polyomavirus-induced tumors.
e. all of the above

8. The minor histocompatibility antigens on cells
a. are detected by reaction with antibodies and complement.
b. are controlled by several genes in the major histocompatibility complex.
c. are unimportant in human transplantation.
d. induce reactions that can cumulatively lead to a strong rejection response.
e. all of the above

9. Individuals of blood group type AB
a. are Rh(d)-negative.
b. are "universal recipients" of transfusions.
c. have circulating anti-A and anti-B antibodies.
d. have the same haplotype.

e. all of the above

10. Graft and tumor rejection are mediated primarily by
a. non-complement-fixing antibodies.
b. phagocytic cells.
c. helper T cells.
d. cytotoxic T cells.
e. all of the above

11. The structural basis of blood group A and B antigen specificity is
a. a single terminal sugar residue.
b. a single terminal amino acid.
c. multiple differences in the carbohydrate portion.
d. multiple differences in the protein portion.
e. all of the above

12. Which one of the following is the BEST method of reducing the effect of graft-versus-host disease in a bone marrow recipient in patients with autoimmune diseases?
a. matching the complement components of donor and recipient
b. administering alpha interferon
c. removing mature T cells from the graft
d. removing pre-B cells from the graft
e. all of the above

13. Loss of which of the following classes of molecules on the surface of a tumor cell target would result in loss of susceptibility to killing by host immune cells in patients with autoimmune diseases?
a. CD3
b. CD4
c. CD8
d. MHC class I
e. MHC class II

14. A 34-year-old woman presents with fatigue, malaise, and swollen, tender joints. Physical examination is significant for a maculopapular eruption over sun-exposed areas, including the face. Examination of a peripheral blood smear reveals mild thrombocytopenia. Which of the following autoantibodies, if present, would be most specific for the diagnosis of the patient's disorder?
a. Anti-centromere antibody
b. Anti-IgG antibody
c. Antinuclear antibody
d. Anti-Sm (Smith antigen) antibody
e. Anti-SS-A (Ro) antibody

15. One year after orthotopic liver transplantation for hepatitis C and cirrhosis, a 53-year-old man develops rising transaminase and bilirubin levels. In order to minimize

chronic rejection injury to hepatic endothelial cells, immunosuppressive therapy is aimed at down-regulating of the following components of the immune response:
a. Autoantibody production
b. Complement protein synthesis
c. HLA antigen expression
d. Mast cell degranulation
e. T-lymphocyte activity

CORRECT ANSWERS:

Initial knowledge level:
1.C; 2.D; 3.E; 4.B; 5.E; 6.B; 7.C; 8. E; 9.C; 10.B; 11.D; 12.E; 13.C; 14. D; 15.B

Final knowledge level:
1.A; 2.C; 3. A; 4.B; 5.C; 6.B; 7.C; 8.D; 9.B; 10.D; 11.A; 12.C; 13.D; 14.D; 15.E

Structure of class.
Plan and organizational structure of the lesson:
Preparatory stage: organization of lesson and test control of incoming level of knowledge (5 academic hours or 225 minutes)

	Content	Level of learning	Methods of the learning control	Materials of the methodological support	Time, min.
1.	<u>The preparatory phase</u> The formation of professional knowledge, skills and abilities: - Know clinic of autoimmune diseases;	I	1. Examination the patients	1. Situational tasks	25
2.	<u>The main stage</u> - Methods of diagnosis of autoimmune diseases; - Methods for the treatment of autoimmune diseases; - Determine the necessity of guidance to the patient with an rheumatologist, clinical immunologist	II II II	2. Analysis of anamnesis 3. Filling immunological cards 4. Solving the common tasks	2. Maps of immunological observation 4. Maps of directing the patients to consulting by an rheumatologist, immunologist	180

3.	<u>The final stage</u> Monitoring and adjustment of professional knowledge and skills - Differential diagnosis of systemic vasculitis, differential diagnosis of systemic autoimmune diseases - Stages of treatment and prevention of autoimmune diseases	III III	1.Solving untypical situation tasks	1. Tests 2.Untypical situation tasks	15
4.	To sum up the lessons. Homework for the next topic.				2 3

Study questions.

1. Reasons for the development, triggers and genetic basis of allergy
2. Immunological mechanisms and types of injury biostructures
3. Non-allergic conditions, causes and mechanisms of formation
4. Basic principles of diagnosis of allergic diseases
5. Principles of treatment of allergic diseases
6. The main types of allergic diseases: clinical features, diagnosis, treatment approaches

Control questions.

1. Definition of autoimmunity, immunologic tolerance, autoaggression
2. The main causes of cancellation of immunologic tolerance to own autoantigenes
3. Modern types of classifications of autoimmune diseases
4. Leading effector mechanisms of autoimmune disease
5. Clinical and laboratory diagnosis of autoimmune diseases
6. Typical approaches to the treatment of autoimmune diseases
7. Modern methods of immunotropic therapy of autoimmune disease

Practical skills and abilities.

Master the methods of clinical laboratory Diagnostics of autoimmune diseases.

To be able to work with maps of immunological surveillance and other medical documentation in the conduct of patients with autoimmune disease

Know the basic group of products for kurac?? patients with autoimmune diseases, to be able to determine the correct tactics of treatment

References:

1. Stephen T. Holgate, Martin K. Church, MPharm, David H. Broide, Fernando D Martinez. Allergy, 4th Edition. – Saunders Ltd. (2012). – 432 pages
2. Abul K. Abbas, Andrew H. H. Lichtman, Shiv Pillai Cellular and Molecular Immunology. - Saunders; 7 edition (2011). – 560 pages
3. Roitt's Essential Immunology, Includes Desktop Edition. Peter J. Delves, Seamus J. Martin, Dennis R. Burton, Ivan M. Roitt. Wiley-Blackwell; 12 edition (2011). – 560 pages
4. How the Immune System Works, Includes Desktop Edition. Lauren M. Sompayrac. Wiley-Blackwell; 4 edition (2012). – 152 pages
5. Lecture Notes: Immunology, 6th Edition. Ian Todd, Gavin Spickett. Wiley-Blackwell (2011). – 480 pages
6. Essentials of Clinical Immunology, 6th Edition. by Helen Chapel, Mansel Haeney, Siraj Misbah, Neil Snowden. Wiley-Blackwell (2014). – 376 pages
7. Brian A. Baldo, Nghia H Pham. Drug Allergy. Clinical Aspects, Diagnosis, Mechanisms, Structure-Activity Relationships. Springer New York (2013). – 447 pages

METHODICAL INSTRUCTION

Practical class №7

3. **THEME. ALLERGY. HYPERSENSITIVITY. ALLERGIC DISEASES**

(5 academic hours).

Actuality of the topic:

In connection with widespread allergic diseases among allergized population, students need to advance their knowledge about the reasons of allergy development, immunopathogenesis, clinics, diagnosis and therapy of the allergic diseases.

4. Aim:

Academic (study): Students have to study the reasons and genetic basis of development of allergic diseases, immunological mechanisms and types of injury biostructures, body;

professionally focused: Students have to know methods of diagnosis, clinical features and approaches in treatment of basic allergic diseases, syndromes, reactions, be able to make a plan of examination and determine the necessity of referral to an allergist;

educational: form students' understanding of the impact of environmental, social and psychogenic factors on the development of allergic diseases and the necessity of early diagnosis of allergic diseases.

3. Equipment for conducting: short/notes information, diagrams, power point presentation; equipment and reagents for the skin test, allergy cards observation, history and outpatients case history, case studies, tests

4. Materials: Equipment to run powerpoint presentation

Main books. Short information due to the topic.

5. Integrative ties of the topic:

5.1. Interdisciplinary integration: The topic of the practical lesson is connected with the topics of the same series of practical lessons "The subject and tasks of clinical immunology and allergology"

5.2. Out-disciplinary integration:

Subject	**To know**	**Be able**
1	**2**	**3**
Physiology	Know the basic parameters of external respiration	Rate normal levels of external respiration and blood indexes
Pathophysiology	Types of hypersensitivity reactions	Name the types of reactions
Pharmacology	Know the basic groups of anti-histamines, anti-serotonin, β_2-agonists, cholinolytic,	Prescribe these drugs

	mucolytic and anti-inflammatory drugs	
Propaedeutic therapy	Features of the examination of patients with immunopathology	Perform palpation, percussion, auscultation of breath, evaluate the results of laboratory and instrumental methods of examination
Dermatology	Diagnosis of allergic skin diseases	Clinically evaluate the prevalence of skin process, the presence of secondary purulent infection
Therapy	Clinical picture, differential diagnosis of bronchial asthma, pollinosis, allergic conjunctivitis, rhinitis	Conduct clinical examination, evaluate the results of laboratory and instrumental examinations, prescribe treatment

The content of the topic

6. Student has to know:

7. Understand the classification of hypersensitivity reactions
8. Know the diseases associated with hypersensitivity reactions
9. Understand the mechanisms of damage in hypersensitivity reactions
10. Know the methods for diagnosing conditions due to hypersensitivity
11. Definition of allergic diseases
12. Classification of allergic diseases
13. Basic principles of allergic diseases

8. Study questions.

6. Reasons for the development, triggers and genetic basis of allergic diseases
7. Immunological mechanisms and types of injury biostructures
8. Basic principles of diagnosis of allergic diseases
9. Principles of treatment of allergic diseases
10. Prevention of allergic diseases

Main part

Allergy, or hypersensitivity, provokes various clinical manifestations, caused by an immune response to one or more environmental antigens and results in tissue inflammation and organ dysfunction. The major clinical events are recurrent or chronic inflammatory disorders of the respiratory mucosa (causing asthma and rhinitis) and skin (causing eczema and urticaria), and on rare occasions an acute systemic disease such as anaphylactic shock (AS).

An allergy is a hypersensitivity disorder of the immune system. Symptoms such as red eyes, itchiness, and runny nose, eczema, hives, or anasthma attack. Allergies can play a major role in conditions such as asthma. In some people, severe allergies to environmental or dietary allergens or to medication may result in life-threatening reactions called anaphylaxis. Food allergies, and reactions to the venom of stinging insects such as waspsand bees are more often associated with these severe reactions. Not all reactions or intolerances are forms of allergy

Hypersensitivity reactions

Hypersensitivity refers to excessive undesirable (damaging, discomfort producing and sometimes fatal) reactions produced by the normal immune system. Hypersensitivity reactions require a pre-sensitized (immune) state of the host. Hypersensitivity reactions can be divided into four types: type I, type II, type III and type IV, based on the mechanisms involved and time taken for the reaction. Frequently, a particular clinical condition (disease) may involve more than one type of reaction.

Type I Hypersensitivity

It is also known as **immediate** or **anaphylactic** hypersensitivity. The reaction may involve skin (urticaria and eczema), eyes (conjunctivitis), nasopharynx (rhinorrhea, rhinitis), bronchopulmonary tissues (asthma) and gastrointestinal tract (gastroenteritis). The reaction may cause from minor inconvenience to death. The reaction takes 15-30 minutes from the time of exposure to the antigen. Sometimes the reaction may have a delayed onset (10-12 hours).

Type I hypersensitivity is mediated by **IgE**. The primary cellular component in this hypersensitivity is **mast cell** or **basophil**. The reaction is amplified and/or modified by platelets, neutrophils and eosinophils. A biopsy of the reaction site demonstrates mainly **mast cells** and **eosinophils**. The mechanism of reaction involves preferential production of IgE, in response to certain antigens, often called **allergens** (Figure 1). The precise mechanism as to why some individuals are more prone to type-I hypersensitivity is not clear. However, it has been shown that such individuals preferentially produce more of TH2 cells that secrete IL-4, IL-5 and IL-13 which in turn favor IgE class switch. IgE has very high affinity for its receptor (Fcε; CD23) on mast cells and basophils. A subsequent exposure to the same allergen cross links the cell-bound IgE and triggers the release of various pharmacologically active substances (Figure 1). Cross-linking of IgE Fc-receptor is important in mast cell triggering. Mast cell degranulation is preceded by increased **Ca++ influx**, which is a crucial process; ionophores which increase cytoplasmic Ca^{++} also promote degranulation of mast cells, whereas, agents which deplete cytoplasmic Ca^{++} suppress degranulation.

The agents released from mast cells and their effects are listed in Table 1. Mast cells may be triggered by other stimuli such as exercise, emotional stress, chemicals (e.g., photographic developing medium, calcium ionophores, codeine, etc.), anaphylotoxins (e.g., C4a, C3a, C5a, etc.). These reactions mediated by agents without IgE-allergen interaction **are not typical hypersensitivity reactions**, although they produce the same symptoms.

Table 1. Pharmacologic Mediators of Immediate Hypersensitivity mediator	Physiological effect
preformed mediators in granules	
histamine tryptase kininogenase ECF-A (tetrapeptides)	bronchoconstriction, mucus secretion, vasodialatation, vascular permeability proteolysis kinins and vasodialatation, vascular permeability, edema attract eosinophil and neutrophils
newly formed mediators	
leukotriene B_4 leukotriene C_4, D_4 prostaglandins D_2 PAF	basophil attractant similar to histamine but 1000x more potent Eosinophil and basophil chemotactic, histamine-like but more potent edema and pain platelet aggregation and heparin release: microthrombi

Type II hypersensitivity reactions: Antibody-mediated cytotoxicity

Type II hypersensitive reactions involve antibody-mediated destruction of cells. This type of reaction is best seen by blood-transfusion reactions, in which host antibodies react with foreign antigens on the incompatible transfused blood cells and mediate destruction of these cells. Antibody can mediate cell destruction by activating the complement system to create pores in the membrane of the foreign cell. Antibody can also mediate cell destruction by antibody-dependent cell-mediated cytotoxicity (ADCC). In this process, cytotoxic cells with Fc-receptors bind to the Fc-region of antibodies on target cells and promote killing of the cells.

ABO incompatibility

If a blood group A individual is accidentally transfused with blood from a blood group A donor, the anti-B isohaemagglutinins bind to the B blood cells and mediate complement-mediated lysis a massive intravascular of the transfused red blood cells follows. Typical symptoms of a transfusion reaction include fever, chills, nausea, and

pain in the lower back. Within hours, free haemoglobin can be detected in the plasma; it is filtered through the kidneys, re¬sulting in haemoglobinuria. Some of the haemoglobin converts to bilirubin, which at high levels is toxic to brain. There is a clotting within blood vessels, too. A treatment involves prompt termination of the transfusion and maintenance of urine flow with a diuretic because the accumulation of haemoglobin in the kidney can cause acute tubular necrosis. Transfusion reactions can be prevented by a proper cross¬-matching between the donor's and the recipient's blood. The cross-matching can reveal the presence of the antibodies in donor or recipient sera that can cause these reactions.

Haemolytic disease of the newborn

Haemolytic disease of the newborn develops when maternal IgG antibodies specific for foetal blood-group antigens cross the pla¬centa and destroy foetal red blood cells. It most commonly develops in the Rh-negative mother bearing her Rh-positive foetus (i.e. the Rh(D) anti¬gens are expressed on its red blood cells). During her first pregnancy the Rh-negative woman is usually not exposed to sufficient quantity of foetal red blood cells (RBC) to activate her Rh(D)-specific B cells. At the time of delivery, however, separation of the placenta from the uterine wall allows larger amounts of foetal umbilical-cord blood to enter the mother's circulation. These foetal red blood cells activate the Rh(D)-specific B cells, resulting in production of the Rh(D)-specific antibodies and appearance of memory B cells in the mother. The secreted IgM antibody clears the Rh(D)+ foetal red blood cells from the mother's circulation and disappear in the time course; however, the memory B cells remain. When the woman is pregnant the second time, the Rh(D)-positive erythrocytes of the foetus cross the placenta and activate the memory B cells what results in production of antibodies. However, this time, they are of the IgG class (the secondary immune response. The IgG anti-Rh(D) antibodies cross the placenta and bind to the Rh(D) antigens; the complement system activation follows resulting in destruction of foetal red blood cells. Depending on the extent of RBC lysis, less severe haemolytic anaemia or more severe, sometimes also fatal, erythroblastosis foetalis, develops.

The development of haemolytic disease of the newborn by Rh(D) incompatibility can be detected by testing materna1 serum at intervals during pregnancy for antibodies to Rh(D) antigen. A rise in the titer of these antibodies as pregnancy progresses indicates that the mother has been exposed to Rh(D) antigens and is producing increasing amounts of antibodies. The presence of maternal IgG on the surface of foetal erythrocytes can be detected by a Coombs test.
The treatment haemolytic disease caused by the Rh(D) incompatibility is based on an exchange transfusion, primary to remove bilirubin; the infant is also exposed to low levels of UV light to break down the bilirubin and prevent any cerebral damage.

To prevent the Rh-isoimmunisations, all Rh-negative women are given anti-Rhesus antibodies 72 h after delivery at the latest. The antibodies originate from immunisation of men by the Rhesus-positive erythrocytes. The antibodies destroy foetus RBC and so prevent of the immunisation of the Rh-negative women.

Drug-induced haemolytic anaemia

Some drugs (e.g. penicillins, cephalosporins, etc.) can adsorb non-specifically to proteins on RBC membranes, forming a complex similar to a hapten-carrier complex. In some patients, such drug-protein complexes induce formation of antibodies, which then bind to the adsorbed drug on red blood cells, inducing complement-mediated lysis and thus progressive anaemia. When the drug is withdrawn, the haemolytic anaemia disappears.

Type III hypersensitivity reactions: Immune complexes induced inflammation

Complexes between antigens and antibodies, so called immune complexes, are formed whenever an antigen binds to its specific antibody; mononuclear phagocytes engulf and degrade them immediately. However, in dependence from relative concentration ratios of antigens and antibodies, respectively, immune complexes can sometimes induce immunopathological reactions. Large immune complexes are insoluble and are rapidly cleared by mononuclear phagocytes; also small complexes fail cause any damage, as they do not activate the complement system. However, when intermediate size immune complexes are formed, they tend to be deposited into tissues and organs where they induce inflammation and their damage.

The extent of immune complex deposition depends from a general capacity of the organism to degrade them, esp. from a physiological status of the monunuclear-phagocytic system and the complement system. Phagocytosis disorders are connected with persistence of immune complexes and their deposition to the tissues. Similarly, the deficiencies of C2 and C2 components of the complement system are associated with immune complex diseases, e.g. with SLE.

When immune complexes are deposited in tissues, they induce an inflammatory process. They activate the complement system what results in formation of C3a and C5a anaphylatoxins. These molecules activate mast cells to release permeability factors permitting localisation of immune complexes along the endothelial cell basement membranes. Neutrophils, macrophages, lymphocytes and other cells with membrane Fc-receptors are activated. The activated neutrophils are especially important. They release proteolytic enzymes and produce reactive oxygen intermediate products (ROI) that cause a damage of the tissue. Platelets can be subsequently activated resulting in blood clotting and microtrombi formation; local ischemy and tissue necrosis follows. As it contains fibrin, the term fibrinoid necrosis was coined. Historically, generalized type III reactions were often observed at the administration of antitoxins containing foreign proteins, such as horse anti-tetanus or anti-diphtheria serum; the condition is known as serum sickness. The clinical symptoms include fever, weakness, generalised vasculitis (rash) with oedema and erythema, lymphadenopathy, arthritis, and sometimes glomerulonephritis. As immune complexes are continuously degraded, the clinical manifestations spontaneously vanish. Formation of circulating immune complexes contributes to the pathogenesis of a number of conditions other than serum sickness. These include SLE (systemic lupus erythematosus), rheumatoid arthritis, Goodpasture's syndrome, poststreptococcal glomerulonephritis and others.

Except of generalised type III hypersensitivity reaction, there is also a localised type. It was **Nicholas Maurice Arthus** who first described it in 1903. Arthus showed that injection of an antigen intradermally or subcutaneously into an animal that had had

high levels of circulating antibody spe¬cific for the antigen produced local inflammation that progressed to a haemorrhagic necrotic ulcerating skin lesion.

Arthus reactions are rare in humans. After an insect bite, a sensitive individual may have a rapid, localized type I reaction at the site. Often, some 48 hrs later, a typical Arthus reaction also develops at the site, pronounced by erythema and oedema.

