Topic №4:

Immunodiagnostics, immunotherapy and prevention of viral infection COVID-19

1. **Background:**

Actuality: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a new member of the coronavirus family that can cause coronavirus disease 2019 (COVID-19). COVID-9 has become a global pandemic with severe health issues around the world. Identifying the accurate immunopathogenesis of the COVID-19 and the immune response against SARS-CoV-2 is necessary for the development of therapeutic approaches and rational drug design. Today it is especially important for students to understand the importance of knowledge on the diagnosis, treatment and prevention of viral infection COVID-19.

2. **Aim:**

Academic – students need to know the clinical, instrumental and laboratory diagnosis of COVID-19, the main approaches to its treatment.

Professionally-oriented - students should be able to establish indications for the use of immunotropic therapy in the treatment of viral infection COVID-19 on the basis of clinical and laboratory data.

Educational - students need to have a sense of responsibility for the timeliness and correctness of professional actions.

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

Subjects	To know	To be able to
Histology and embryology	Histological changes in lung tissue in patients with COVID-19	Be able to determine the inflammatory process in the tissue according to the morphological picture
Medical biochemistry	Basic methods of isolation and purification of proteins with antigenic properties	To choose methods of biochemical purification to isolate protein preparations from blood plasma
Therapy	Diagnostic criteria for SARS-CoV-2 virus infection	To diagnose the severity of COVID-19, establish indications for the use of biological therapy
Pediatrics	SARS-CoV-2 infection in children	To determine indications for the use of biological therapy for the treatment of COVID-19 in children
Molecular biology	Basic biotechnologies (hybridoma, recombinant)	Using of modern biotechnologies in the production of vaccines for the prevention of COVID-19

5. Study questions.:

1. Epidemiology of SARS-CoV-2 virus infection
2. The structure of the SARS-CoV-2 virus
3. Pathogenesis of COVID-19
4. Ways of SARS-CoV-2 infection and anti-epidemic quarantine measures
5. Symptoms of COVID-19
6. Features of COVID-19.
7. Diagnosis of SARS-CoV-2 and COVID-19.
8. Is there a specific etiotropic therapy for coronavirus infection?
9. Types of vaccines against SARS-CoV-2

Main part

In December 2019, the World Health Organization (WHO) was informed about an outbreak of pneumonia in Wuhan, Hubei Province, China, and the etiology was not identified. On January 30, 2020, WHO declared that the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) epidemic is a public health emergency of international concern (PHEIC). On February 11, 2020, the WHO officially named the current outbreak of coronavirus disease as Coronavirus Disease-2019 (COVID-19) and the International Committee on Taxonomy of Viruses (ICTV) named the virus as SARS-CoV-2.

Coronavirus disease 2019 is a newly emerging infectious disease currently spreading across the world. It is caused by a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The spike (S) protein of SARS-CoV-2, which plays a key role in the receptor recognition and cell membrane fusion process, is composed of two subunits, S1 and S2. The S1 subunit contains a receptor-binding domain that recognizes and binds to the host receptor angiotensin-converting enzyme 2, while the S2 subunit mediates viral cell membrane fusion by forming a six-helical bundle via the two-heptad repeat domain. In this review, we highlight recent research advance in the structure, function and development of antivirus drugs targeting the S protein.

SARS-CoV-2 belongs to the β coronavirus family. It is the seventh known coronavirus to infect humans; four of these coronaviruses (229E, NL63, OC43, and HKU1) only cause slight symptoms of the common cold. Conversely, the other three, SARS-CoV, MERS-CoV, and SARS-CoV-2, are able to cause severe symptoms and even death, with fatality rates of 10%, 37%, and 5%, respectively.

SARS-CoV-2 is a single-stranded RNA-enveloped virus. An RNA-based metagenomic next-generation sequencing approach has been applied to characterize its entire genome, which is 29,881 bp in length (GenBank no. MN908947), encoding 9860 amino acids. Gene fragments express structural and nonstructural proteins. The S, E, M, and N genes encode structural proteins, whereas nonstructural proteins, such as 3-chymotrypsin-like protease, papain-like protease, and RNA-dependent RNA polymerase, are encoded by the ORF region.

A large number of glycosylated S proteins cover the surface of SARS-CoV-2 and bind to the host cell receptor angiotensin-converting enzyme 2 (ACE2), mediating viral cell entry [8]. When the S protein binds to the receptor, TM protease serine 2 (TMPRSS2), a type 2 TM serine protease located on the host cell membrane, promotes virus entry into the cell by activating the S protein. Once the virus enters the cell, the viral RNA is released, polyproteins are translated from the RNA genome, and replication and transcription of the viral RNA genome occur via protein cleavage and assembly

of the replicase–transcriptase complex. Viral RNA is replicated, and structural proteins are synthesized, assembled, and packaged in the host cell, after which viral particles are released

The S protein on the surface of the virus is a key factor involved in infection. It is a trimeric class I TM glycoprotein responsible for viral entry, and it is present in all kinds of HCoVs, as well as in other viruses such as HIV (HIV glycoprotein 160, Env), influenza virus (influenza hemagglutinin, HA), paramyxovirus (paramyxovirus F), and Ebola (Ebola virus glycoprotein). Similar to other coronaviruses, the S protein of SARS-CoV-2 mediates receptor recognition, cell attachment, and fusion during viral infection.