Type IV hypersensitive reactions: Delayed type of hypersensitivity

Type IV hypersensitive reactions (delayed type of hypersensitivity - DTH) develop when antigen activates sensitised T_{DTH} cells; these cells belong to T_H1 subset, although sometimes cytotoxic T cells (CTLs) are involved. Activation of T cells by antigen on appropriate antigen-presenting cells results in the secretion of various cytokines, including IL-2, IFN-gama, MIF (macrophage migration inhibitory factor, and TNF (tumour necrosis factor). The overall effect of these cytokines is to draw macrophages into the area and activate them, promoting increased phagocytic activity and increased concentrations of lytic enzymes for more effective killing. As lytic enzymes leak out of the activated macrophages into the surrounding tissue, localised tissue destruction can ensue. These reactions typically take 48 to 72 h to develop, the time required for initial T cell activation and cytokine secretion to mediate accumulation of macrophages and the subsequent release of their lytic enzymes. The hallmarks of a type IV reaction are the delay in time required for the reaction to develop and the recruitment of macrophages as opposed to neutrophils, as found in a type III reaction. Macrophages are the major component of the infiltrate that surrounds the site of inflammation.

The type IV reaction is important in host defence against parasites and bacteria that can live within cells, such as Mycobacterium tuberculosis, Mycobacterium leprae, Brucela species and others. Once these organisms are inside the host's cells, circulating antibodies cannot reach them. However, the heightened phagocytic activity and the build up of lytic enzymes from macrophages in the area of infection lead to non-specific destruction of cells, and thus of the intracellular pathogen. When this defence process is not entirely effective, the continued presence of the pathogen's antigens can provoke a chronic DTH reaction, which is characterised by excessive numbers of macrophages, continual release of lytic enzymes, granuloma formation and consequent tissue destruction. T cells mediate many contact dermatitis reactions, including the responses to formaldehyde, trinitrophenol, nickel, various cosmetics and hair dyes, poison oak, poison ivy, and others. Most of these substances are small molecules that can complex with skin proteins. This complex is internalised by antigen-presenting cells in the skin (i.e. Langerhans cells), processed and presented together with class II MHC molecules, causing activation of sensitised T cells. In the reaction to poison oak, for example, a pentadecacatechol compound from leafs of the plant complexes with skin proteins. When T_H cells react with this compound appropriately displayed by local antigen presenting cells, they differentiate into sensitised T cells; a subsequent exposure to pentadecacatechol will elicit activation of T cells and induce cytokine production. Approximately 48-72 h after this secondary exposure, the secreted cytokines cause macrophages to accumulate at the site. Activation of these macrophages and release of their lytic enzymes results in the redness and pustules that characterise a reaction to poison oak.

Type V hypersensitivity reactions

Type V hypersensitivity reactions were additionally added to the scheme originally described by Coombs and Gell. Contrary to type IV and in agreement with types I, II, and III, respectively, they are mediated by antibodies too. The type V reactions are sometimes considered as a subtype of the type II hypersensitivity. As its mechanisms do not destroy target cells, they are responsible for induction of organ/tissue dysfunctions only most of authors prefer it to be and independent, the 5th type of hypersensitivity reactions.

Cells receive information from their microenvironment in which they live; they sense signals that process and transduce into the cell nucleus by means of second signals. The specificity of binding between the signal and its receptor is mediated by complementarities of structures. For instance, thyroid-stimulating hormone (TSH) released from the adenohypophysis, by binding to its receptors in membranes of the thyroid gland stimulates adenylate-cyclase system what results in production of hormones.

Morbus Graves is characterised by production of antibodies directed against the TSH binding receptor that subsequently stimulate the thyroid gland, resulting in production of hormones (thyroxine and triiodothyronine). Contrary to physiological situation, there is no feedback mechanism – the increased levels of the thyroid gland hormones do not stop its hormones production as at the physiological condition when elevated amounts of thyroxines switch off the production of TSH and subsequent synthesis of hormones. The result is the hormone overproduction and appearance of clinical symptoms of hyperthyroidism. As antibodies increase the function of a target organ, this type of hypersensitivity is called stimulatory.

Autoantibodies cannot only stimulate cells of a target organ/tissue, however, on the contrary, also to inhibit it (hence the designation - inhibitory hypersensitivity reactions). A prototype of such a situation is **myastenia gravis**. It is an autoimmune disease characterised by production of autoantibodies directed against the acetylcholine receptors (AchR) present in neuro-muscular plates. By occupying the receptors, they inhibit a physiological binding of acetylcholine to, resulting in precluding signal transmission and muscle activation. The result of the events is a paralysis of striated muscles. In some cases the anti-acetylcholine receptors antibodies activate the complement system; a destruction of cell present in neuro-muscular plates follows; the condition is more severe than in the previous situation and is incurable.

Pernicious anemia (PA) is a disease is characterised by vitamin B12 deficiency caused by the absence of intrinsic factor. Vitamin B12 cannot be produced by the human body and must be obtained from the diet. When foods containing B12 are eaten, the vitamin is usually bound to protein and is released by stomach acid. Following its release, most B12 is absorbed by the body in the ileum after binding to a protein known as **intrinsic factor**. It is produced by parietal cells of the gastric mucosa and the intrinsic factor-B12 complex is absorbed by receptors on the ileum epithelial cells. In patients suffering from PA, antibodies to parietal cells cause the destruction of the

gastric mucosa, in which the parietal cells are located, leading to the subsequent loss of intrinsic factor synthesis. In other subgroup of PA patients, antibodies to intrinsic factor are directly induced. Without intrinsic factor, the ileum can no longer absorb the B12 and the disease develops.

Type VI Hypersensitivity
Antibody Dependant Cell Mediated Cytotoxicity (ADCC)
Type VI reaction according to the **Gell and Coombs Classification** . A phenomenon in which target cells, coated with antibody, are destroyed by specialized killer cells (**NK cells** , **Killer T-cells** and **macrophages**), which bear receptors for the Fc portion of the coating antibody (Fc receptors). These receptors allow the killer cells to bind to the anti-body-coated target.**Eosinophils** kill helminths (parasitic larvae infections) by ADCC.

Signs and symptoms of allergic diseases

Common symptoms	
Affected organ	**Symptom**
Nose	swelling of the nasal mucosa (allergic rhinitis)
Sinuses	allergic sinusitis
Eyes	redness and itching of the conjunctiva (allergic conjunctivitis)
Airways	Sneezing, coughing, bronchoconstriction, wheezing and dyspnea, sometimes outright attacks of asthma, in severe cases the airway constricts due to swelling known as laryngeal edema
Ears	feeling of fullness, possibly pain, and impaired hearing due to the lack of eustachian tube drainage.
Skin	rashes, such as eczema and hives (urticaria)
Gastrointestinal tract	abdominal pain, bloating, vomiting, diarrhea

Many allergens such as dust or pollen are airborne particles. In these cases, symptoms arise in areas in contact with air, such as eyes, nose, and lungs. For instance, allergic rhinitis, also known as hay fever, causes irritation of the nose, sneezing, itching, and redness of the eyes.Inhaled allergens can also lead to asthmatic symptoms, caused by narrowing of the airways (bronchoconstriction) and increased production of mucus in the lungs, shortness of breath (dyspnea), coughing and wheezing.
Aside from these ambient allergens, allergic reactions can result from foods, insect stings, and reactions to medications like aspirin and antibiotics such as penicillin. Symptoms of food allergy include abdominal pain, bloating, vomiting, diarrhea, itchy skin, and swelling of the skin during hives. Food allergies

rarely cause respiratory (asthmatic) reactions, or rhinitis.[7] Insect stings, antibiotics, and certain medicines produce a systemic allergic response that is also called anaphylaxis; multiple organ systems can be affected, including the digestive system, the respiratory system, and the circulatory system. Depending on the rate of severity, it can cause cutaneous reactions, bronchoconstriction, edema, hypotension, coma, and evendeath. This type of reaction can be triggered suddenly, or the onset can be delayed. The severity of this type of allergic response often requires injections of epinephrine, sometimes through a device known as the EpiPen or Twinject auto-injector. The nature of anaphylaxis is such that the reaction can seem to be subsiding, but may recur throughout a prolonged period of time.

Substances that come into contact with the skin, such as latex, are also common causes of allergic reactions, known as contact dermatitis or eczema. Skin allergies frequently cause rashes, or swelling and inflammation within the skin, in what is known as a "wheal and flare" reaction characteristic of hives and angioedema.

Cause

Risk factors for allergy can be placed in two general categories, namely host and environmental factors. Host factors include heredity, gender, race, and age, with heredity being by far the most significant. However, there have been recent increases in the incidence of allergic disorders that cannot be explained by genetic factors alone. Four major environmental candidates are alterations in exposure to infectious diseases during early childhood, environmental pollution, allergen levels, and dietary changes.

Foods

A wide variety of foods can cause allergic reactions, but 90% of allergic responses to foods are caused by cow's milk, soy, eggs, wheat, peanut, tree nuts, fish and shellfish. Other food allergies, affecting less than 1 person per 10,000 population, may be considered "rare".

The most common food allergy in the population is a sensitivity to crustacea.Although peanut allergies are notorious for their severity, peanut allergies are not the most common food allergy in adults or children. Severe or life-threatening reactions may be triggered by other allergens and are more common when combined with asthma.

Rates of allergies differ between adults and children. Peanut allergies can sometimes be outgrown by children. Egg allergies affect one to two percent of children but are outgrown by about two-thirds of children by the age of 5. The sensitivity is usually to proteins in the white rather than the yolk.

Milk-protein allergies are not Immunoglobulin E reactions, and are usually attributable to proctocolitis. They are most prevalent in children.[19] Some people are unable to tolerate milk from goats or sheep as well as cows, and many are also unable to tolerate dairy products such as cheese. A small portion of children with a milk allergy, roughly ten percent, will have a reaction to beef. Beef contains a small amount of protein that is present in cow's milk. Lactose intolerance, a common reaction to milk, is not a form of allergy at all, but rather due to the absence of an enzyme in the digestive tract.

Those with tree nut allergies may be allergic to one or many tree nuts, including pecans, pistachios, pine nuts, and walnuts. Also seeds, including sesame seeds and poppy seeds, contain oils where protein is present, which may elicit an allergic reaction.
Allergens can be transferred from one food to another through genetic engineering; however genetic modification can also remove allergens. Little research has been done on the natural variation of allergen concentrations in the unmodified crops.

Non-food proteins

Latex can trigger an IgE-mediated cutaneous, respiratory, and systemic reaction. The prevalence of latex allergy in the general population is believed to be less than one percent. In a hospital study, one in 800 surgical patients (0.125 percent) report latex sensitivity, although the sensitivity among healthcare workers is higher, between seven and ten percent. Researchers attribute this higher level to the exposure of healthcare workers to areas with significant airborne latex allergens, such as operating rooms, intensive-care units, and dental suites. These latex-rich environments may sensitize healthcare workers who regularly inhale allergenic proteins.
The most prevalent response to latex is an allergic contact dermatitis, a delayed hypersensitive reaction appearing as dry, crusted lesions. This reaction usually lasts 48 to 96 hours. Sweating or rubbing the area under the glove aggravates the lesions, possibly leading to ulcerations.Anaphylactic reactions occur most often in sensitive patients, who have been exposed to the surgeon's latex gloves during abdominal surgery, but other mucosal exposures, such as dental procedures, can also produce systemic reactions.
Latex and banana sensitivity may cross-react; furthermore, those with latex allergy may also have sensitivities to avocado, kiwifruit, and chestnut.These patients often have perioral itching and local urticaria. Only occasionally have these food-induced allergies induced systemic responses. Researchers suspect that the cross-reactivity of latex with banana, avocado, kiwifruit, and chestnut occurs because latex proteins are structurally homologous with some plant proteins.

Toxins interacting with proteins

Another non-food protein reaction, urushiol-induced contact dermatitis, originates after contact with poison ivy, eastern poison oak, western poison oak, or poison sumac. Urushiol, which is not itself a protein, acts as a hapten and chemically reacts with, binds to, and changes the shape of integral membrane proteins on exposed skin cells. The immune system does not recognize the affected cells as normal parts of the body, causing a T-cell-mediated immune response.Of these poisonous plants, sumac is the most virulent. The resulting dermatological response to the reaction between urushiol and membrane proteins includes redness, welling, papules, vesicles, blisters, and streaking.
Estimates vary on the percentage of the population that will have an immune system response. Approximately 25 percent of the population will have a strong allergic response to urushiol. In general, approximately 80 percent to 90 percent of adults will develop a rash if they are exposed to .0050 milligrams ($7.7?10^{-5}$ gr) of purified urushiol, but some people are so sensitive that it takes only a molecular trace on the skin to initiate an allergic reaction.

Genetic basis

Allergic diseases are strongly familial: identical twins are likely to have the same allergic diseases about 70% of the time; the same allergy occurs about 40% of the time in non-identical twins. Allergic parents are more likely to have allergic children, and those children's allergies are likely to be more severe than those in children of non-allergic parents. Some allergies, however, are not consistent along genealogies; parents who are allergic to peanuts may have children who are allergic to ragweed. It seems that the likelihood of developing allergies is inherited and related to an irregularity in the immune system, but the specific allergen is not.

The risk of allergic sensitization and the development of allergies varies with age, with young children most at risk. Several studies have shown that IgE levels are highest in childhood and fall rapidly between the ages of 10 and 30 years. The peak prevalence of hay fever is highest in children and young adults and the incidence of asthma is highest in children under 10. Overall, boys have a higher risk of developing allergies than girls, although for some diseases, namely asthma in young adults, females are more likely to be affected. These differences between the sexes tend to decrease in adulthood.Ethnicity may play a role in some allergies; however, racial factors have been difficult to separate from environmental influences and changes due tomigration. It has been suggested that different genetic loci are responsible for asthma, to be specific, in people of European origins.

Hygiene hypothesis

Allergic diseases are caused by inappropriate immunological responses to harmless antigens driven by a TH2-mediated immune response. Many bacteria and viruses elicit a TH1-mediated immune response, which down-regulates TH2 responses. The first proposed mechanism of action of the hygiene hypothesis was that insufficient stimulation of the TH1 arm of the immune system leads to an overactive TH2 arm, which in turn leads to allergic disease. In other words, individuals living in too sterile an environment are not exposed to enough pathogens to keep the immune system busy. Since our bodies evolved to deal with a certain level of such pathogens, when they are not exposed to this level, the immune system will attack harmless antigens and thus normally benign microbial objects — like pollen — will trigger an immune response.

The hygiene hypothesis was developed to explain the observation that hay fever and eczema, both allergic diseases, were less common in children from larger families, which were, it is presumed, exposed to more infectious agents through their siblings, than in children from families with only one child. The hygiene hypothesis has been extensively investigated by immunologists and epidemiologists and has become an important theoretical framework for the study of allergic disorders. It is used to explain the increase in allergic diseases that have been seen sinceindustrialization, and the higher incidence of allergic diseases in more developed countries. The hygiene hypothesis has now expanded to include exposure to symbiotic bacteria and parasites as important modulators of immune system development, along with infectious agents.

Epidemiological data support the hygiene hypothesis. Studies have shown that various immunological and autoimmune diseases are much less common in the developing world than the industrialized world and that immigrants to the industrialized world

from the developing world increasingly develop immunological disorders in relation to the length of time since arrival in the industrialized world. Longitudinal studies in the third world demonstrate an increase in immunological disorders as a country grows more affluent and, it is presumed, cleaner. The use of antibiotics in the first year of life has been linked to asthma and other allergic diseases. The use of antibacterial cleaning products has also been associated with higher incidence of asthma, as has birth by Caesarean section rather than vaginal birth.

Other environmental factors

International differences have been associated with the number of individuals within a population have allergy. Allergic diseases are more common in industrialized countries than in countries that are more traditional or agricultural, and there is a higher rate of allergic disease in urban populations versus rural populations, although these differences are becoming less defined.

Exposure to allergens, especially in early life, is an important risk factor for allergy. Alterations in exposure to microorganisms is another plausible explanation, at present, for the increase inatopic allergy. Endotoxin exposure reduces release of inflammatory cytokines such as TNF-α, IFNγ, interleukin-10, and interleukin-12 from white blood cells (leukocytes) that circulate in theblood. Certain microbe-sensing proteins, known as Toll-like receptors, found on the surface of cells in the body are also thought to be involved in these processes.

Gutworms and similar parasites are present in untreated drinking water in developing countries, and were present in the water of developed countries until the routine chlorination and purification of drinking water supplies. Recent research has shown that some common parasites, such as intestinal worms (e.g., hookworms), secrete chemicals into the gut wall (and, hence, the bloodstream) that suppress the immune system and prevent the body from attacking the parasite.This gives rise to a new slant on the hygiene hypothesis theory — that co-evolution of man and parasites has led to an immune system that functions correctly only in the presence of the parasites. Without them, the immune system becomes unbalanced and oversensitive. In particular, research suggests that allergies may coincide with the delayed establishment of gut flora in infants. However, the research to support this theory is conflicting, with some studies performed in China and Ethiopia showing an increase in allergy in people infected with intestinal worms. Clinical trials have been initiated to test the effectiveness of certain worms in treating some allergies. It may be that the term 'parasite' could turn out to be inappropriate, and in fact a hitherto unsuspected symbiosis is at work. For more information on this topic, seeHelminthic therapy.

In the early stages of allergy, a type I hypersensitivity reaction against an allergen encountered for the first time and presented by a professionalAntigen-Presenting Cell causes a response in a type of immune cell called a T_H2 lymphocyte, which belongs to a subset of T cells that produce acytokine called interleukin-4 (IL-4). These T_H2 cells interact with other lymphocytes called B cells, whose role is production of antibodies. Coupled with signals provided by IL-4, this interaction stimulates the B cell to begin production of a large amount of a particular type of antibody known as IgE. Secreted IgE circulates in the blood and binds to an IgE-specific receptor (a kind of Fc

receptor called FcεRI) on the surface of other kinds of immune cells called mast cells and basophils, which are both involved in the acute inflammatory response. The IgE-coated cells, at this stage, are sensitized to the allergen.

If later exposure to the same allergen occurs, the allergen can bind to the IgE molecules held on the surface of the mast cells or basophils. Cross-linking of the IgE and Fc receptors occurs when more than one IgE-receptor complex interacts with the same allergenic molecule, and activates the sensitized cell. Activated mast cells and basophils undergo a process called degranulation, during which they release histamine and other inflammatory chemical mediators (cytokines, interleukins, leukotrienes, and prostaglandins) from their granules into the surrounding tissue causing several systemic effects, such as vasodilation, mucous secretion, nerve stimulation, and smooth muscle contraction. This results in rhinorrhea, itchiness, dyspnea, andanaphylaxis. Depending on the individual, allergen, and mode of introduction, the symptoms can be system-wide (classical anaphylaxis), or localized to particular body systems; asthma is localized to the respiratory system and eczema is localized to the dermis.

Late-phase response

After the chemical mediators of the acute response subside, late-phase responses can often occur. This is due to the migration of other leukocytes such as neutrophils, lymphocytes, eosinophils and macrophages to the initial site. The reaction is usually seen 2–24 hours after the original reaction.Cytokines from mast cells may play a role in the persistence of long-term effects. Late-phase responses seen in asthma are slightly different from those seen in other allergic responses, although they are still caused by release of mediators from eosinophils and are still dependent on activity of T_H2 cells.

Allergy undergoes dynamic changes over time. Regular allergy testing of relevant allergens provides information on if and how patient management can be changed, in order to improve health and quality of life. Annual testing is often the practice for determining whether allergy to milk, egg, soy, and wheat have been outgrown and the testing interval is extended to 2 to 3 years for allergy to peanut, tree nuts, fish, and crustacean shellfish. Results of follow-up testing can guide decision-making regarding whether and when it is safe to introduce or re-introduce allergenic food into the diet.

Differential diagnosis

Before a diagnosis of allergic disease can be confirmed, other possible causes of the presenting symptoms should be considered. Vasomotor rhinitis, for example, is one of many maladies that shares symptoms with allergic rhinitis, underscoring the need for professional differential diagnosis. Once a diagnosis of asthma, rhinitis, anaphylaxis, or other allergic disease has been made, there are several methods for discovering the causative agent of that allergy.

Management

In recent times, there have been enormous improvements in the medical practices used to treat allergic conditions. With respect to anaphylaxis and hypersensitivity reactions to foods, drugs, and insects and in allergic skin diseases, advances have included the identification of food proteins to which IgE binding is associated with severe reactions and development of low-allergen foods, improvements in skin prick test predictions;

evaluation of the atopy patch test; in wasp sting outcomes predictions and a rapidly disintegrating epinephrine tablet, and anti-IL-5 for eosinophilic diseases.

Traditional treatment and management of allergies consisted simply of avoiding the allergen in question or otherwise reducing exposure. For instance, people with cat allergies were encouraged to avoid them. However, while avoidance of allergens may reduce symptoms and avoid life-threatening anaphylaxis, it is difficult to achieve for those with pollen or similar air-borne allergies. Nonetheless, strict avoidance of allergens is still considered a useful treatment method, and is often used in managing food allergies.

New technology approaches to decreasing IgE overproduction, and regulating histamine release in allergic individuals have demonstrated statistically significant reduction on Total Nasal Symptom Scores.

Medication

Several antagonistic drugs are used to block the action of allergic mediators, or to prevent activation of cells and degranulation processes. These include antihistamines, glucocorticoids,epinephrine (adrenaline), theophylline and cromolyn sodium. Anti-leukotrienes, such as montelukast (Singulair) or zafirlukast (Accolate), are FDA approved for treatment of allergic diseases. Anti-cholinergics, decongestants, mast cell stabilizers, and other compounds thought to impair eosinophil chemotaxis, are also commonly used. These drugs help to alleviate the symptoms of allergy, and are imperative in the recovery of acute anaphylaxis, but play little role in chronic treatment of allergic disorders.

Epidemiology

Many diseases related to inflammation such as type 1 diabetes, rheumatoid arthritis, and allergic diseases — hay fever and asthma — have increased in the Western world over the past 2-3 decades. Although genetic factors fundamentally govern susceptibility to atopic disease, increases in atopy have occurred within too short a time frame to be explained by a genetic change in the population, thus pointing to environmental or lifestyle changes. Several hypotheses have been identified to explain this increased prevalence; increased exposure to perennial allergens due to housing changes and increasing time spent indoors, and changes in cleanliness or hygiene that have resulted in the decreased activation of a common immune control mechanism, coupled with dietary changes, obesity and decline in physical exercise.The hygiene hypothesis maintains that high living standards and hygienic conditions exposes children to fewer infections. It is thought that reduced bacterial and viral infections early in life direct the maturing immune system away from T_H1 type responses, leading to unrestrained T_H2 responses that allow for an increase in allergy.

Changes in rates and types of infection alone however, have been unable to explain the observed increase in allergic disease, and recent evidence has focused attention on the importance of the gastrointestinal microbial environment. Evidence has shown that exposure to food and fecal-oral pathogens, such as hepatitis A, Toxoplasma gondii, and Helicobacter pylori (which also tend to be more prevalent in developing countries), can reduce the overall risk of atopy by more than 60%, and an increased prevalence of parasitic infections has been associated with a decreased prevalence of asthma. It is speculated that these infections exert their effect by critically altering T_H1/T_H2

regulation. Important elements of newer hygiene hypotheses also include exposure to endotoxins, exposure to pets and growing up on a farm.

Common Allergy Diagnostic Testing

Percutaneous skin test ranks first in confirming the presence of IgE-mediated sensitization in the allergist's office. This should come as no surprise, as it has many advantages. Skin testing is minimally invasive, and when it is performed correctly it has good reproducibility, is easily quantified, and allows the evaluation of multiple allergens at one session. The results correlates with in vivo challenges. in vitro testing is an alternative, usually a back up tool for diagnosing allergic illness. Skin testing alone or in combination with in vitro testing is relied upon for the evaluation of allergic rhinitis, asthma, eczema, food allergy, insect sting allergy, drug allergy (especially beta-lactam and local anesthetic allergy), occupational disease and anaphylaxis. However, the reliability of these tests depends on a number of factors. In the case of skin testing, it is important that the technician performing the skin tests and the clinician ordering or interpreting these tests are aware of the advantages and pitfalls of the type of skin testing, the device used, the location of the tests on the body, the extracts used and the potential for suppression of the skin response by medications used to treat allergies or depression. These issues have been reviewed elsewhere in greater detail. For in vitro testing, it is imperative that quality standards be met. These include calibration of the assay, training and experience of the technician and the use of quality allergens in the solid phase. As in any diagnostic test, it is of paramount importance that the clinician consider the positive and negative predictive value of the tests performed. These tests should always be considered as adjuncts to the medical history and physical exam in formulating the diagnosis in each individual case, bearing in mind that both test types can yield false positive or, less commonly, false negative results.

Methods of Skin Testing

Skin testing may be performed using either the prick/puncture (percutaneous) or intradermal (intracutaneous) technique. Intradermal testing is far more sensitive than prick/puncture testing, which means that it requires about 1000-fold less concentrated extracts than those used for prick/puncture testing to achieve a similar response. Although direct comparisons indicate that intradermal testing is more reproducible than percutaneous testing, there are many factors that favor the routine use of percutaneous allergy tests. These include economy of time, patient comfort and patient safety. Percutaneous testing allows the use of extract in 50% glycerin, which provides greater extract stability. Intradermal testing cannot use this diluent, as it may incite a false-positive irritant response. However, the most important consideration is that results of percutaneous testing correlate better with clinical allergy. The higher sensitivity of intradermal skin tests does not usually offer added benefit, since the results of skin prick tests performed with potent extracts are of sufficient sensitivity for use in clinical practice.

Two studies reinforce this concept.Each study compared intradermal with skin prick tests by correlating their results with patients' responses to natural exposure to allergen as well as by allergen challenge testing. In the first study, three groups of patients with seasonal rhinitis were compared. These subjects were classified into 3 groups based on

their degree of sensitization to Timothy grass pollen. They were either skin prick test positive, only intradermal test positive, or were negative by both skin prick and intradermal testing. Both nasal allergen provocation testing and symptom scores during the pollen season correlated best with a positive skin prick test (>60% of subjects with positive skin prick tests had symptoms on allergen exposure). The frequency of positive nasal provocation (11%) and symptom scores (21%) in subjects with positive intradermal testing alone were not different from subjects who were skin prick test and intradermal test negative. The authors conclude that under the conditions of this study, the presence of a positive intradermal skin test response to Timothy grass in the presence of a negative skin prick test did not indicate the presence of clinically significant sensitivity to this grass.

In the second study, patients were challenged with cat exposure for one hour. Both positive skin prick tests and in vitro tests to cat were highly predictive of the development of symptoms upon allergen exposure in the cat challenge room. Subjects with a negative skin prick test were just as likely to have a positive challenge result if they had a negative intradermal skin test (31%) as subjects with a positive intradermal skin test (24%). The authors conclude that, at least with regard to cat allergy, major therapeutic decisions, such as environmental control or immunotherapy, should never be based on a positive intradermal skin test alone.

Both of these studies were performed in adults and both relied upon skin testing with relatively potent allergens (Timothy grass and cat). The clinical applicability of these results to less potent allergens, such as dog, or to younger patients (especially infants) is a matter of clinical judgment, because no specific evidence is available for these groups.

Skin Testing Devices

Whereas intradermal skin tests are always performed using a hypodermic syringe and needle, percutaneous tests may be performed with a variety of devices. Comparisons of percutaneous devices have been reviewed elsewhere in greater detail. Some devices have a single stylus with one or several points, whereas others have multiple heads and allow up to 10 tests to be accomplished with one application. The degree of skin trauma created by these devices for percutaneous testing varies and so may result in differences in the size of positive reactions, and the likelihood of producing a reaction at the site of the negative control.

Recording and Scoring of Skin Test Results

Skin test results are often reported by clinicians in semiquantitative terms. They may record results only as positive or negative, or express them on a 0 to 4+ scale without any indication of the size of the reactions that these numbers represent. However, allergy patients may have to change their allergist for numerous reasons, and it is important that records of prior allergy testing be interpretable by the receiving physician. At the very least, a record of skin testing should contain sufficient information to allow another physician to interpret the results and avoid the need to repeat skin testing. Although the area of the wheal and erythema are the most accurate measurements, the longest diameter or two diameters at right angles to each other correlate with area ($r > 0.9$). The importance of performing such measurements is exemplified by McCann and Ownby in which allergists were asked to interpret

photographs of skin test reactions. The scoring and interpretation of the skin test results varied greatly. The authors of this study reinforce the idea that the most reliable method of reporting a skin test reaction is to measure and record the reaction size. At the very minimum, skin test results should be graded 0 to 4+, and the criteria for each grade of reaction clearly stated along with the skin test results.

Various investigators suggest different criteria for interpreting a skin test response as positive. To assess the reliability of different means of interpreting the results of skin prick testing, Vanto and colleagues studied a group of 202 children sensitive to dogs. A determination of sensitivity to dog was based on a composite score derived from the history, RAST, and bronchial or conjunctival allergen challenges. Although in this study the overall efficacy was greatest with the histamine reference method (in which the allergy skin test response is compared to a histamine control, with a positive response considered to be a response at least as great as that of the histamine control), maximal sensitivity was achieved when using a cutoff of a wheal 3 mm. If a clinician wishes to maximize sensitivity, the latter criterion would be most useful; however, adjustment must be made for the device used. Therefore, the criteria for a positive test should be the larger of: 1) 3 mm mean wheal diameter or 2) equal to or greater than the 99th percentile reaction with that device at negative control sites.

Proficiency Testing

Like all other laboratory tests, it is imperative that quality assurance standards be met to ensure that the testing technique produces accurate results. To confirm such standards, it is recommended that all technicians performing skin testing undergo evaluation of their technique. European publications suggest a coefficient of variation of less than 20% following repeated skin test control applications, and the Childhood Asthma Management Program study requires that a coefficient of variation of less than 30% be attained with repeated testing with histamine and consistently negative reactions to saline to confirm proficiency in skin testing.

The National Committee for Clinical Laboratory Standards recommends quality control procedures for daily performance of in vitro allergy testing, with a recommended coefficient of variation of less than or equal to 15%. Even with such calibration and the increased use of automation, in vitro assays still have flaws. Williams and colleagues examined the performance of 6 large commercial laboratories on tests of blinded samples of the same sera, both diluted and non-diluted. They found that only two of the laboratories demonstrated acceptable precision and accuracy.

Comparing in vivo to in vitro Testing:

The preponderance of comparative studies demonstrate skin tests to be more sensitive than in vitro tests. However, the majority of these studies were performed with earlier generation in vitro tests. The newer in vitro tests produce higher test sensitivity and specificity by using a matrix capsule containing antigen bound to a hydrophilic carrier to produce enhanced specific IgE binding with lower nonspecific IgE binding. Levels of specific IgE measured by different commercial assays are not equivalent, as each assay differs in the composition of allergen reagents, methods of measurement and standardization procedures.

The advantages of in vitro testing are largely related to use in patients with extensive dermatoses (e.g., atopic dermatitis), resulting in an inability to perform tests on

unaffected skin, or in patients who are unable to discontinue medicines that block the histamine response, i.e., antihistamines or tricyclic antidepressants. The disadvantages of in vitro testing include a potential decrease in sensitivity, added cost, and lack of immediate and visible response. Performing both in vitro and in vivo tests may yield improved sensitivity.

"Gold Standard" Confirmation of Allergy

Although there are challenge protocols available in the research setting to confirm allergic rhinitis and asthma, the standard tool available to the clinician is a careful history and physical exam. Skin testing correlates with results of nasal challenge or bronchial challenges, when allowance is made for nonspecific airway responsiveness.

When evaluating potential food allergy, the clinical history is the initial screening, with skin testing or in vitro tests used to corroborate the history. Oral food challenges represent the "gold standard" for the confirmation of food allergy. These can be performed as open challenges or in a single- or double-blind fashion. Food challenges are not without risk and thus require that appropriate supportive care be available. Several studies demonstrate that the magnitude of the in vitro test or the skin test reaction size may be useful in determining the utility of performing a food challenge. One additional advantage of skin testing for food allergies is the ability to perform skin testing with the fresh food, "prick-prick" test. Several reports demonstrate that fresh foods provide greater sensitivity for certain foods.This is particularly important in assessing allergy to fruit; however, useful results have also been demonstrated for other foods, including seafoods, peanut, tree nuts, vegetables, milk and eggs.

Skin testing is also known as "puncture testing" and "prick testing" due to the series of tiny puncture or pricks made into the patient's skin. Small amounts of suspected allergens and/or their extracts (pollen, grass, mite proteins, peanut extract, etc.) are introduced to sites on the skin marked with pen or dye (the ink/dye should be carefully selected, lest it cause an allergic response itself). A small plastic or metal device is used to puncture or prick the skin. Sometimes, the allergens are injected "intradermally" into the patient's skin, with a needle and syringe. Common areas for testing include the inside forearm and the back. If the patient is allergic to the substance, then a visible inflammatory reaction will usually occur within 30 minutes. This response will range from slight reddening of the skin to a full-blown hive (called "wheal and flare") in more sensitive patients similar to a mosquito bite. Interpretation of the results of the skin prick test is normally done by allergists on a scale of severity, with +/- meaning borderline reactivity, and 4+ being a large reaction. Increasingly, allergists are measuring and recording the diameter of the wheal and flare reaction. Interpretation by well-trained allergists is often guided by relevant literature.Some patients may believe they have determined their own allergic sensitivity from observation, but a skin test has been shown to be much better than patient observation to detect allergy.

If a serious life threatening anaphylactic reaction has brought a patient in for evaluation, some allergists will prefer an initial blood test prior to performing the skin prick test. Skin tests may not be an option if the patient has widespread skin disease or has taken antihistamines sometime in the last several days.

Blood testing

An allergy blood test is quick and simple and can be ordered by a licensed health care provider e.g. an allergy specialist. Unlike skin-prick testing, a blood test can be performed irrespective of age, skin condition, medication, symptom, disease activity and pregnancy. Adults and children of any age can take an allergy blood test. For babies and very young children, a single needle stick for allergy blood testing is often more gentle than several skin tests.

An allergy blood test is available through most laboratories, and a sample of the patient's blood is sent to a laboratory for analysis and the results are sent back a few days later. Multiple allergens can be detected with a single blood sample.

Allergy blood tests are very safe, since the person is not exposed to any allergens during the testing procedure.

The test measures the concentration of specific IgE antibodies in the blood. Quantitative IgE test results increase the possibility of ranking how different substances may affect symptoms. A general rule of thumb is that the higher the IgE antibody value, the greater the likelihood of symptoms. Allergens found at low levels that today do not result in symptoms can nevertheless help predict future symptom development. The quantitative allergy blood result can help determine what a patient is allergic to, help predict and follow the disease development, estimate the risk of a severe reaction and explain cross-reactivity.

A low total IgE level is not adequate to rule out sensitization to commonly inhaled allergens. Statistical methods, such as ROC curves, predictive value calculations, and likelihood ratios have been used to examine the relationship of various testing methods to each other. These methods have shown that patients with a high total IgE have a high probability of allergic sensitization, but further investigation with allergy tests for specific IgE antibodies for a carefully chosen of allergens is often warranted.

NEW APPROACH TO THE ALLERGY DIAGNOSIS

In the late 1960s, the discovery of the **immunoglobulin (IgE)** antibody provided a specific biomarker that could be used to identify allergic diseases triggered by environmental allergens (i.e., generally proteins). Traditional IgE antibody tests such as **skin prick tests (SPT)** or in vitro specific IgE (sIgE) tests are based on crude extracts composed of allergenic and non-allergenic molecules obtained from an allergenic source. With the application of DNA technology in the late 1980's, allergenic molecules were characterized and cloned in order to resolve the determinants of various allergic diseases. The availability of allergenic molecules in the last decade has ushered in a new phase of diagnostics, **termed molecular-based allergy (MA) diagnostics**, that allows for improved management of allergic diseases.

Today, many of the most common allergenic molecules have been cloned or purified, have had their three-dimensional structures elucidated, and can be consistently produced. Because of the growing number of allergens identified, a systematic allergen nomenclature, approved by the World Health Organization and International Union of Immunological Species (WHO/IUIS) Allergen Nomenclature Subcommittee, has been established. Allergenic molecules are named using their Latin family name (genus and species). For example, allergens that begin with **Phl p**are

from **Phl**eum **p**ratense (timothy grass). A number is added to the name to distinguish the various allergens from the same species (e.g., Phl p 1, Phl p 2, etc.). The numbers are assigned to the allergens in the order of their identification. Allergenic molecules are classified into protein families, according to their structure and biological function. Many different molecules share common epitopes (antibody binding sites) and the same IgE antibody can bind and induce an immune response to allergenic molecules with similar structures from various allergen sources. These cross-reactive allergens give valuable information regarding sensitization to several different sources. In contrast, some molecules are unique markers for specific allergen sources, allowing for the identification of the primary sensitizer.

MA diagnostics is increasingly entering routine care and can improve management of allergic patients. This is particularly evident in food allergy. Knowledge of the allergenic molecules the patient is sensitized to can help to discriminate between likelihood of local versus systemic reactions and persistence of clinical symptoms. For example, some allergens such as storage proteins in peanuts (e.g. Ara h 2) and nuts (e.g. Cor a 9) have been shown to be associated with severe reactions, while other allergens cause sensitization mostly without a clinical reaction. Another important aspect, difficult to elucidate using traditional tests, is the stability of the allergen. Allergens that are stable to heat and digestion (e.g., Ara h 2 from peanut) are more likely to cause severe clinical reactions, whereas heat and digestion labile molecules (e.g., Ara h 8 from peanut) are more likely to cause milder, local reactions or be tolerated. Similarly, identifying whether the sensitization is genuine in nature or due to cross-reactivity help to evaluate the likelihood of reaction on exposure to different allergen sources. Molecular diagnostics may also improve the selection of both patients and specific allergens for specific immunotherapy (SIT) for inhalant allergies (e.g., for pollen) and hymenoptera venom allergy. An ever increasing number of studies focusing on different allergenic molecules or allergic diseases are rapidly being published. However, the search for more, clinically relevant molecules is needed and ongoing.

The presence of IgE antibodies against allergenic molecules may be determined using a singleplex (one assay per sample) or multiplex (multiple assays per sample) measurement platform. A singleplex platform allows the doctor to select those allergenic molecules necessary for an accurate diagnosis defined by the clinical history of the patient. The multiplex approach allows for characterization of the IgE response against a broad array of pre-selected allergens on a chip independently of the clinical history. There is one commercially available multiplex immuno-solid phase allergen chip (ISAC) which contains more than 100 allergens from about 50 allergen sources. The large number of allergens provides extensive and detailed information about a patient's sensitization profile. ISAC is especially suited for use in patients with complex sensitization pattern or symptoms. The ISAC technology is a promising MA approach for improved diagnosis, prognosis, and selection of patients for SIT. While it is a commercial product, it has been the mainstay of many investigator studies so far.

Allergen source

A tissue, particle, food or organism inducing allergy (e.g. cat dander, D. pteronyssinus, milk, Aspergillus fumigatus, Phleum pratense pollen, etc.).

Allergen extract

A crude, unfractionated mixture of allergenic and non-allergenic proteins, polysaccharides, and lipids obtained by extraction from an allergen source (e.g., pollen grains).

Allergenic molecule (allergen component)

A molecule (i.e., protein or glycoprotein) derived from a given allergen source that is identified by sIgE antibodies (hereafter referred to as allergen). Allergens can be isolated from natural allergen sources (native, purified allergen) or can be produced by using recombinant DNA technology (recombinant allergen).

Stability of allergens

Allergens that are susceptible to **acid pH** in context with peptic digestion (relevant at the gastric level) are not able to cross the gastric barrier (except possibly in patients treated with antacid drugs). **Temperature** (cooking or boiling) susceptibility indicates that the allergen does not maintain its allergenicity after cooking/heating procedures. Heating may occur in the industrial processing of food for production and in domestic cooking. The structure of allergens susceptible to**protease digestion** is affected by gastric and pancreatic enzymes. Accordingly, allergens sensitive to these factors are considered labile, while those that are not are considered to be stable.

More about allergenic molecules

A **genuine** allergen causes specific sensitization to its corresponding allergen source. **Major** allergens are defined as those that bind to IgE in 50% or more of patients with the same allergy; in other words, the majority of patients (≥50%) with the same allergy are sensitized to the allergen in question. A **primary** allergen is the original sensitizing molecule (i.e., the driving trigger; in contrast to secondary sensitization due to cross-reactivity). In general, major allergens are also genuine and primary. Finally, the **abundance** of a molecule present in the allergen source is also a parameter to take into consideration.

Cross-reactivity: the phenomenon of an IgE antibody recognizing, binding, and inducing an immune response to similar allergenic molecules (homologues) present in different species; for example, an IgE antibody that binds and reacts to both Bet v 1 in birch pollen and Cor a 1 in hazelnut due to their structural similarity (generally characterized by greater than 50%-70% sequence homology between the primary structures of the proteins). IgE cross-reactivity often occurs between the following:

a) Allergenic molecules in closely related species (e.g., between grass or between mite allergens);

b) Well preserved molecules with similar function present in widely different species that belong to the same protein family (e.g., members of the tropomyosin protein family, such as Der p 10 in house dust mite and Pen m 1 in black tiger shrimp).

Component resolved diagnostics (CRD)

See molecular-based allergy diagnostics.

Co-sensitization

Genuine sensitization to more than one allergen source (e.g., timothy grass and birch), where the sensitization is not due to cross-reactivity.

CCD

Cross-reactive carbohydrate determinant. CCDs are carbohydrate moieties of glycoproteins. The most commonly described is MUXF3.

Epitope

The region of the protein recognized and bound by an antibody (i.e., the antibody binding site).

Molecular-based allergy (MA) diagnostics

A diagnostic approach to define the allergen sensitization of a patient at the molecular level using purified natural or recombinant allergen on singleplex or multiplex measurement platforms.

Pan-allergen

A cross-reactive allergen, belonging to a protein family well preserved throughout many widely different species, able to trigger IgE antibody binding (e.g., profilins or serum albumins).

Recombinant allergen

An allergenic molecule produced using DNA cloning and protein purification techniques. Recombinant allergens can be produced with consistency in terms of quality and amounts, and without CCD structures. Allergen extracts cannot be produced by recombinant techniques.

sIgE concentration/level

a) High level: represents a high concentration of sIgE antibodies specific for an allergenic extract or molecule. Generally, the higher the sIgE level the higher the probability of clinical reactions. Some allergens also have a high probability of inducing severe reactions at low sIgE concentrations (e.g., storage proteins and **lipid transfer proteins [LTPs**]), while others typically do not result in any clinical reactions despite high sIgE concentrations (e.g., **cross-reactive carbohydrate determinants [CCD]**).

b) Low level: Represents a low concentration of sIgE antibodies specific for an allergen extract or molecule.

sIgE sensitization

Presence of allergen-specific IgE (sIgE) antibodies in the blood that may occur in the presence or absence of clinical symptoms.

a) **Mono-sensitization**: Sensitization to one allergen source (Dermatophagoides pteronyssinus) or to a closely related taxonomical family or group of allergen sources (i.e., mites).

b) **Poly- (or multi-) sensitization:** Sensitization to three or more allergen sources (e.g., mite, birch, and grass pollen).

sIgE detection based on allergen extracts

Singleplex or multiplex platforms for in vitro measurement of sIgE reactivity to allergen extracts. Terms such as CAP, radioallergosorbent test (RAST), sIgE and in vitro-test are often used interchangeably for this technique. However, the performance of different measurement platforms differs and this should be taken into consideration when reporting and comparing results. This approach cannot identify cross-reacting molecules.

sIgE detection based on allergenic molecules

Singleplex or multiplex platforms for in vitro measurement of sIgE reactivity to allergenic molecules.

Increase accuracy and resolve cross-reactivity

•One of the most important implications of molecular-based allergy (MA) diagnosis is its ability to distinguish genuine sensitization from sensitization due to cross-reactivity.

•This information helps the clinician to determine whether a single, a few closely related, or several widely different allergen sources need to be considered.

Allergic individuals may produce IgE antibodies to allergens that are either unique to a single species or common to many. Thus, the individual may show a genuine sensitivity to a given allergen or may show sensitivity to many unrelated species as a consequence of immunological cross-reactivity to structurally related allergens. In general, the closer the taxonomical relationship between species, the higher the degree of structural and immunological similarity between the allergens.

Specific allergens are markers for their respective allergen sources, allowing identification of the primary sensitizer. One of the most important clinical uses of MA is its ability to identify the offending allergenic molecule and to distinguish specific molecules from markers of cross-reactivity. Thus, the probability of a clinical reaction on exposure to different allergen sources may be defined, in some cases, by the pattern of sensitization to different allergens.

In the field of pollen-related food allergy, MA has demonstrated its ability to play an important role by increasing the accuracy of diagnosis. As an example of this, in peanut allergic patients, sensitization to Ara h 2 is considered a genuine marker for peanut that may induce systemic reactions, while Ara h 8 is a marker for cross-reactivity among food allergens and Fagales tree pollen and is mainly associated with mild, oral reactions. Therefore, measuring IgE responses to certain food allergens may reduce the need for food challenges. In patients sensitized to different pollen species, MA diagnostics is able to improve the resolution of a conventional diagnostics obtained by skin tests in a substantial number of cases, either by detecting new relevant sensitizations or by ruling out clinically irrelevant sensitizations caused by non-symptomatic cross-reactive allergens. For example, MA diagnostics can help to distinguish baker's asthma from pollen or wheat allergy.

When testing a limited panel of molecules, only what is measured can be detected; in other words, when using Phleum pratense positive SPT or sIgE test, Phl p 1 and Phl p5 will be able to define genuine sensitization, while Phl p 7 and Phl p 12 will identify sIgE for polcalcins and profilins, respectively. The presence of other molecules such as Phl p 2 and Phl p 4 could improve the accuracy of the diagnosis. If all of these molecules are studied, a fairly representative IgE profile for P. pratense would be obtained; if only one or a few molecules are evaluated, the characterization of the IgE profile would be less accurate. Thus, the descriptive quality of the sIgE profile will be based on the choice of tests prescribed by the clinician.

Nevertheless, it must be kept in mind that all allergy diagnostics, including MA, should be evaluated within the framework of a patient's clinical history, because IgE sensitization towards a given allergen does not necessarily imply clinical responsiveness. This is of particular importance, since allergic patients respond in an

individualized manner to exposure to allergens from various sources, i.e., every individual produces their own unique IgE antibody profile at the molecular level.

Assess the risk and type of reaction

•Molecular-based allergy (MA) diagnostics have emerged into routine care due to its ability to improve risk assessment, particularly for food allergies.

•Different foods contain unique allergenic molecules that are stable or labile to heat and digestion. The stability of a molecule and a patient's clinical history help the clinician evaluate the risk of systemic versus local reactions. Labile allergens are linked to local reactions (typically oral symptoms) and cooked food is often tolerated, whereas stable allergens tend to be associated with systemic reactions in addition to local reactions.

•MA diagnostics may decrease the need for provocation testing and improve recommendations for allergen avoidance.

Risk assessment of allergic individuals is one potential application of MA diagnostics. Since patient sensitization profiles may differ with regard to disease expression and severity, detecting "low-risk" versus "high-risk" molecules is an area of major interest that could reduce the use of potentially harmful diagnostic procedures such as challenge tests. Such knowledge may also improve allergy management recommendations to patients (e.g., exposure reduction). This has been shown with the use of MA diagnostics in food, venom, respiratory and latex allergy. In addition, the sensitization profile of a patient may impact overall symptomatology, as poly-sensitization to several different allergens from a single allergen source may increase symptom severity.

Nevertheless, it must be noted that information may only be applicable to the specific population which has been studied, since it is known that both food and inhalant sensitization profiles and disease expression differ according to local exposures patterns characteristic of the geographical region.

Food allergens

Generally, allergens resistant to heat and digestion often trigger more severe allergic reactions (i.e., anaphylaxis) compared to labile allergens, the latter which typically induce local symptoms such as oral allergy syndrome (OAS) (Table 1). In addition, the amount of a molecule present in a food source is also a parameter to take into consideration. The following text provides a few examples of how IgE sensitization to different allergens from a food allergen source can result in clinically unique reactions.

High- versus low-risk molecules from foods giving rise to anaphylaxis

Peanut

Allergen sensitization profiles in peanut-allergic individuals have been extensively studied. IgE antibodies against storage proteins such as Ara h 1, 2, and 3 have been associated with genuine peanut reactions; in contrast, isolated sensitization to Ara h 8 (PR-10 protein and Bet v 1- homologue) is a marker of milder or local symptoms. Finally, patients with profilin or CCD sensitization to peanut alone usually react with no or local oral symptoms, and heated peanuts may be tolerated.

Soy

Sensitization to Gly m 5 and/or Gly m 6 has been associated with severe reactions in allergic patients. Patients with profilin or CCD sensitization to soy alone usually exhibit no, or local oral symptoms, and heated soy may be tolerated.

Hazelnut

While sensitization to Cor a 1 (PR-10) is associated with local reactions like OAS, Cor a 8 (LTP) and storage proteins (e.g. Cor a 9 and Cor a 14) are more frequently recognized by IgE antibodies from patients with severe symptoms. Patients with profilin (Cor a 2) or CCD sensitization to hazelnut alone usually exhibit no or local oral symptoms and heated hazelnuts may be tolerated.

Walnut

Severe reactions in walnut-allergic patients are associated with storage protein (Jug r 1, Jug r 2) or LTP (Jug r 3) sensitization. Walnut allergens have not been available on the market until recently, as is reflected by the lack of recent clinical studies. Patients with profilin or CCD sensitization to walnut alone usually exhibit no, or local oral, symptoms and heated walnut may be tolerated.

Wheat

Sensitization to ω-5-gliadin (Tri a 19) is a risk factor for immediate allergic reactions in children and for systemic exercise-induced reactions in adults. The wheat LTP (Tri a 14) shows some degree of cross-reactivity with other food LTPs, however more knowledge is needed about its prevalence and clinical implication. Patients with profilin or CCD sensitization to wheat alone usually exhibit no, or local oral, symptoms and heated wheat may be tolerated.

Rosaceae fruits

Apple, peach, and other stone fruits are members of the Rosaceae family. In patients allergic to these fruits, particularly to allergens such as PR-10 proteins (Mal d 1, Pru p 1) or profilins (Pru p 4), local, oral reactions are more frequent, since these protein families are sensitive to heat and digestion. In contrast, sensitization to LTP (Pru p 3), typical of the Mediterranean area, is associated with a wide range of clinical expressions (from asymptomatic to anaphylaxis), and is generally considered a risk marker for severe reactions including co-factor (e.g., exercise, alcohol or drugs) dependent anaphylaxis.

Egg

High levels of sIgE antibodies to ovomucoid (Gal d 1) have been identified as a risk factor for persistent egg allergy, including reactions to cooked/heated egg, while undetectable levels indicate tolerance to cooked egg.

Milk

Casein (Bos d 8) and beta-lactoglobulin (Bos d 5) sIgE antibodies are markers of persistent allergy to milk, including heated milk, in milk allergic patients while undetectable levels indicate tolerance to baked milk products.

Fish

Parvalbumins (e.g., Gad c1 and Cyp c 1) are the major allergens in fish and are typically stable to heat and digestion. Parvalbumins show a high degree of cross-reactivity whereby patients sensitized to one parvalbumin may also react to parvalbumins from other fish, including carp, cod, herring, plaice, mackerel, tuna, salmon, perch, and eel.

Shellfish

Allergic reactions to crustaceans may be caused by tropomyosin, which shows high degree of cross-reactivity across a wide variety of species, including mites. Shrimp and other shellfish also contain other clinically relevant allergens, like sarcoplasmic calcium-binding protein and arginine kinases.

Meat allergy

Galactose-α-1,3-galactose (α-Gal) is a sugar structure found on glycoproteins and glycolipids of non-primate mammals and new world monkeys, but not on humans. IgE-antibodies specific for α-Gal (anti-α-Gal-IgE) may be associated with severe allergic symptoms and with delayed-type anaphylaxis. α-Gal is also present on cat IgA which does not show high allergenic activity, and on gelatine containing material. It is assumed that sensitization to α-Gal can be induced by tick bites or certain parasite infections. Clinically, α-Gal–sensitized patients may experience delayed immediate type reactions to red meat (beef, pork, goat, deer) anaphylaxis.

α-Gal is also present on the chimeric antibody cetuximab (cancer drug), and patients sensitized to α-Gal may react with anaphylactic reaction after the administration of cetuximab. Testing for α-Gal before administration of cetuximab should therefore be considered.

Bovine serum albumin (e.g. Bos d 6) is a heat labile allergen present both in milk and beef, which may cause cross-reactivity between different mammalian meat.

Inhalants

Pet dander

Higher levels of sIgE antibodies against Fel d 1 are associated with asthma in cat-allergic individuals. Recognition of more than three animal-derived allergens such as lipocalins (Mus m 1, Equ c 1, Fel d 4, Can f 1, 2), kallikrein (Can f 5), and secretoglobin (Fel d 1) has been associated with severe asthma in Swedish children. More knowledge is needed in the area of pet allergy where many of the patients are poly-sensitized to several pets and the clinical history is often inconclusive, in addition the cross-reactivities between e.g. cat, dog and horse is not fully clarified at the MA level.

Pollen

Research in pollen allergy has focused on distinguishing genuine allergens from those that are cross-reactive, however, little is known regarding specific markers of severe reactions. Nevertheless, some sensitivities to specific allergens may be markers of more severe symptoms in pollen allergy, increasing the risk of systemic reactions during immunotherapy, such as Ole e 9 and the pollen LTP Ole e 7.

Profilin sensitization is common among pollen allergic patients and it is usually associated with mild or no clinical symptoms. However, for a minority of patients, profilin may be a risk factor for more severe reactions in olive pollen-allergic individuals and in patients allergic to certain plant foods like melon or citrus.

Mites

Although no specific sensitization profile has been described as a risk factor for lower airway disease or disease severity, a higher sIgE/IgG4 ratio for Der p 2 has been associated with asthma. Der p 10 (tropomyosin) is a minor allergen in mite-allergic patients, however it may still indicate a risk for allergic reactions to shellfish or snail, which can be severe.

Molds

In hypersensitivity reactions to Aspergillus fumigatus, the presence of IgE antibody reactivity to Asp f 2, 4, and 6 may suggest allergic bronchopulmonary aspergillosis (ABPA), whereas sensitization to Asp f 1 and/or Asp f 3 may be more indicative of allergic asthma. These associations must still be confirmed in other patient populations.

Cockroach

It was recently described that sensitization to Per a 2 correlates with severity of airway allergy in cockroach-allergic patients. Per a 2 is currently not commercially available for in vitro testing; however, the Per a 2 homologue Bla g 2 is available. Cockroaches also contain cross-reactive tropomyosin (Bla g 7), which indicates a risk for allergic reactions to shellfish or snail, which can be severe.

Other allergens

Latex

Sensitization to Hev b 8 (profilin) seems to be clinically irrelevant and not related to clinical latex reactions. The other latex allergens are linked to clinical reactions; however, no association between allergens and severity of reactions has been identified so far. The cross-reactive allergen responsible for the so called latex-fruit syndrome are not fully clarified, although data indicate that Hev b 5, 6 and 11 play a role.

Hymenoptera venoms

Most hymenoptera venom allergens possess CCDs that are responsible for a portion of clinically irrelevant IgE antibody cross-reactivity between bee and wasp venom. Detection of recombinant venom allergens can discriminate between genuine venom sensitization and cross-reactivity due to CCDs in patients with double-positive IgE results from traditional venom tests that are based on allergen extract.

Therapy of allegic diseases :

Allergy treatments include:

Allergen avoidance. Your doctor will help you take steps to identify and avoid your allergy triggers. This is generally the most important step in preventing allergic reactions and reducing symptoms.

Medications to reduce symptoms. Allergy medications can help reduce your immune system reaction and ease symptoms. The drugs you use depend on the type of allergy you have. They can include over-the-counter or prescription medications in the form of oral medications, nasal sprays or eyedrops. Some common allergy medications include corticosteroids, antihistamines, decongestants, cromolyn sodium and leukotriene modifiers.

Immunotherapy. For severe allergies or allergies not completely relieved by other treatment, your doctor may recommend allergy shots (immunotherapy). This treatment involves a series of injections of purified allergen extracts, usually given over a period of a few years.

Emergency epinephrine. If you have a severe allergy, your doctor may give you an emergency epinephrine shot to carry with you at all times. Given for severe allergic reactions, an epinephrine shot (EpiPen, EpiPen Jr, Twinject) can reduce symptoms until you get emergency treatment.

Specific immunotherapy

•Molecular-based allergy (MA) diagnostics represents a useful tool to distinguish genuine sensitisations from cross-reactions in poly-sensitized patients, when traditional diagnostic tests and clinical history are unable to identify the relevant allergen(s) for specific immunotherapy (SIT).
•Given that SIT is an expensive treatment typically used over longer periods of time (3 to 5 years), correct diagnosis, selection of truly eligible patients, and identification of primary sensitizing allergen(s) are important for optimal and cost-effective patient management.
Specific immunotherapy (SIT) involves the administration, either subcutaneously or sublingually, of an extract of the allergen responsible for clinical symptoms to induce tolerance and reduce reactivity (i.e., symptoms) to the allergen itself . This is achieved through complex immune modifications that involve both humoral and cell-mediated immunity. As a paradigm, allergen immunotherapy is "specific", meaning that it only modifies the immune response against the allergen for which the vaccination is being performed. As a consequence, a precise etiological diagnosis is required for the prescription of SIT, whereby the allergen responsible for clinical symptoms must be unequivocally identified. In some patients, a detailed clinical history and traditional extract-based IgE testing (SPT and/or in vitro sIgE) is sufficient to identify the relevant allergen(s). This is especially true in the case of allergy to plants with a well-defined pollen season, which does not overlap significantly with that of other plants or other allergen sources.
However, the complexity of diagnosis increases when the patient demonstrates poly-sensitization by traditional diagnostic tests based on allergen extracts and their clinical history is not sufficient to clarify the nature of the sensitization. This may occur in a relatively high proportion of patients. In the United States, for instance, such cases would involve preparing a vaccine for SIT by mixing together all of the allergens that a patient tests positive for. Mixing numerous allergens appears to achieve good clinical efficacy; however, there may be an inability to identify the responsible allergen in the case of adverse events.
It is well recognized that, in many cases, multiple positive results obtained with allergen extracts (i.e., SPT and/or in vitro sIgE) are due to the presence of cross-reactive allergens in the diagnostic extracts. Certain proteins (e.g., profilins, polcalcins, LTPs, PR10, tropomyosins) are highly conserved in a wide variety of species. For instance, a patient who is primarily sensitized to grasses may also test positive for birch with SPT. This cross-reactivity occurs because the birch extract used in SPT contains profilin (e.g., Bet v 2), which are largely similar to those in grasses (e.g., Phl p 12). Indeed, the use of recombinant/purified allergens would allow for the discrimination between genuine sensitizations and cross-reactivities. In the example mentioned above a patient with sIgE antibodies against Phl p 1 and Phl p 5 but no sIgE to Bet v 1 is truly sensitized to grass. If sIgE antibodies to Phl p 12 (profilin) were also detected, profilin sensitization would probably be responsible for the positive SPT result obtained with birch extract, which contains profilin as well. Thus, using knowledge gained through the identification of allergens, SIT would be prescribed for grass only. Similarly, if a patient is sensitized to a traditional house dust mite extract, but their IgE antibodies are specifically directed against Der p 10 (tropomyosin) and not to Der p 1, 2/ Der f 1, 2,

SIT for mites should not be given, because mite extracts mainly contain Der p 1, 2/Der f 1, 2 and have variable or low amounts of Der p 10. Molecular diagnostics can also improve the selection of patients for hymenoptera venom SIT. Sensitization to the major allergens Api m 1 of honeybee and Ves v 5 and/or Ves v 1 of yellow jackets may be helpful in discriminating between true double bee and wasp sensitization and cross-reactivity due to CCDs.

In addition, most commercial allergen extracts used in SIT are well standardized for major allergens, but contain only minimal or variable amounts of minor allergens. Thus, patients with sensitization to minor allergens alone will likely not receive sufficient amounts of allergen to achieve a successful outcome by SIT. A recent study reported that patients receiving a 2-year course of SIT with either birch or grass pollen had a much more favourable outcome with SIT when sensitization to the marker allergens of birch or grass pollen were detected compared to patients sensitized to only minor, cross-reactive allergens.

In poly-sensitized patients, the most relevant sensitizing allergens for which SIT should be prescribed can be more clearly identified with MA diagnostics. A recent study reported that the use of MA diagnostics modified the prescription of SIT compared to SPT in more than 50% of patients, suggesting that poly-sensitized patients are at risk of incorrect SIT prescription.

Theoretically, a detailed identification of molecules to which IgE antibodies are directed against would allow for tailored SIT based only on allergens with a documented IgE response for each patient. In practice, this does not seem feasible. First, the number of possible combination of sensitization profiles is large when taking into consideration all allergenic sources; second, recombinant vaccines do not perform better than traditional allergen extracts, as observed in some studies; and third, each single recombinant/purified allergen would need to be individually tested and registered, which carries a substantial financial burden for manufacturers. Thus, the reality of patient-tailored SIT is still a distant prospect.

Patients most likely to benefit from molecular-based allergy diagnostics

•Molecular-based allergy (MA) diagnosis is most useful for selection of SIT, evaluation of cross-reactivity, and assessment of severity of reaction associated with various allergens.

•Patients who are poly-sensitized, have an unclear symptom and/or sensitization pattern, or who do not respond to their treatment may be routinely evaluated using MA diagnostics when available.

•Mono-sensitized patients with a clear case history and symptom profile may not benefit from MA diagnostics compared to traditional diagnostic tests.

MA diagnostics offers several advantages useful for the examination of allergic patients with symptoms like e.g., asthma, rhinitis, eczema, urticaria, gastrointestinal, oral allergy syndrome or anaphylaxis. Identification of genuine sensitization is as important as the identification of secondary sensitizations caused by cross-reacting allergens.

MA diagnostics, based on either the physician’s choice of single allergens or the use of a microarray, offers a large amount of information pertaining to the IgE profile of sensitized patients. This information is mainly useful for three purposes. First, MA

diagnostics is helpful in the identification of a genuine sensitization to an allergen source, particularly when SIT is being considered. MA diagnostics is often essential to the accuracy of prescribed SIT for a large proportion of allergic patients. Second, MA diagnostics can detect sensitization to certain cross-reacting protein families of allergens, thereby contributing to identify the triggering allergen source and to improve the recommendations made to patients regarding exposure avoidance. Finally, MA diagnostics helps to assess the risk associated with certain allergens (i.e., type of reaction, local or systemic). For example, sensitization to LTP or storage proteins may cause severe, systemic reactions in allergic patients while profilin, CCD and PR-10 proteins generally are associated with mild, local reactions in food allergy.

From among those potentially eligible for MA diagnostics, different patient categories can be defined. In most patients, MA diagnostics may be considered a useful and interesting, but not essential, tool, particularly when only symptomatic treatment is prescribed. Mono-sensitized patients (e.g., to pet or mite allergens) and patients with a clear case history and symptom profile do not generally seem to derive benefit from MA diagnostics compared to traditional diagnostic tests.

Previously, patients who were sensitized to one or two allergen sources were the most prevalent patient type in clinical practice; currently, they are becoming a minority, mainly in developed countries. In fact, poly-sensitized pediatric and adult patients with complex symptoms, as well as patients in whom sensitization to cross-reacting allergens is suspected, should be carefully considered for MA evaluation. Within this population, patients with documented poly-sensitization to one or more inhalants, but also suffering from food allergy (i.e., from less severe manifestations such as OAS to more severe, including anaphylaxis, asthma or eczema) should be routinely considered for evaluation using MA diagnostics. In addition, MA diagnostics may offer additional information for early diagnosis of allergies and may aid in the monitoring of the evolution of the allergic disease, useful for preventive indications to the patient.

In conclusion, current guidelines of allergy diagnosis should recommend a thorough clinical investigation as a first-line approach, followed by allergen extract testing using in vitro sIgE or SPT tests as a second-line approach, and as a third step MA diagnostics. For experienced users MA may be included in second-line testing.

Unmet needs

•Molecular-based allergy (MA) diagnostics enhances the clinical utility of specific IgE (sIgE) antibody-based allergy diagnostics nevertheless; a number of unmet needs have yet to be addressed.

Molecular analysis of allergen sensitization patterns can enhance the clinical utility of allergy tests based on extracts. In selected cases it may also reduce the need for challenge testing for food allergies and may also improve the selection of SIT prescription. However, there are a number of unmet needs pertaining to MA diagnostics:

1) Large-scale, population-based multicenter studies are needed to further define in which categories of patients MA diagnostics may be beneficial.

2) The practical use and selection of allergens in MA diagnostics need to be evaluated in large studies that include well-characterized patients and healthy, sensitized controls representative of different geographical regions.

3) Evaluation of the incremental benefits relative to the incremental costs for MA diagnostics, by way of cost-utility studies, is needed. These studies should compare the effectiveness of MA diagnostics with the traditional in vitro sIgE or SPT techniques that are currently available.
4) Identification and clinical evaluation of the most relevant allergens have to be further investigated in many allergen sources.(e.g., nuts, molds, tree and weed pollen).
5) Training efforts in both the clinical and research settings is warranted, with a focus on developing this new "molecular" era in allergology.
6) Development of clinical decision support is needed to prevent misinterpretation and improve knowledge as the amount of information obtained from MA diagnostics may be complex, especially as the evidence for MA is rapidly progressing.
There are additional needs in the field of allergy diagnostics including traditional tests based on extracts. Currently there is one published cost effectiveness analysis on food allergy diagnostics . In this guideline document, economic evidence shows that both IgE antibody testing and skin prick testing are cost effective compared to clinical anamneses without testing. Since MA diagnostics increase the accuracy in selected food allergies (e.g. peanut allergy) and selection of SIT prescription compared to traditional tests based on extracts, the cost effectiveness should logically increase for these particular scenarios. However, the fact that only one cost effectiveness analysis in food allergy is available underlines the need for more cost effectiveness analysis in allergy diagnostics.
There is also a need for characterization and standardization of allergen concentrations in allergen extracts that are used in diagnostic testing and treatment.
Summary and conclusions
•International guidelines recommend a thorough clinical case history as a first-line approach and allergen extract-based IgE tests (in vitro specific IgE or skin prick test) as a second-line investigation.
•Molecular-based allergy (MA) diagnostics is considered a third-line approach to be used for patients in whom first- and second-line investigations were inconclusive. For experienced users MA may be included in second-line testing.
•MA diagnosis is a new and complex procedure that, in the near future, will represent a standard tool in the allergist's armamentarium. Educational programs on MA diagnostics for allergists are needed.
MA diagnostics was developed more than a decade ago. The recent availability of a greater number of allergens has substantially modified the diagnostic approach used by many allergists. Currently, international guidelines recommend a thorough clinical case history as a first-line approach and allergen extract-based IgE tests (in vitro specific IgE or skin prick test) as a second-line investigation for the identification of the allergen source responsible for a patient's symptoms. SPT and in vitro sIgE tests provide similar information and the associated advantages and disadvantages of both types of tests are dependent on the clinical case. For the majority of patients, first- and second-line investigation is sufficient to define the nature of a patient's allergy. Molecular-based allergy (MA) diagnostics is considered a third-line approach to be used for select patients in whom first- and second-line investigations were inconclusive. For experienced users MA may be included in second-line testing.

Traditional diagnostic tests have been considered sufficient for the identification of the best SIT prescription in the majority of patients. With the identification of specific and cross-reacting allergens, a number of new diagnostic and therapeutic options are available to allergists, including the ability to choose the allergen composition for SIT. MA diagnostics is relatively expensive compared with traditional tests, especially with regard to the microarray technology. Economic consideration or budget limitations may influence the decision in the individual patient, whether using a singleplex or multiplex approach. The number of allergens to be tested may influence this decision, both for economical reason, amount of information gained and for the overall serum volume required (especially in young children).

When making the choice to use the microarray diagnostics, it is important to consider the primary advantage which is that with a small serum or blood sample, a broad spectrum analysis of a patient's IgE profile can be performed. However, a disadvantage is that patients may be at risk of revealing unanticipated sensitivities, possibly to potentially harmful molecules. Although this could also be considered as an advantage, the interpretation of such sensitization in clinically unresponsive patients is difficult or even impossible.

Some allergy symptoms improve with home treatment.

Sinus congestion and hay fever symptoms. These symptoms often improve with saline nasal irrigation — rinsing out the sinuses with a salt and water solution. You can use a neti pot or a specially designed squeeze bottle to flush out thickened mucus and irritants from your nose. Use water that's distilled, sterile, previously boiled and cooled, or filtered using a filter with an absolute pore size of 1 micron or smaller to make up the irrigation solution. Prepare the saline solution with the appropriate water, using the mixture supplied by the neti pot or one you prepared yourself. Be sure to rinse the irrigation device after each use with similarly distilled, sterile, previously boiled and cooled, or filtered water and leave open to air dry.

Household airborne allergy symptoms. Symptoms such as those caused by dust mites or pet dander, may improve by taking steps to reduce your exposure to allergens. Steps include frequently washing bedding and stuffed toys in hot water, maintaining low humidity, regularly using a vacuum with a fine filter such as a high-efficiency particulate air (HEPA) filter, and replacing carpeting with hard flooring.

Mold allergy symptoms. These may be alleviated by reducing moisture in damp areas, such as your bath and kitchen, by using ventilation fans and dehumidifiers, and fixing any leaks inside and outside of your home.

Alternative medicine

The following herbs may be of some benefit in treating allergies:

Butterbur may help relieve symptoms of allergic rhinitis.

Milk thistle may improve symptoms of allergic rhinitis.

Phleum pratense may help relieve symptoms associated with asthma.

If you're interested in adding alternative therapies to your treatment plan, always talk with your doctor first. He or she can help you understand which therapies are most likely to help in your case. Your doctor can also provide information about potential health risks and drug interactions.

Allergy prevention

Preventing allergic reactions depends on the type of allergy you have. General measures include the following:

Avoid known triggers. Even if you get treatment for your allergy symptoms, you still need to try and avoid your triggers. Common triggers include airborne allergens outdoors, at home or at work, and certain foods, insects or medications. Some allergic reactions are triggered or worsened by temperature extremes or emotional stress.

Keep a diary. When trying to identify exactly what causes or worsens your allergic symptoms, try to track all of your activities, note when symptoms occur, and write down what seems to help. This may help you and your doctor identify triggers and the best steps to prevent and treat them.

Wear a medical alert bracelet. If you've ever had a severe allergic reaction. A medical alert bracelet (or necklace) lets others know that you have a serious allergy in case you have a reaction and you're unable to communicate.

Final part Test-control
Initial knowledge level

1. Which category of hypersensitivity BEST describes hemolytic disease of the newborn caused by Rh incompatibility?

a. atopic or anaphylactic
b. cytotoxic
c. immune complex
d.delayed
e. correct answer - absent

2. The principal difference between cytotoxic (type II) and immune complex (type III) hypersensitivity is
a. the class (isotype) of antibody.
b. the site where antigen-antibody complexes are formed.
c. the participation of complement.
d. the participation of T cells
e. correct answer - absent

3. A child stung by a bee experiences respiratory distress within minutes and lapses into unconsciousness. This reaction is probably mediated by
a. IgE antibody.
b. IgG antibody.
c. sensitized T cells.
d. complement.
e. IgM antibody.

4. A patient with rheumatic fever develops a sore throat from which beta-hemolytic streptococci are cultured. The patient is started on treatment with penicillin, and the sore throat resolves within several days. However, 7 days after initiation of penicillin therapy the patient develops a fever of 103°F, a generalized rash, and proteinuria. This MOST probably resulted from
a. recurrence of the rheumatic fever.
b. a different infectious disease.
c. an IgE response to penicillin.
d. an IgG-IgM response to penicillin.
e. a delayed hypersensitivity reaction to penicillin.

5. A kidney biopsy specimen taken from a patient with acute glomerulonephritis and stained with fluorescein-conjugated anti-human IgG antibody would probably show
a. no fluorescence.
b. uniform fluorescence of the glomerular basement membrane.
c. patchy, irregular fluorescence of the glomerular basement membrane.
d. fluorescent B cells.
e. fluorescent macrophages.

6. A patient with severe asthma gets no relief from antihistamines. The symptoms are MOST likely to be caused by
a. interleukin-2.
b. slow-reacting substance A (leukotrienes).
c. serotonin.
d. bradykinin.
e. correct answer - absent

7. Hypersensitivity to penicillin and hypersensitivity to poison oak are both
a. mediated by IgE antibody.
b. mediated by IgG and IgM antibody.
c. initiated by haptens.
d. initiated by Th-2 cells.
e. correct answer - absent

8. A recipient of a 2-haplotype MHC-matched kidney from a relative still needs immunosuppression to prevent graft rejection because
a. graft-versus-host disease is a problem.
b. minor histocompatibility antigens will not be matched.
c. minor histocompatibility antigens will not be matched.
d. complement components will not be matched.
e. correct answer - absent

9. Which major problem does the bone marrow transplantation in immunocompromised patients present?
a. potentially lethal graft-versus-host disease
b. high risk of T cell leukemia
c. inability to use a live donor
d. delayed hypersensitivity
e. correct answer - absent

10. What is the role of class II MHC proteins on donor cells in graft rejection?
a. They are the receptors for interleukin-2, which is produced by macrophages when they attack the donor cells.
b. They are recognized by helper T cells, which then activate cytotoxic T cells to kill the donor cells.
c. They induce the production of blocking antibodies that protect the graft.
d. They induce IgE which mediates graft rejection
e. correct answer – absent

11. Grafts between genetically identical individuals (i.e., identical twins)
a. are rejected slowly as a result of minor histocompatibility antigens.
b. are subject to hyperacute rejection.
c. are not rejected, even without immunosuppression.
d. are not rejected if a kidney is grafted, but skin grafts are rejected.

e. correct answer- absent

12. A large variety of urticaria variants exist in the form of:
   a. urticaria multiforme
   b. neutrophilic urticaria
   c. cholinergic urticaria
   d. cold urticaria
   e. all of the above

13. Urticaria may be confused with a variety of other dermatologic diseases that could be similar in appearance, including:
a. insect bites
b. erythema multiforme
c. pityriasis rosea
d. a,b,c – correct
e. a,b – correct

14. Respiratory symptoms of anaphylaxy include all EXCEPT:
   a. dyspnea
   b. hoarseness,
   c. urticaria
   d. shortness of breath
   e. cough

15. The primary gastrointestinal symptoms by anaphylaxy include:
a. dysphagia
b. nausea
c. vomiting
d. diarrhea
e. all of rhe above

Final knowledge level

1. Respiratory findings by anaphylaxis include:
a. severe angioedema of the tongue and lips
b. tachypnea
c. stridor air hunger
d. loss of voice
e. all of the above
2. A large variety of urticaria variants exist in the form of:
a. Muckle-Wells syndrome
b. mastocytosis
c. cholinergic urticaria
d. cold urticaria
e. all of the above

3. A youth, aged 15, from childhood suffers from atopic dermatitis and allergy to the shell fish. In the last 3 months after acquiring aquarium fish rhinitis, conjunctivitis, itching in the nose developed. Level of what immunologic index should be defined in this case?
a. IgG
b. Circulating immunocomplexes
c. IgM
d. IgE
e. IgA

4. A 15 y.o. boy was twice attacked by bees, as a result he had severe anaphylactic shock. What is the most effective preventive method?
a. Long-term prophylactic treatment with antihistamines
b. Desensibilisation by means of bee venom extract
c. Limitation of outside staying during summer months
d. Prescription of corticosteroids for summer
e. Protective clothing

5. A 32 y.o. woman has got the Laiel's syndrome after taking the biceptol. What immunotrope medicines are to be prescribed in this situation?
a. Non-steroid immunosupressants
b. Interferons
c. Specific immune modulators
d. Steroid immunosupressants
e. Non-specific immune modulators

6. In the development of the inflammation processes glucocorticoids reduce the level of a certain most important active enzyme. It results also in the reducing of the synthesis of prostaglandins and leukotrienes which has a key-role in the development of the inflammation processes. Give the exact term of this enzyme.

a. Phospholipase A2
b. Arachidonic acid
c. Lipoxygenase
d. cyclooxygenase – 1
e. Cyclooxygenase – 2

7. A 6-year old asthmatic child is brought to the emergency room because of severe coughing and wheezing during the prior 24 h. The child had been taking theophylline without relief. Physical examination reveals a child who is anxious, has intercostal and suprasternal retractions, expiratory wheezing throughout all lung fields, and a respiratory rate of 60 breaths per minute. Initial treatment may include the administration of

a. Subcutaneous epinephrine
b. Parenteral phenobarbital
c. Intravenous fluids in the first 2 h to correct a water deficiency.
d. N-acetyl cysteine and cromolyn by inhaler
e. Parenteral gentamicyn

8. A 2 year old boy has been vomiting intermittently for 3 weeks and has been irritable, listless, and anorectic. His use of language has regressed to speaking single words. In your evaluation of this patient, the LEAST likely, diagnosis to consider is:

a. Food allergy
b. Lead poisoning
c. Tuberculous meningitis
d. Brain tumor
e. Subdural hematoma

9. A college student sitting in the stands at a football game suddenly begins breathing hard and complains to his friends of tightness in his chest. Minutes later, he is sweating profusely and faints. It is discovered that he had been stung by a bee. Paramedics arrive, assess the situation, then successfully treat the young man. Which one of the following drugs was most likely initially administered in this case?

a. Diphenhydramine
b. Blocking antibody
c. Cromolyn sodium
d. Epinephrine
e. Theophylline

10. Which of the following class II antigens would be most likely to play a contributing role in hay fever?

a. DR2
b. DR3
c. DR4
d. DR5
e. DR7

11. Which of the following could an allergen prevent from reacting with a specific IgE molecule present on the mast cell membrane?

a. Antihistamine
b. Blocking antibody
c. Cromolyn sodium
d. Epinephrine
e. Theophylline

12. A 24-year-old man presents with complaints of itching on his arms and face. Physical examination reveals well-circumscribed wheals with raised, erythematous borders and blanched centers. Which form of hypersensitivity is this patient probably exhibiting?

a. Acute serum sickness (Type III)
b. Antibody-dependent cell-mediated cytotoxicity (Type II)
c. Anti-receptor antibodies (Type II)
d. Delayed type hypersensitivity (Type IV)
e. Immediate type hypersensitivity (Type I)

13. A 26-year-old systems analyst presents for evaluation of a bee sting allergy. He describes an episode in which he was stung on the forearm by a bee and, within 5 minutes, experienced pruritus, urticaria, and mild wheezing. The effector cell in this type of hypersensitivity is a(n)

a. eosinophil
b. mast cell
c. megakaryocyte
d. neutrophil
e. TH1 CD4+ lymphocyte

14. A 27-year-old woman presents to the emergency department complaining of wheezing and swelling of the lips. The symptoms began acutely just after eating a chicken and nut dish at a nearby Chinese restaurant. She also complains of tightness in her chest and difficulty breathing. On examination, she has a temperature of 36.8 C, a heart rate of 110/min, a blood pressure of 90/50 mm Hg, and a respiratory rate of 24/min. She is anxious and in mild respiratory distress. There is diffuse facial erythema, with swelling of the lips and tongue. Wheezing is noted bilaterally, and oxygen saturation is 92%. Which of the following cytokines is necessary for production of the antibody that mediates this response?

a. Interferon-γ
b. Interleukin 1
c. Interleukin 2
d. Interleukin 3
e. Interleukin 4

15. A 34-year-old woman comes to the office complaining of an itchy rash on her eyelids that has recurred with the spring. She has had similar problems for many years

and has always had trouble controlling it during the spring months. She has no other medical problems and does not take any medications on a regular basis, but has started an over-the-counter diphenhydramine formulation to control the itch. She takes 25 mg at nighttime because otherwise it makes her drowsy and interferes with daily functions. The patient's medical history is remarkable for childhood asthma and seasonal allergies. She has two children, both of whom have moderately severe atopic dermatitis. On physical examination, she is in no acute distress. Her vital signs are within normal limits. Inspection of the skin reveals edema and erythema of the upper and lower eyelids with scant scale and incipient lichenification. The skin folds of the lower eyelids are pronounced. There is also slight conjunctival injection secondary to frequent rubbing. The rest of the physical examination is within normal limits. You propose a new medication named pimecrolimus as the most adequate for thin eyelid skin. Which of the following best describes the mechanism of action this medication?

a. Immune deviation from Th1 to Th2 cytokine secretion pattern
b. Immune suppression through inhibition of T-cell activation
c. Immune suppression through decreasing the number of antigen-presenting cells
d. Immune suppression through T cell apoptosis
e. Vasoconstriction

CORRECT ANSWERS:

Initial knowledge level:

1.A; 2.B; 3.A; 4.D; 5.C; 6.B; 7.C; 8. C; 9.A; 10.B; 11.C; 12.E; 13.D; 14. C; 15.E

Final knowledge level:

1.E; 2.E; 3. D; 4.B; 5.D; 6.A; 7.A; 8.A; 9.D; 10.A; 11.B; 12.E; 13.B; 14.E; 15.B

Structure of class.

Plan and organizational structure of the lesson:

Preparatory stage: organization of lesson and test control of incoming level of knowledge (5 academic hours or 225 minutes)

	Content	Level of learning	Methods of the learning control	Materials of the methodological support	Time, min.
1.	<u>The preparatory phase</u> The formation of professional knowledge, skills and abilities.	III	1. Front rapid survey	Tests. Scheme.	25
2.	<u>The main stage</u> - Know clinic of allergic diseases;		1. Examination the patients	1. Situational tasks	180
	- Methods of diagnosis of allergic diseases; - Methods for the treatment of allergic diseases; - Determine the necessity of guidance to the patient with an allergist, clinical immunologist	III III III	2. Analysis of anamnesis 3. Filling immunological cards 4. Solving the common tasks	2. Maps of immunological observation 4. Maps of directing the patients to consulting by an allergist, immunologist	180
3.	<u>The final stage</u> Monitoring and adjustment of professional knowledge and skills - Differential diagnosis of respiratory allergies, differential diagnosis of allergic dermatitis - Stages of treatment and prevention of allergic diseases	IV III III	1. Solving untypical situation tasks	Tests 2. Untypical situation tasks	15
4.	To sum up the lessons. Homework for the next topic.				2 3

Study questions.

7. Reasons for the development, triggers and genetic basis of allergy
8. Immunological mechanisms and types of injury biostructures
9. Non-allergic conditions, causes and mechanisms of formation
10. Basic principles of diagnosis of allergic diseases
11. Principles of treatment of allergic diseases
12. The main types of allergic diseases: clinical features, diagnosis, treatment approaches

Control questions

1. Role of triggers in the formation of allergic diseases.
2. Mechanisms of atopy.
3. Differential diagnosis of allergic and non-allergic reactions.
4. The sequence of pathoallergic diagnosis.
5. Approaches to the treatment of allergic diseases.
6. Specific immunotherapy mechanism of action, indications and contraindications for its implementation.
7. Allergic rhinitis, conjunctivitis, polinosis: symptoms, diagnosis and treatment.
8. Bronchial asthma: etiology, clinical features, stepwise approach to treatment.
9. Diagnosis and treatment of insect and food allergy.
10. Diagnosis and treatment of dryg alleegy.

Practical skills:

1. Be able to collect allergic anamnesis and diagnose allergic rhinitis, atopic and allergic dermatitis.
2. Know instrumental and laboratory methods for diagnosis of allergic diseases.
3. Master the modern principles of diagnosis and treatment of bronchial asthma.
4. To be able to evaluate the specific skin tests.
5. Know and be able to carry out specific immunotherapy.

The conclusions.

1. To be able to clarify modern knowledge about the mechanisms of immunological reactions of biostructures damage, genetic and environmental bases of allergic diseases.
2. To form the basic principles of clinical and laboratory and instrumental diagnostics of allergic diseases.
3. To determine the basic methods of laboratory diagnosis of allergic diseases (the role of specific IgE and its connection with specific immunotherapy)
4. To determine the basic group of antihistamines and approaches to the treatment of allergic diseases.
5. To know new moecular-based allergy diagnostics, that is allowed for improved management of allergic diseases.

References:

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5. Lecture Notes: Immunology, 6th Edition. Ian Todd, Gavin Spickett. Wiley-Blackwell (2011). – 480 p.
6. Essentials of Clinical Immunology, 6th Edition. by Helen Chapel, Mansel Haeney, Siraj Misbah, Neil Snowden. Wiley-Blackwell (2014). – 376 p.
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METHODICAL INSTRUCTION

Practical class №8

1. **THEME. ACUTE ALLERGIC DISEASES** (5 academic hours).

2. Actuality of the topic:

In connection with widespread allergic diseases and rising allergization of population, students need to obtain advanced knowledge of the reasons of their development, immunopathogenesis, clinics, diagnosis and therapy.

3. **Aim:**
   - *academic(study):* Students have to study the reasons and genetic basis of development of allergic diseases, immunological mechanisms and types of injury biostructures, body;
   - *professionally focused*: Students have to know methods of diagnosis, clinical features and approaches in treatment of basic allergic diseases, syndromes, reactions, be able to make a plan of examination and determine the necessity of referral to an allergist;
   - *educational*: form students' understanding of the impact of environmental, social and psychogenic factors on the development of allergic diseases and the necessity of early diagnosis of allergic diseases.

4. **Equipment for conducting:** short-notes information, diagrams, power points presentation; equipment and reagents for skin test, allergy cards observation, history and outpatients case histories, case studies, tests
5. Integrative ties of the topic:

5.1. *Interdiscipline integration*: The topic of the practical lesson is connected with the topics of the same series of practical lessons "The subject and tasks of clinical immunology and allergology"

5.2. *Out-disciplinary integration*:

Subject	To know	Be able
1	**2**	**3**
Physiology	Know the basic parameters of external respiration	Rate normal levels of external respiration and blood indexes
Pathophysiology	Types of hypersensitivity reactions	Name the types of reactions
Pharmacology	Know the basic groups of anti-histamines, anti-serotonin, β_2-agonists, cholinolytic, mucolytic and anti-inflammatory drugs	Prescribe these drugs
Propaedeutic therapy	Features of the examination of patients with immunopathology	Perform palpation, percussion, auscultation of breath, evaluate the results of

		laboratory and instrumental methods of examination
Dermatology	Diagnosis of allergic skin diseases	Clinically evaluate the prevalence of skin process, the presence of secondary purulent infection
Therapy Emergency department	Clinical picture, differential diagnosis of bronchial asthma, pollinosis, allergic conjunctivitis, rhinitis Diagnosis of acute allergic reactions	Conduct clinical examination, evaluate the results of laboratory and instrumental examinations, prescribe treatment Conduct clinical examination and provide emergency medical care in anaphylactic shock, acute toxic-allergic reactions

6. The content of the topic

Student has to know:

6.1.1. Definition of acute allergic diseases

6.1.2. Classification of acute allergic diseases

6.1.3. Basic principles of allergic diseases

Study questions.

1. Reasons for the development, triggers and genetic basis of acute allergic diseases
2. Immunological mechanisms and types of injury biostructures
3. Basic principles of diagnosis of acute allergic diseases
4. Principles of treatment of acute allergic diseases
5. Prevention of acute allergic diseases

Main part

Allergy, or hypersensitivity, provokes various clinical manifestations, caused by an immune response to one or more environmental antigens and results in tissue inflammation and organ dysfunction. The major clinical events are recurrent or chronic inflammatory disorders of the respiratory mucosa (causing asthma and rhinitis) and skin (causing eczema and urticaria), and on rare occasions an acute systemic disease such as anaphylactic shock (AS).

Urticaria and Angioedema

Urticaria is a skin rash, also called hives, or nettle rash, which is often accompanied by swelling and itching of the skin.

Angioedema (in the past this was called giant urticaria or angioneurotic edema) is a condition involving swelling in the deeper layers of the skin, caused by a build up of fluid leaking from thin-walled blood vessels. It can accompany hives or occur alone.

Symptoms

Hives are itchy and have a central, raised white wheal surrounded by an area of redness. Hives whiten if pressure is applied to the rash. The rash generally disappears within 24 hours.

Swelling of deeper layers of the skin, angioedema, is often seen with hives (click for picture). The redness that accompanies hives isn't seen, but the swelling is very obvious. The swelling generally occurs on the fingers and toes, as well as areas of the head, neck, face, and, in men, the reproductive organs, and is often described as painful or burning.

Classification

Hives and angioedema are described by the length of time that symptoms last. A rash and/or swelling lasting less than six weeks is called **acutehives/angioedema**. Episodes that last more than six weeks are described as **chronic hives/angioedema**. The causes and the body's reactions that lead to development of hives are different in acute and chronic hives/angioedema, and so treatment is also different.

Acute Hives

Acute hives can be divided into two general types, depending on the rate at which hives develop and the length of time the rash lasts. In one type, the rash lasts 1-2 hours; this is usually the type found in physically induced hives (see below). The second type can last as long as 36 hours; this is the type commonly seen in food or drug reactions.

Chronic Hives and Angioedema

Chronic hives and angioedema are diagnosed when hives and swelling are present for more than six weeks (click for picture). Before the diagnosis is made, it is important to make sure that what seems to be a long-lasting attack of hives is not really a series of short attacks occurring close together.

Chronic Idiopathic Hives and Idiopathic Angioedema

This is a common disorder, and the diagnosis of idiopathic hives and angioedema is made when no cause can be found. The skin symptoms may vary from severe to mild or may intermittently subside, and routine blood tests show no obvious abnormalities. Chronic hives does not appear to be a true allergic reaction, because IgE antibody is not involved, and no contact with an allergen is needed to bring on the symptoms.

Causes

Allergic Hives

Acute hives caused by an allergic reaction is a common condition in children and adults. When an allergen (for example, a food or insect sting) to which the person is allergic enters the bloodstream, it starts a series of reactions in the body's immune system. These reactions lead to the release of histamine and other chemicals into the blood and can result in hives and/or other allergic symptoms. Common allergens that can cause acute hives include foods, drugs (particularly antibiotics such as penicillin), and venoms from the stings of insects such as bee, wasp, yellow jacket, hornet, or fire ant, but virtually any allergen has the potential to cause hives.

In general, if an allergen causes hives or swelling, it is usually eaten (food, drug taken by mouth) or injected (drugs, stings). Allergens that are inhaled tend to cause asthma or rhinitis and may contribute to the development of eczema in children.

If an allergen can penetrate the skin, hives will develop at the site of exposure. For example, contact hives may occur following exposure to latex gloves if sufficient latex penetrates through the skin.

Non-specific Causes

Acute hives can result from causes other than true (IgE-mediated) allergies. An example is exposure to certain dyes used in X-ray procedures, which can cause a whole-body reaction called anaphylaxis which includes hives. Acute viral illnesses in children can be associated with hives which last a few weeks and then spontaneously subside. This usually occurs in association with the symptoms of a common cold, sore throat, or bronchitis. If these patients are given an antibiotic, the cause of the hives becomes confused, because a reaction to the antibiotics may be causing the hives. If penicillin or related antibiotics have been taken, the doctor may perform an allergy skin test, or blood tests for IgE antibodies against the antibiotic, because it is important to know whether or not the patient has had an allergic reaction to the antibiotic. Hepatitis B, glandular fever and intestinal parasites may all be associated with the development of hives. Hives and angioedema can also result from drug treatments. These include codeine and opiate-derived medications, as well as aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs). The responses to NSAIDs can be life-threatening because the angioedema can lead to serious swelling of the tongue and/or throat. Drugs

used to treat high blood pressure, known as ACE inhibitors, can cause recurrent episodes of angioedema.

If chronic hives do not appear to be associated with any other disease, and are not due to one of the physically induced urticarias described below, they are called idiopathic, that is, of unknown origin. Research suggests that in 35-45% of patients with idiopathic hives the cause may be autoimmunity – that is, the patient's immune system working against itself. These autoimmune types of hives are not serious and usually respond to treatment with antihistamines.

Physical Hives

Hives and/or angioedema can be caused by environmental factors, such as a change in temperature, or pressure on the skin. Two rare causes of hives are exposure to sunlight, or contact with water.

Cold-dependent Disorders

Cold urticaria is the rapid onset of itching, redness, and swelling of the skin after exposure to cold. The symptoms of cold urticaria may occur for the first time some weeks after a viral infection, and only affect those parts of the body that have been exposed to cold. To test for this, an ice-cube can be placed on the forearm for 4-5 min. A positive reaction leads to a hive in the shape of the ice cube within 10 minutes after the source of cold has been removed (click for picture).

Cold urticaria can be restricted to certain areas of the body, for example, where there has been a cold injury, or at the sites of allergen immunotherapy (desensitization) injections, or insect bites. Another skin condition which is related to cold is cold-dependent dermatographism where hives form if the skin is scratched and then chilled (click for picture).

Exercise-induced Disorders

Cholinergic or generalized heat urticaria is the onset of small wheals surrounded by a large area of redness, associated with exercise, hot showers, sweating and anxiety (click for picture). The rash first appears on the neck and upper chest, giving a flushed appearance. This is accompanied by intense itching. The rash spreads gradually to the face, back, and extremities, and the wheals increase in size. In some people the hives join up and resemble angioedema. Watering eyes, increased saliva production and diarrhea can occur at the same time. Cholinergic urticaria is the only form of hives that can be caused by emotional responses. Exercise-induced anaphylaxis was first described in a series of people who experienced combinations of itching, skin rash, swelling, wheezing, and low blood pressure as a result of exercise. The hives seen with exercise-induced anaphylaxis are large, in contrast to the small hives seen in cholinergic urticaria. A type of exercise-induced anaphylaxis has been described that

is related to food, and occurs only if exercise takes place 5-24 hours after eating a food to which the individual is allergic.

Pressure-induced Hives/Angioedema

Pressure-induced hives/angioedema occurs 4-6 hours after pressure has been applied to the skin. There may be either a rash or swelling, or both, occurring around tight clothing; the hands may swell with activity such as hammering; foot swelling is common after walking; and buttock swelling may occur after sitting for a few hours.

Solar Urticaria

Solar urticaria is a rare disorder in which brief exposure to light causes the development of hives within 1-3 minutes. It starts with itching about 30 seconds after exposure to sunlight, and is followed by swelling and redness of the light-exposed area. The symptoms usually disappear within 1-3 hours.

Aquagenic Urticaria

Individuals develop small wheals after contact with water, regardless of its temperature.

Association with Autoimmune Thyroid Disease

Patients with chronic hives have an increased frequency of Hashimoto's Disease (thyroiditis), and tests of thyroid function and thyroid antibody levels can be performed to see if this is responsible for the skin symptoms.

Treatment

Treatment of Acute Hives and Angioedema

Acute episodes of hives and/or swelling can be treated with antihistamines, and 1% menthol in aqueous cream may help control itching. If the allergens causing hives and/or swelling have been identified, either from the description of the attacks, or by blood testing for specific IgE antibodies, allergen avoidance will help to prevent further attacks. If the hives or swelling have resulted from taking medications, the patient's physician will be able to identify different types of medications for future treatment. Tightly-fitting clothes should be avoided, as wheals often occur in areas of pressure. As the itching associated with hives can be more severe in warm conditions, it may help to keep the home cool, and to ensure that the bedroom is not too hot. Urticaria and angioedema can be symptoms of a systemic reaction called anaphylaxis and may require urgent administration of intramuscular epinephrine (adrenaline).

Treatment of Chronic Hives

Antihistamines are valuable in the treatment of chronic hives and are more effective on the itching than the wheals. If the symptoms continue when the maximum recommended amount of antihistamines has been given, a short course of corticosteroid tablets may be helpful.

Epidemiology: Who Develops Urticaria and Angioedema, and Why?

Urticaria and angioedema are thought to affect 20% of the population at some time during their lifetime. Hives alone or associated with the swelling of angioedema are more common in women, while angioedema alone, in the absence of hives, is more common in men. Less than 10% of hives develop into a chronic problem. Very often an attack of hives occurs without anyone understanding why it has happened, with little or no risk of the symptoms recurring.

Anaphylaxis

Definition of Anaphylaxis

Anaphylaxis is an acute, potentially life-threatening hypersensitivity reaction, involving the release of mediators from mast cells, basophils and recruited inflammatory cells. Anaphylaxis is defined by a number of signs and symptoms, alone or in combination, which occur within minutes, or up to a few hours, after exposure to a provoking agent. It can be mild, moderate to severe, or severe. Most cases are mild but any anaphylaxis has the potential to become life-threatening. Anaphylaxis develops rapidly, usually reaching peak severity within 5 to 30 minutes, and may, rarely, last for several days.

Classification

The term **anaphylaxis** is often reserved to describe immunological, especially IgE-mediated reactions. A second term, **non-allergic anaphylaxis**, describes clinically identical reactions that are not immunologically mediated. The clinical diagnosis and management are, however, identical.

Symptoms and Signs of Anaphylaxis

The initial manifestation of anaphylaxis may be loss of consciousness. Patients often describe "a sense of doom." In this instance, the symptoms and signs of anaphylaxis are isolated to one organ system, but since anaphylaxis is a systemic event, in the vast majority of subjects two or more systems are involved.
Gastro-intestinal: Abdominal pain, hyperperistalsis with faecal urgency or incontinence, nausea, vomiting, diarrhea.
Oral: Pruritus of lips, tongue and palate, edema of lips and tongue.
Respiratory: Upper airway obstruction from angioedema of the tongue, oropharynx or larynx; bronchospasm, chest tightness, cough, wheezing; rhinitis, sneezing, congestion, rhinorrhea.

Cutaneous: Diffuse erythema, flushing, urticaria, pruritus, angioedema.
Cardiovascular: Faintness, hypotension, arrhythmias, hypovolemic shock, syncope, chest pain.
Ocular: Periorbital edema, erythema, conjunctival erythema, tearing.
Genito-urinary: Uterine cramps, urinary urgency or incontinence.
Severe initial symptoms develop rapidly, reaching peak severity within 3-30 minutes. There may occasionally be a quiescent period of 1–8 hours before the development of a second reaction (a biphasic response). Protracted anaphylaxis may occur, with symptoms persisting for days. Death may occur within minutes but rarely has been reported to occur days to weeks after the initial anaphylactic event.

Causes of Anaphylaxis

1. IgE-Mediated Reactions

Foods

In theory, any food glycoprotein is capable of causing an anaphylactic reaction. Foods most frequently implicated in anaphylaxis are:

- Peanut (a legume)
- Tree nuts (walnut, hazel nut/filbert, cashew, pistachio nut, Brazil nut, pine nut, almond)
- Fish
- Shellfish (shrimp, crab, lobster, oyster, scallops)
- Milk (cow, goat)
- Chicken eggs
- Seeds (cotton seed, sesame, mustard)
- Fruits, vegetables

Food sensitivity can be so severe that a systemic reaction can occur to particle inhalation, such as the odors of cooked fish or the opening of a package of peanuts. A severe allergy to pollen, for example, ragweed, grass or tree pollen, can indicate that an individual may be susceptible to anaphylaxis or to the oral allergy syndrome (pollen/food syndrome) (manifested primarily by severe oropharyngeal itching, with or without facial angioedema) caused by eating certain plant-derived foods. This is due to homologous allergens found between pollens and foods. The main allergen of all grasses is profilin, which is a pan-allergen, found in many plants, pollens and fruits, and grass-sensitive individuals can sometimes react to many plant-derived foods.

Typical aero-allergen food cross-reactivities are:

- Birch pollen: apple, raw potato, carrot, celery and hazelnut
- Mugwort pollen: celery, apple, peanut and kiwifruit
- Ragweed pollen: melons (watermelon, cantaloupe, honeydew) and banana
- Latex: banana, avocado, kiwifruit, chestnut and papaya

Food-associated, exercise-induced anaphylaxis may occur when individuals exercise within 2-4 hours after ingesting a specific food. The individual is, however, able to exercise without symptoms, as long as the incriminated food is not consumed before exercise. The patient is likewise able to ingest the incriminated food with impunity as long as no exercise occurs for several hours after eating the food.

Antibiotics and Other Drugs

PENICILLIN, CEPHALOSPORIN, AND SULPHONAMIDE ANTIBIOTICS

Penicillin is the most common cause of anaphylaxis, for whatever reason, not just drug-induced cases. Penicillin and other antibiotics are haptens, molecules that are too small to elicit immune responses but which may bind to serum proteins and produce IgE antibodies. Serious reactions to penicillin occur about twice as frequently following intramuscular or intravenous administration versus oral administration, but oral penicillin administration may also induce anaphylaxis. Neither atopy, nor a genetic history of allergic rhinitis, asthma or eczema, is a risk factor for the development of penicillin allergy.

MUSCLE RELAXANTS

Muscle relaxants, for example, suxamethonium, alcuronium, vecuronium, pancuronium and atracurium, which are widely used in general anesthesia, account for 70-80% of all allergic reactions occurring during general anesthesia. Reactions are caused by an immediate IgE-mediated hypersensitivity reaction.

Insects

Hymenoptera venoms (bee, wasp, yellow-jacket, hornet, fire ant) contain enzymes such as phospholipases and hyaluronidases and other proteins which can elicit an IgE antibody response.

Latex

Latex is a milky sap produced by the rubber tree Hevea brasiliensis. Latex-related allergic reactions can complicate medical procedures, for example, internal examinations, surgery, and catheterization. Medical and dental staff may develop occupational allergy through use of latex gloves.

Miscellaneous

Examples of miscellaneous agents which cause anaphylaxis are insulin, seminal proteins, and horse-derived antitoxins, the latter of which are used to neutralize venom

in snake bites. Individuals who have IgA deficiency may become sensitized to the IgA provided in blood products. Those selective IgA deficient subjects (1:500 of the general population) can develop anaphylaxis when given blood products, because of their anti-IgA antibodies (probably IgE-anti-IgA).

Elective Medical Procedures

Allergen immunotherapy

2. Cytoxic and Immune Complex – Complement-Mediated Reactions

Whole Blood, Serum, Plasma, Fractionated Serum Products, Immunoglobulins, Dextran

Anaphylactic responses have been observed after the administration of whole blood or its products, including serum, plasma, fractionated serum products and immunoglobulins. One of the mechanisms responsible for these reactions is the formation of antigen-antibody reactions on the red blood cell surface or from immune complexes resulting in the activation of complement. The active by-products generated by complement activation (anaphylatoxins C3a, C4a and C5a) cause mast cell (and basophil) degranulation, mediator release and generation, and anaphylaxis. In addition, complement products may directly induce vascular permeability and contract smooth muscle. Cytotoxic reactions can also cause anaphylaxis, via complement activation. Antibodies (IgG and IgM) against red blood cells, as occurs in a mismatched blood transfusion reaction, activate complement. This reaction causes agglutination and lysis of red blood cells and perturbation of mast cells resulting in anaphylaxis.

3. Non-immunologic Mast Cell Activators

Radiocontrast Media, Low-molecular Weight Chemicals

Mast cells may degranulate when exposed to low-molecular-weight chemicals. Hyperosmolar iodinated contrast media may cause mast cell degranulation by activation of the complement and coagulation systems. These reactions can also occur, but much less commonly, with the newer contrast media agents.

Narcotics

Narcotics are mast cell activators capable of causing elevated plasma histamine levels and non-allergic anaphylaxis. They are most commonly observed by anesthesiologists.

4. Modulators of Arachidonic Acid Metabolism

Aspirin, Ibuprofen, Indomethacin and other Non-steroidal Anti-inflammatory Agents (NSAIDs)

IgE antibodies against aspirin and other NSAIDs have not been identified. Affected individuals tolerate choline or sodium salicylates, substances closely structurally related to aspirin but different in that they lack the acetyl group.

5. Sulfiting Agents

Sodium and Potassium Sulfites, Bisulfites, Metabisulfites, and Gaseous Sulfur Dioxides

These preservatives are added to foods and drinks to prevent discoloration and are also used as preservatives in some medications. Sulfites are converted in the acid environment of the stomach to SO_2 and H_2SO_3, which are then inhaled. They can produce asthma and non-allergic hypersensitivity reactions in susceptible individuals.

6. Idiopathic Causes

Exercise

Exercise alone can cause anaphylaxis as can food-induced anaphylaxis, exercise-induced anaphylaxis can occur during the pollinating season of plants to which the individual is allergic.

Catamenial Anaphylaxis

Catamenial anaphylaxis is a syndrome of hypersensitivity induced by endogenous progesterone secretion. Patients may exhibit a cyclic pattern of attacks during the premenstrual part of the cycle.

Idiopathic Anaphylaxis

Flushing, tachycardia, angioedema, upper airway obstruction, urticaria and other signs and symptoms of anaphylaxis can occur without a recognizable cause. Diagnosis is based primarily on the history and an exhaustive search for causative factors. Serum tryptase and urinary histamine levels may be useful, in particular, to rule out mastocytosis.

Emergency Treatment of Anaphylaxis

A = Airway

Ensure and establish a patent airway, if necessary, by repositioning the head and neck, endotracheal intubation or emergency cricothyroidotomy. Place the patient in a supine position and elevate the lower extremities. Patients in severe respiratory distress may be more comfortable in the sitting position.

B = Breathing

Assess adequacy of ventilation and provide the patient with sufficient oxygen to maintain adequate mentation and an oxygen saturation of at least 91% as determined by pulse oximetry. Treat bronchospasm as necessary. Equipment for endotracheal intubation should be available for immediate use in event of respiratory failure and is indicated for poor mentation, respiratory failure, or stridor not responding immediately to supplemental oxygen and epinephrine.

C = Circulation

Minimize or eliminate continued exposure to causative agent by discontinuing the infusion, as with radio-contrast media, or by placing a venous tourniquet proximal to the site of the injection or insect sting. Assess adequacy of perfusion by taking the pulse rate, blood pressure, mentation and capillary refill time. Establish I.V. access with large bore (16- to 18-gauge) catheter and administer an isotonic solution such as normal saline. A second I.V. may be established as necessary. If a vasopressor, such as dopamine becomes necessary, the patient requires immediate transfer to an intensive care setting.

The same ABC mnemonic can be used for the pharmacologic management of anaphylaxis:

A = Adrenalin = epinephrine

Epinephrine is the drug of choice for anaphylaxis. It stimulates both the beta-and alpha-adrenergic receptors and inhibits further mediator release from mast cells and basophils. Animal and human data indicate that platelet activating factor (PAF) mediates life-threatening manifestations of anaphylaxis. The early use of epinephrine in vitro inhibits the release of PAF in a time-dependent manner, giving support to the use of this medication with the first signs and symptoms of anaphylaxis. The usual dosage of epinephrine for adults is 0.3-0.5 mg of a 1:1000 w/v solution given intramuscularly every 10-20 minutes or as necessary. The dose for children is 0.01 mg/kg to a maximum of 0.3 mg intramuscularly every 5-30 minutes as necessary. Lower doses, e.g., 0.1 mg to 0.2 mg administered intramuscularly as necessary, are usually adequate to treat mild anaphylaxis, often associated with skin testing or immunotherapy. Epinephrine should be given early in the course of the reaction and the dose titrated to the clinical response. For severe hypotension, 1 cc of a 1:10,000 w/v dilution of epinephrine given slowly intravenously is indicated. The patient's response determines the rate of infusion.

B = Benadryl (diphenhydramine)

Antihistamines are not useful for the initial management of anaphylaxis but may be helpful once the patient stabilizes. Diphenhydramine may be administered

intravenously, intramuscularly or orally. Cimetidine offers the theoretical benefit of reducing both histamine-induced cardiac arrhythmias, which are mediated via H2 receptors, and anaphylaxis-associated vasodilation, mediated by H1 and H2 receptors. Cimetidine, up to 300 mg every 6 to 8 hours, may be administered orally or slowly I.V. Doses must be adjusted for children.

C = Corticosteroids

Corticosteroids do not benefit acute anaphylaxis but may prevent relapse or protracted anaphylaxis. Hydrocortisone (100 to 200 mg) or its equivalent can be administered every 6 to 8 hours for the first 24 hours. Doses must be adjusted for children.

Prevention of Anaphylaxis

Agents causing anaphylaxis should be identified when possible and avoided. Patients should be instructed how to minimize exposure. Beta-adrenergic antagonists, including those used to treat glaucoma, may exacerbate anaphylaxis and should be avoided, where possible. Angiotensin-converting enzyme (ACE) inhibitors may also increase susceptibility to anaphylaxis, particularly with insect venom-induced anaphylaxis. Epinephrine is the drug of choice to treat anaphylaxis. Individuals at high risk for anaphylaxis should be issued epinephrine syringes for self-dministration and instructed in their use. Intramuscular injection is recommended since it results in prompt elevation of plasma concentrations and has prompt physiological effects. Subcutaneous injection results in delayed epinephrine absorption. Patients must be alerted to the clinical signs of impending anaphylaxis and the need to carry epinephrine syringes at all times and to use it at the earliest onset of symptoms. Unused syringes should be replaced immediately when they reach their use-by/expiration date, as epinephrine content and bioavailability of the drug decreases in proportion to the number of months past the expiration date. Pre-treatment with glucocorticosteroids and H1 and H2 antihistamines is recommended to prevent or reduce the severity of a reaction where it is medically necessary to administer an agent known to cause anaphylaxis, for example, radio-contrast media.

Other important patient instructions include:

a) Personalized written anaphylaxis emergency action plan
b) Medical Identification (e.g., bracelet, wallet card)
c) Medical record electronic flag or chart sticker, and emphasis on the importance of follow-up investigations by an allergy/immunology specialist

Differential Diagnosis

The differential diagnosis for anaphylaxis includes:

- respiratory difficulty or circulatory collapse, including vasovagal reactions
- globus hystericus

- status asthmaticus
- foreign body aspiration
- pulmonary embolism
- epiglottitis
- myocardial infarction
- carcinoid syndrome
- hereditary angioedema
- pheochromocytoma
- hypoglycemia
- seizures
- overdose of medication
- cold urticaria
- cholinergic urticaria
- sulfite or monosodium glutamate ingestion

Upper airway obstruction, bronchospasm, abdominal cramps, pruritus, urticaria and angioedema are absent in vasovagal reactions. Pallor, syncope, diaphoresis and nausea usually indicate a vaso-vagal reaction but may occur in either condition.

If a reaction occurs during a medical procedure, it is important to consider a possible reaction to latex or medication used for or during anesthesia.

Epidemiology

Food-induced anaphylaxis

The prevalence of food-induced anaphylaxis varies with the dietary habits of a region. A United States survey reported an annual occurrence of 10.8 cases per 100,000 person years. By extrapolating this data to the entire population of the USA, this suggests approximately 29,000 food-anaphylactic episodes each year, resulting in approximately 2,000 hospitalizations and 150 deaths. Similar findings have been reported in the United Kingdom and France. Food allergy is reported to cause over one-half of all severe anaphylactic episodes in Italian children treated in emergency departments and for one-third to one-half of anaphylaxis cases treated in emergency departments in North America, Europe and Australia. It is thought to be less common in non-Westernized countries. A study in Denmark reported a prevalence of 3.2 cases of food anaphylaxis per 100,000 inhabitants per year with a fatality rate of approximately 5%.

Risk factors for food anaphylaxis include asthma and previous allergic reactions to the causative food.

Food-associated, exercise-induced anaphylaxis

This is more common in females, and over 60% of cases occur in individuals less than 30 years of age. Patients sometimes have a history of reacting to the food when younger and usually have positive skin tests to the food that provokes their anaphylaxis.

Anaphylaxis caused by radio-contrast media

Mild adverse reactions are experienced by approximately 5% of subjects receiving radio-contrast media. U.S. figures suggest that severe systemic reactions occur in 1:1000 exposures with death in 1:10,000-40,000 exposures.

Penicillin-induced anaphylaxis

One percent to 5% of courses of penicillin therapy are complicated by systemic hypersensitivity reactions. Point two percent is associated with anaphylactic shock, and mortality occurs in 0.02% of the cases. If a patient has a strongly positive skin test or circulating IgE antibody to penicillin, there is a 50-60% risk of an anaphylactic reaction upon subsequent challenge. In patients with a case history suggestive of penicillin allergy and negative skin tests, the risk of anaphylaxis is very low. Atopy and mold sensitivity are not risk factors for the development of penicillin allergy.

Muscle relaxants

Anaphylaxis to muscle relaxants occurs in approximately 1 in 4,500 of general anesthesia, with fatalities occurring in 6% of these cases. Risk factors are female sex (80% of cases). Atopy is not a risk factor; previous drug allergy may be a risk factor. In patients with a history of anaphylaxis, skin tests to different muscle relaxants may be helpful. If the test result is positive, the muscle relaxant should not be used. A negative result provides evidence that the muscle relaxant can probably be administered safely.

Insect venom anaphylaxis

Studies from Australia, France, Switzerland and the USA suggest incidences of systemic reactions to Hymenoptera stings ranging from 0.4% to 4% of the population. In the USA, at least 40 deaths occur each year as a result of Hymenoptera stings.

Management

Allergy / immunology specialists play a uniquely important role to confirm the etiology of anaphylaxis, prepare the patient for self administration of epinephrine, educate the patient and/or family about allergen avoidance, and rule out any underlying condition, such as mastocytosis, which can predispose a patient to develop anaphylaxis. Referral to an allergist / immunologist is indicated for patients with this disease.

Stevens-Johnson syndrome

Definition

Stevens-Johnson syndrome is a rare, serious disorder in which your skin and mucous membranes react severely to a medication or infection. Often, Stevens-Johnson syndrome begins with flu-like symptoms, followed by a painful red or purplish rash that spreads and blisters, eventually causing the top layer of your skin to die and shed.

Stevens-Johnson syndrome presents a medical emergency that usually requires hospitalization. Treatment focuses on eliminating the underlying cause, controlling symptoms and minimizing complications.

Recovery after Stevens-Johnson syndrome can take weeks to months, depending on the severity of your condition. If your doctor determines that your case of Stevens-Johnson syndrome was caused by medication, you'll need to permanently avoid the medication and all others related to it.

Signs and symptoms of Stevens-Johnson syndrome include:

- Facial swelling
- Tongue swelling
- Hives
- Skin pain
- A red or purple skin rash that spreads within hours to days
- Blisters on your skin and mucous membranes, especially in your mouth, nose and eyes
- Shedding (sloughing) of your skin

If you have Stevens-Johnson syndrome, several days before the rash develops you may experience:

- Fever
- Sore throat
- Cough
- Burning eyes

When to see a doctor

Stevens-Johnson syndrome requires immediate medical attention. Seek emergency medical care if you experience any of the following signs or symptoms:

- Unexplained widespread skin pain
- Facial swelling
- Blisters on your skin and mucous membranes
- Hives
- Tongue swelling
- A red or purple skin rash that spreads
- Shedding of your skin

The exact cause of Stevens-Johnson syndrome can't always be identified. Usually, the condition is an allergic reaction in response to medication, infection or illness.

Medication causes

Medications are most often the cause of Stevens-Johnson syndrome. Drugs commonly associated with Stevens-Johnson syndrome include:

- Anti-gout medications, such as allopurinol
- Nonsteroidal anti-inflammatory drugs (NSAIDs), often used to treat pain
- Penicillins, which are used to treat infections
- Anticonvulsants, which are used to treat seizures

Infectious causes

Infections that can cause Stevens-Johnson syndrome include:

- Herpes (herpes simplex or herpes zoster)
- Influenza
- HIV
- Diphtheria
- Typhoid
- Hepatitis

Other causes

In some cases, Stevens-Johnson syndrome may be caused by physical stimuli, such as radiation therapy or ultraviolet light.

Risk factors

Stevens-Johnson syndrome is a rare and unpredictable reaction. No test is available to help predict who is at risk. Some factors, however, may increase your risk of developing Stevens-Johnson syndrome, including:

- **Existing medical conditions.** Viral infections, diseases that decrease your immunity, human immunodeficiency virus (HIV) and systemic lupus erythematosus — a chronic inflammatory disease — increase your risk of developing Stevens-Johnson syndrome.
- **Genetics.** Carrying a gene called HLA-B12 may make you more susceptible to Stevens-Johnson syndrome.

Possible complications of Stevens-Johnson syndrome include:

- **Secondary skin infection (cellulitis).** This acute infection of your skin can lead to life-threatening complications, including meningitis — an infection of the membranes and fluid surrounding your brain and spinal cord — and sepsis.

- **Sepsis.** Sepsis occurs when bacteria from a massive infection enter your bloodstream and spread throughout your body. Sepsis is a rapidly progressing, life-threatening condition that can cause shock and organ failure.
- **Eye problems.** The rash caused by Stevens-Johnson syndrome can lead to inflammation in your eyes. In mild cases, this may cause irritation and dry eyes. In severe cases, it can lead to extensive tissue damage and scarring within your eyes that can result in blindness.
- **Damage to internal organs.** Stevens-Johnson syndrome can cause lesions on your internal organs, which can result in inflammation of your lungs (pneumonitis), heart (myocarditis), kidney (nephritis) and liver (hepatitis).
- **Permanent skin damage.** When your skin grows back following Stevens-Johnson syndrome, it may have abnormal bumps and coloring (pigmentation). Scars may remain on your skin, as well. Lasting skin problems may cause your hair to fall out, and your fingernails and toenails may not grow normally.

Stevens-Johnson syndrome is an emergency medical condition. If you have signs and symptoms, call 911 or emergency medical help, or go to an emergency room immediately. If you have time before you go:

- **Put all the medications you're taking in a plastic bag,**including prescription and over-the-counter drugs. This may help your doctor figure out what triggered Stevens-Johnson syndrome.
- **Ask a family member or friend to come along,** if they're available immediately. Someone who knows you well can help inform the medical staff of your medical history and can help take in information about your current illness.

The emergency room doctor is likely to ask you about your other medical conditions and whether you've experienced a flu-like illness recently. If possible, give that some thought on your way to the hospital, and share important information with anyone who is accompanying you to the emergency room.

Doctors often can identify Stevens-Johnson syndrome based on your medical history, a physical exam and the disorder's distinctive signs and symptoms. To confirm the diagnosis, your doctor may take a tissue sample of your skin (biopsy) for examination under a microscope.

Stevens-Johnson syndrome requires hospitalization, often in an intensive care unit or burn unit.

Stopping medication causes

The first and most important step in treating Stevens-Johnson syndrome is to discontinue any medications that may be causing it. Because it's difficult to determine exactly which drug may be causing the problem, your doctor may recommend that you stop taking all nonessential medications.

Supportive care

Currently there are no standard recommendations for treating Stevens-Johnson syndrome. Supportive care you're likely to receive while hospitalized includes:

- **Fluid replacement and nutrition.** Because skin loss can result in significant loss of fluid from your body, replacing fluids is an important part of treatment. You may receive fluids and nutrients through a tube placed through your nose and advanced into your stomach (nasogastric tube).
- **Wound care.** Cool, wet compresses will help soothe blisters while they heal. Your health care team may gently remove any dead skin, and then place a dressing with a topical anesthetic over the affected areas, if needed.
- **Eye care.** Because of the risk of eye damage, your treatment should include consultation with an eye specialist (ophthalmologist).

Medications

Medications commonly used in the treatment of Stevens-Johnson syndrome include:

- Pain medication to reduce discomfort
- Antihistamines to relieve itching
- Antibiotics to control infection, when needed
- Topical steroids to reduce skin inflammation

In addition, you may receive one of the following types of medications that are currently being studied in the treatment of Stevens-Johnson syndrome:

- **Intravenous corticosteroids.** For adults, these drugs may lessen the severity of symptoms and shorten recovery time if started within a day or two of when symptoms first appear. For children, they may increase risk of complications.
- **Immunoglobulin intravenous (IVIG).** This medication contains antibodies that may help your immune system halt the process of Stevens-Johnson syndrome.

Skin grafting

If a large area of your body is affected, skin grafting — removing skin from one area of your body and attaching it to another or using a synthetic skin substitute — may be necessary to help you heal. This treatment is only rarely required.

If the underlying cause of Stevens-Johnson syndrome can be eliminated and the skin reaction stopped, your skin may begin to grow again within several days. In severe cases, full recovery may take several months.

Lifestyle and home remedies

If you have had Stevens-Johnson syndrome, be sure to:

- **Know what caused your reaction.** If your case of Stevens-Johnson syndrome was caused by a medication, learn the name of that medication and any other closely related medications that may cause the same reaction.
- **Inform your health care providers.** Tell all your health care providers that you have a history of Stevens-Johnson syndrome. If the reaction was caused by medication, provide your caregivers with the name of that medication.
- **Wear a medical information bracelet or necklace.** Have information about your condition and what caused it inscribed on a medical information bracelet or necklace, and wear it at all times.

Prevention

It's difficult to prevent an initial attack of Stevens-Johnson syndrome because you don't know what will trigger it. However, if you had Stevens-Johnson syndrome once, and your doctor determined that it was caused by medication, be sure to avoid that medication and others in the same class to prevent another attack. If the herpes virus caused your reaction, you may need to take daily antiviral medications to prevent a recurrence.

A recurrence of Stevens-Johnson syndrome is usually more severe than the first episode and, in many cases, it can be fatal.

Lyell's syndrome

Background

Toxic epidermal necrolysis (TEN) is a potentially life-threatening dermatologic disorder characterized by widespread erythema, necrosis, and bullous detachment of the epidermis and mucous membranes, resulting in exfoliation and possible sepsis and/or death. Mucous membrane involvement can result in gastrointestinal hemorrhage, respiratory failure, ocular abnormalities, and genitourinary complications.

Diffuse maculopapular rash in toxic epidermal necrolysis (TEN).
TEN is most commonly drug induced. However, the disorder has other potential etiologies, including infection, malignancy, and vaccinations. TEN is idiosyncratic, and its occurrence is not easily predicted.

Some authors believe that Stevens-Johnson syndrome (SJS; also known as erythema multiforme major) is a manifestation of the same process involved in TEN, with the latter involving more extensive necrotic epidermal detachment. TEN involves more than 30% of the body surface, whereas SJS involves less than 10%.

A classification system, based largely on the extent of epidermal detachment and morphology of the skin lesions, aids in differentiating opposite spectrums of the same disease entity. This system comprises the following:

- TEN with spots

- TEN without spots
- Overlap Stevens-Johnson syndrome and TEN (SJS-TEN)

TEN with spots is defined as widespread, irregularly shaped erythematous or purpuric macules with blistering that occurs on all or part of the macule. Blisters become more confluent and result in detachment of the epidermis and erosions on greater than 30% of the body surface area. Mucosal surfaces are usually involved.

TEN without spots is defined as widespread, large areas of erythema with no discrete lesions. Epidermal detachment is greater than 10% of the body surface area. Mucosal surfaces are usually involved.

Overlap Stevens-Johnson syndrome and TEN (SJS-TEN) is characterized by widespread, irregularly shaped erythematous or purpuric macules with blistering that occurs on all or part of the macule. Blisters become confluent and result in detachment of the epidermis and erosions on 10-29% of the body surface area.

TEN is a clinical diagnosis, confirmed by histopathologic analysis of lesional skin (see Clinical and Workup). The mainstay of treatment is supportive care until the epithelium regenerates. Early transfer of patients to a burn or intensive care unit has been shown to reduce the risk of infection, mortality rate, and length of hospitalization.

Historical background

Alan Lyell provided an early description of TEN in 1956, describing the condition as "an eruption resembling scalding of the skin." This dermatologic condition is characterized by extensive epidermal loss suggestive of severe scalding. In that same year, Lang and Walker reported a case of TEN.[3] The disorder was originally described by Debre et al in 1939 in French as l'erythrodermie bulleuses avec epidermolyse.

Lyell later reclassified the conditions of 2 of his patients as having staphylococcal scalded skin syndrome, which is due to Staphylococcus aureus infection rather than to a probable drug hypersensitivity-type reaction. Histopathologic analysis of the skin remains the main tool for discrimination between the two conditions.

Patient education

Patients who have had TEN must be counseled regarding the likely causative medication or agent, and they must be advised to avoid these medications and those of the same or similar classes in the future. Cross-reactivity may occur with agents that chemically resemble the causative agent. Patients must call a pharmacist whenever they start a new prescription.

Genetic factors are suspected in drug-induced blistering disorders, and blood relatives of the patient also should not use the suspected drug.

Pathophysiology

The pathophysiology of TEN has not been fully elucidated; however, various theories have received wide acceptance. TEN is believed to be an immune-related cytotoxic

reaction aimed at destroying keratinocytes that express a foreign antigen. TEN mimics a hypersensitivity reaction, with its characteristic delayed reaction to an initial exposure and an increasingly rapid reaction with repeated exposure. The widespread epidermolysis and blistering of TEN results from keratinocyte apoptosis—an organized series of biochemical reactions leading to cell changes and cell death. However, the number of inflammatory T cells in the skin of patients with TEN is variable and perhaps too low to explain the widespread destruction. There is evidence supporting several immunopathologic pathways leading to keratinocyte apoptosis in TEN, including the following:

- Fas ligand activation on keratinocyte membranes leading to death receptor–mediated apoptosis
- Release of destructive proteins (perforin and granzyme B) from cytotoxic T lymphocytes (CTLs) generated from an interaction with cells expressing major histocompatability complex (MHC) class I
- Overproduction of T cell– and/or macrophage-derived cytokines (interferon-γ [INF-γ], tumor necrosis factor-α [TNF-α], and various interleukins)
- Drug-induced secretion of granulysin from CTLs, natural killer cells, and natural killer T cells

  Precisely how the inciting agent triggers the proposed pathways is yet to be elucidated.

Etiology

TEN can be induced by drugs or infection or can be idiopathic. Medications are the major precipitating cause. Numerous medications have been implicated,including antibiotics, antiepileptic drugs, nonsteroidal anti-inflammatory drugs (NSAIDs), ampicillin, allopurinol, corticosteroids (topical and systemic), and the antiretroviral drugs nevirapine and abacavir.

Antibacterial drugs associated with TEN include the following:

- Sulfonamides (4.5 cases per million users per week)
- Chloramphenicol
- Macrolides (eg, erythromycin)
- Penicillins
- Quinolones (eg, ciprofloxacin, trovafloxacin)

Anticonvulsants associated with TEN include the following:

- Phenobarbital
- Phenytoin
- Carbamazepine
- Valproic acid
- Lamotrigine

TEN in patients taking anticonvulsants has most often been reported within 2 months of starting the drug. However, some cases associated with long-term use have been reported.

NSAIDs associated with TEN include the following:

- Phenylbutazone and oxybutazone - Implicated most commonly, although they are no longer available in the United States
- Oxicams (eg, piroxicam, tenoxicam) - Implicated more often than other NSAIDs
- Ibuprofen
- Indomethacin
- Sulindac
- Tolmetin

With allopurinol, risk is not constant over time. Patients have a 5.5 relative risk. However, during the first 2 months of therapy, the relative risk is 52, and the long-term therapy risk is 0.5.

No laboratory test is able to confirm a specific drug etiology. A causal link is suggested when TEN occurs during the first 4 weeks of medication therapy, usually between 1 and 3 weeks. Drugs with longer half-lives and those with circulating active metabolites may result in more fulminant disease.

Infectious agents (ie, Mycoplasma pneumoniae, herpes virus, hepatitis A), immunizations, and bone marrow or solid organ transplantation have also been associated with TEN.

Epidemiology

In the United States, the annual frequency of TEN is reported to be 0.22-1.23 cases per 100,000 population. In the HIV-positive population, the incidence of TEN increases to 1 case per thousand per year.

Worldwide, the average annual incidence of TEN is 0.4-1.3 cases per million population. In 1992, the cumulative incidence of TEN and SJS in Germany was 1.9 cases per million population. A French survey of dermatologists and health care facilities reported an annual incidence of 1 case per million population.

Race-, sex-, and age-related demographics

A genetic predilection toward carbamazepine-induced TEN has been observed in HLA-B*1502–positive Han Chinese patients. The US Food and Drug Administration recommends screening for the HLA-B*1502 allele before initiating carbamazepine in patients of Asian ancestry.

For unclear reasons, TEN appears to have a predilection for females. The female-to-male ratio is 1.5:1.

TEN may occur in all age groups; however, the mean age of patients with TEN is reported to be between 46 and 63 years. Infection is more commonly implicated as an etiology in children, whereas medication exposure is more common in adults. Elderly persons may be at greater risk because of their tendency to use multiple medications.

Prognosis

The estimated mortality associated with TEN varies widely in different reports, from 10-70%. Outcome depends in part on the quality of care and the rapidity with which treatment is initiated.

Septicemia and multisystem organ failure are the primary causes of death. Epithelial loss results in vulnerability to bacterial and fungal infections. Sloughing of stratified epithelium of mucosal membranes can result in GI hemorrhage, respiratory failure, ocular abnormalities, and genitourinary lesions. Significant fluid loss from extensive skin exfoliation and an inability to tolerate oral intake can lead to hypovolemia, acute tubular necrosis, and shock.

Age, extent of epidermal involvement, and serum urea level are said to be the most important prognostic factors in TEN. Mortality rates in children are much lower than in adults. Elderly patients have a poor prognosis.

Other negative prognostic factors include the following:

- Elevated blood urea nitrogen (BUN) and serum creatinine levels
- Respiratory failure
- Multiple drugs
- Thrombocytopenia
- Lymphopenia
- Neutropenia
- Leukopenia
- Sepsis

Severity-of-illness score

A severity-of-illness score that estimates the risk of death in TEN (SCORTEN) has been developed and validate. Each of the following independent prognostic factors is given a score of 1:

- Age >40 years
- Heart rate >120 beats per minute
- Cancer or hematologic malignancy
- Involved body surface area >10%
- Blood urea nitrogen level >10 mmol/L (28 mg/dL)
- Serum bicarbonate level < 20 mmol/L (20 mEq/L)
- Blood glucose level >14 mmol/L (252 mg/dL)

The number of positive criteria and the corresponding mortality rates are as follows:

- 0: 1 to 3%
- 2: 12%
- 3: 35%
- 4: 58%
- 5 or more: 90%

Sequelae

Major sequelae are generally limited to the affected organ systems (ie, the skin and mucosal membranes).

Cutaneous sequelae of TEN include the following:

- Changes in skin pigment (hypopigmentation or hyperpigmentation; sun exposure must be avoided for several months because ultraviolet light can worsen hyperpigmentation; sunblock is recommended)
- Nail loss and nail dystrophy
- Hypohidrosis (inability to sweat)
- Scarring, alopecia, and hypertrophic scarring
- Dermal desiccation, causing deep dermal wounds
- Chronic xerostomia
- Esophageal strictures
- Vulvovaginal synechiae
- Phimosis
- Chronic erosion of the mouth and genitalia

Ocular complications generally result from abnormal keratinization of the tarsal conjunctiva. A Sjogrenlike syndrome with decreased lacrimal secretion causes dry eye and predisposes to corneal abrasions and corneal scarring with neovascularization. In addition, patients have been reported to have palpebral synechiae, entropion, or symblepharon (adhesion of the eyelids).

A study by Power and colleagues found that 50% of patients with TEN developed ocular complications. Patients treated with steroids fared no better than those treated without steroids. Therefore, TEN remains a common cause of visual loss in a significant number of patients. Ultimately, 5-9% of patients can become blind as a result of some of these complications.

3. Final part Test-control
Initial knowledge level

1. Which types of the angioedema are the most common?
a. Drug-induced
b. Hereditary
c. Idiopathic
d. A, B,C - correct
e. A,B – correct

2. The most important therapeutic agents used to treat anaphylaxis are:
a. Oxygen and diphenhydramine
b. Epinephrine and diphenhydramine
c. Albuterol and epinephrine
d. Oxygen and epinephrine
e. all of the above

3. Signs and symptoms of anaphylaxis include all of the following EXCEPT:
a. Vomiting
b. Flushing
c. Bradycardia
d. Syncope
e. all of the above

4. If a patient is experiencing anaphylaxis, the first step in management is:
a. Assessing airway, breathing and circulation
b. Administering epinephrine
c. Calling the PICU
d. Placing patient in recumbent position
e. all of the above

5. Which of the following therapeutic agents is correctly paired with the symptom it can relieve?
a. H1-antihistamine….bronchospasm
b. Inhaled beta-2 agonist…airway edema
c. Glucocorticosteroid…acute edema
d. H1-antihistamine…urticaria
e. all of the above

6. The recommended route of administration of epinephrine in the setting of anaphylaxis is:
a. Intramuscular
b. Subcutaneous
c. Endotracheal
d. Intravenous
e. all of the above

7. Which of the following scenarios does NOT fit the diagnostic criteria for anaphylaxis? Sudden onset:
a. flushing with lip and tongue swelling and dyspnea
b. urticaria after starting amoxicillin for acute otitis media
c. pruritis and hypotension after starting ceftriaxone
d. hypotension after exposure to latex in a patient with latex allergy
e. all of the above

8. Acute urticaria, commonly referred to as hives, is the most frequent dermatologic disorder seen in the:
a. emergency department (ED)
b. therapeutic department
c.diagnostic department
d. rheumatologic department
e. all of the above

9. Urticaria may be acute:
a. lasting less than 6 weeks
b. lasting less than 8 weeks
c. lasting less than 7 weeks
d. lasting less than 12 week
e. lasting more than 6 weeks

10. Urticaria may be chronic:
a. lasting less than 6 weeks
b. lasting less than 2 weeks
c. lasting less than 3 weeks
d. lasting less than 1 week
e. lasting more than 6 weeks

11. A large variety of urticaria variants exist in the form of:
a. acute immunoglobulin E (IgE)–mediated urticaria
b. chemical-induced urticaria (non-IgE-mediated)
c. urticarial vasculitis
d. autoimmune urticaria
e. all of the above

12. Urticaria may be confused with a variety of other dermatologic diseases that could be similar in appearance, including:
a. atopic dermatitis (eczema)
b. maculopapular drug eruptions
c. contact dermatitis
d. a,b,c – correct
e. a,b – correct

13. Acute IgE-mediated urticaria usually occurs independently, but it may be accompanied by the more serious clinical manifestations of anaphylaxis:
a. angioedema
b. anaphylactic shock
c. weakness
d. usually a,b - correct
e. usually a,c – correct

14. The process of the swelling in patients with urticaria is caused by several mechanisms:
a. the type I allergic immunoglobulin E response, initiated by antigen-mediated IgE immune complexes
b. the type II allergic response, mediated by cytotoxic T cells
c. the type III immune-complex disease
d. b,c – correct

e. a,b,c - correct

15. Anaphylaxis most commonly affects:
a. the cutaneous system
b. the respiratory system
c. the cardiovascular system
d. the gastrointestinal system
e. all of the above

Final knowledge level

1. Which of the of the following independent prognostic factors are connected with the severity-of-illness score that estimates the risk of death in Toxic epidermal necrolysis (TEN) EXCEPT:

a. age >40 years
b. heart rate >120 beats per minute
c. cancer or hematologic malignancy
d. involved body surface area >3%
e. answer d

2. Complement-mediated urticarias include:

a. viral infections
b. bacterial infections
c. serum sickness
d. transfusion reactions
e. all of the above

3. In which cases drugs could be connected with significant cross-reactivity among the drug in causing urticaria and anaphylaxis:

a. nonsteroidal anti-inflammatory drugs
b. opioids
c. vecuronium
d. succinylcholine
e. vancomycin

4. Respiratory symptoms of anaphylaxy include all EXCEPT:

a. throat tightness
b. wheezing
c. urticaria
d. shortness of breath
e. cough

5. The primary gastrointestinal symptoms by anaphylaxy include all EXCEPT:

a. dysphagia
b. nausea
c. throat tightness
d. diarrhea
e. cramps

6. Signs and symptoms of Stevens-Johnson syndrome include:

a. hedding (sloughing) of your skin
b. facial swelling
c. tongue swelling
d. a,b,c -correct
e. a,c - correct

7. Several days before the rash develops by Stevens-Johnson syndrome some symptoms may experience:

a. fever
b. sore throat
c. cough
d. burning eyes
e. all of the above

8. Infections that can cause Stevens-Johnson syndrome include:

a. herpes (herpes simplex or herpes zoster)
b. influenza
c. HIV
d. b,c – correct
e. a,b,c – correct

9. Drugs commonly associated with Stevens-Johnson syndrome include:

a. anti-gout medications
b. nonsteroidal anti-inflammatory drugs
c. penicillins
d. a,b,c – correct
e. b,c - correct

10. Existing medical conditions that may increase the risk of developing Stevens-Johnson syndrome:

a. viral infections
b. diseases that decrease immunity
c. human immunodeficiency virus (HIV)
d. systemic lupus erythematosus
e. all of the above

11. Possible complications of Stevens-Johnson syndrome include:

a. sepsis
b. eye problems
c. pneumonitis
d. nephritis
e. all of the above

12. Toxic epidermal necrolysis (TEN) is a potentially life-threatening dermatologic disorder characterized by:

a. widespread erythema
b. necrosis
c. bullous detachment of the epidermis and mucous membranes
d. a,b,c – correct
e. b,c – correct

13. There is evidence supporting several immunopathologic pathways leading to keratinocyte apoptosis in toxic epidermal necrolysis TEN, including the following:

a. Fas ligand activation on keratinocyte membranes leading to death receptor–mediated apoptosis

b. release of destructive proteins (perforin and granzyme B) from cytotoxic T lymphocytes (CTLs) generated from an interaction with cells expressing major histocompatability complex (MHC) class I

c. overproduction of T cell– and/or macrophage-derived cytokines (interferon-γ [INF-γ], tumor necrosis factor-α [TNF-α], and various interleukins)

d. drug-induced secretion of granulysin from CTLs, natural killer cells, and natural killer T cells

e. all of the above

14. Toxic epidermal necrolysis (TEN) can be induced by:

a. drugs
b. infection
c. can be idiopathic
d. a,c – correct
e. a,b,c, - correct

15. Which of the of the following independent prognostic factors are connected with the severity-of-illness score that estimates the risk of death in Toxic epidermal necrolysis (TEN) EXCEPT:

a. age >20 years
b. heart rate >120 beats per minute
c. cancer or hematologic malignancy
d. involved body surface area >10%
e. answer a

CORRECT ANSWERS:

Initial knowledge level:

1.D; 2.D; 3.C; 4.A; 5.D; 6.A; 7.B; 8. A; 9.A; 10.E; 11.E; 12.D; 13.D; 14. E; 15.E

Final knowledge level:

1.E; 2.E; 3. A; 4.C; 5.C; 6.D; 7.E; 8.E; 9.D; 10.E; 11.E; 12.D; 13.E; 14.E; 15.E

Structure of class.

Plan and organizational structure of the lesson:
Preparatory stage:

- organization of lesson with test control of incoming level of knowledge 135 min.
- final module control 90 min

	Content	Level of learning	Methods of the learning control	Materials of the methodological support	Time, min.
1.	<u>The preparatory phase</u> The formation of professional knowledge, skills and abilities: - Know clinic of acute allergic diseases; - Methods of diagnosis acute of allergic diseases; - Methods for the treatment of acute allergic diseases;	I I-II I-II	1. Examination the patients 2. Analysis of anamnesis 3. Filling immunological cards 4. Solving the common tasks	Tests. Scheme. 1. Situational tasks 2. Maps of immunological observation 4. Maps of directing the patients to consulting by an allergist, immunologist	25
2.	<u>The main stage</u> - Determine the necessity of guidance to the patient with an allergist, clinical immunologist	II-III	5. Tests	5. Special open questions	85
3.	Monitoring and adjustment of professional knowledge and skills	II-III			
4.	<u>The final stage</u> Final modul control	I	1. Open questions	1. Tests 2. Untypical situation tasks	90

Control questions

1. Role of triggers in the formation of acute allergic diseases.
2. Types of immunological damage of biological structures in patients with acute allergic diseases.
3. Anaphylaxis: definition, triggers, symptoms, diagnosis, management to the treatment.
4. Urticaria: definition, triggers, symptoms, diagnosis, management to the treatment.
5. Angioedema: definition, triggers, symptoms, diagnosis, management to the treatment.
6. Stevens-Johnson syndrome: definition, triggers, symptoms, diagnosis, management to the treatment.
7. Lyell's syndrome (toxic epidermal necrolysis): definition, triggers, symptoms, diagnosis, management to the treatment.

Practical skills:

4. To be able to collect allergic anamnesis and diagnose of different acute allergic conditions.
5. To know instrumental and laboratory methods for diagnosis of acute allergic diseases.
6. To formulate the modern principles of diagnosis and treatment of different types of anaphylaxis.
7. To formulate the modern principles of diagnosis and treatment of different types of Stevens-Johnson syndrome.
8. To formulate the modern principles of diagnosis and treatment of different types of Lyell's syndrome (toxic epidermal necrolysis).

The conclusions.

5. Possessed modern knowledge about the mechanisms of immunological reactions of biostructures damage, genetic and environmental bases of acute allergic diseases.
6. Formed basic principles of clinical and laboratory and instrumental diagnostics of acute allergic diseases.
7. Determined the basic methods of laboratory diagnosis of acute allergic diseases.
8. Determined the basic approaches to the treatment of acute allergic conditions in the emergency department.

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4. How the Immune System Works, Includes Desktop Edition. Lauren M. Sompayrac. Wiley-Blackwell; 4 edition (2012). – 152 p.
5. J. Wyatt, R. N. Illingworth, C. A. Graham, K. Hogg. Oxford Handbook of Emergency Medicine. Oxford University Press (2012). – 768 p.
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7. A.Brian Baldo, N.H. Pham. Drug Allergy. Clinical Aspects, Diagnosis, Mechanisms, Structure-Activity Relationships. Springer New York (2013). – 447 p.