Cell invasion includes the viral entry steps (receptor recognition, endocytosis and viral- membrane fusion) and post-entry steps (genomic RNA translation and replication, virion assembly, maturation, and exocytosis). The host immune response is initiated as soon as the type I interferon (IFN-I) response is triggered by intracellular genomic RNA. IFN-I release leads to the transcription of hundreds of interferon-stimulated genes (ISGs) and to the recruitment of CD4 + T-helper cells, further responsible for the Th1/Th2 response and humoral immunity.

DETECTION OF SARS-COV-2 IN GENERAL FEATURES

The first step in managing COVID-19 is the rapid and accurate detection of SARS-CoV-2 enabled by real-time reverse transcription–polymerase chain reaction (RT–PCR). RT–PCR detects SARS-CoV-2 nucleic acids present in nasopharyngeal fluids. Testing is used to prevent infectious spread between persons and communities that include asymptomatic infected persons, whose viral shedding can inadvertently spread the infection to the elderly and those with disease comorbidities[9]. Accurate viral detection is a starting point to contain the COVID-19 pandemic. Lapses affect public safety, enabling infection spread aided by false-negative test results. Improving test sensitivity and specificity remains an urgent need[7]. Serological testing complements virus detection, indicating past infection, which could be harnessed for therapeutic gain. Antibodies are detected by enzyme-linked immunosorbent assay using a qualitative detection of IgG or IgM antibodies. Such tests determine an immune response against the viral spike (S) protein and may be helpful to assess protection against subsequent viral exposure and/or for contact tracing purposes. Thus, the importance of such tests cannot be overstated. This is also true for epidemiological evaluations and broad global therapeutic needs[14]. Future work includes the development of diagnostic tests to improve immunoassay sensitivity and specificity. Indeed, such testing will ultimately reveal viral protection as reinfections emerge. Inducing immunity against SARS-CoV-2 is the next frontier for COVID-19 control. To this end, our intent in this Review is to summarize the clinical disease presentation with a focus on how to best deploy nanomaterial-based and other diagnostic tests at individual, community and societal levels. The Review outlines current and future nanomaterial diagnostics for COVID-19. The intent is to facilitate the containment of the virus's global spread.

Detection of SARS-CoV-2 viral shedding

In throat swabs and sputum, the viral shedding peaks at five to six days after symptom onset and ranges from 10^4 to 10^7 copies ml^{-1}. This reflects higher virus levels in the respiratory tract. The viral RNA detection rate in nasal swabs of infected people has approached 100%. The positivity rates for blood, saliva and tears are 88, 78 and 16%, respectively. The self-collection of naso- or oropharyngeal swabs facilitates large-scale population field testing employing the chemiluminescence immunoassay and the enzyme-linked immunosorbent and lateral-flow immunochromatographic assays. The lateral-flow immunochromatographic assay uses gold nanoparticles (AuNPs) and a colorimetric label to provide a rapid platform for point-of-contact serological detection. Here, SARS-CoV-

2-specific antigen is conjugated with nanoparticles. By blood or saliva specimen loading, SARS-CoV-2 IgG and IgM can bind to the SARS-CoV-2 antigen and antibody, which is detected colorimetrically. The assay is completed in 20 min with a ~90% accuracy. To date, the minimum length of viral shedding is 7 d after symptom onset, with viral infectivity observed within 24 h. SARS-CoV-2 detection declines to undetectable levels, paralleling the presence of serum neutralizing antibodies. Even among cases with concurrent high viral loads, the live virus could not be propagated in cell culture 8 d after symptom onset. These studies warrant the use of quantitative viral RNA load and serological assays when deciding whether to discontinue infection control precautions.

RT–PCR

Current diagnostic tests for the SARS-CoV-2 pandemic use nucleic acid, antibody and protein-based detections, but viral nucleic acid detection by RT–PCR remains the gold standard. Nucleic acid tests have improved sensitivity and specificity for viral detection over the now available serological tests. The recognition of SARS-CoV-2 over common respiratory pathogens is contingent on RT–PCR serving as a sensitive, precise and specific viral detection. Despite the test's accuracy, results have not yet enabled the containment of viral infection. In February 2020, the US Food and Drug Administration (FDA) permitted licensed laboratories to report in-house SARS-CoV-2 diagnostic tests. The procedure begins with the isolation and conversion of viral RNA to complementary DNA (cDNA). Next, the cDNA is amplified using *Taq* DNA polymerase. The RT–PCR test's final overall workflow, which quantifies the viral load. The total turnaround time can exceed 2 d and runs the risk of reduced specificity through cross-contamination6. The tests are commonly performed in hospital laboratories.

Detection of SARS-CoV-2 antibodies

The synthesis of antibodies against SARS-CoV-2 is a primary immune response to infection. Neutralizing antibodies are found in up to 50% of infected individuals by day 7 and in all infected individuals by day 14. Serological studies are an alternative to RT–PCR for SARS-CoV-2 diagnostics. Combining real-time PCR and serological testing significantly increases positive viral detection rates. IgM levels increase during the first week after SARS- CoV- 2 infection, peak after 2 weeks and then fall back to near-background levels in most individuals. IgG is detectable after 1 week and is maintained at a high level for a long period. In contrast, IgG becomes detectable after 1 week, remains elevated for an extended period, sometimes even more than 48 d, and may serve to protect against reinfection. IgA responses appear between 4 and 10 d after infection. Notably, a diagnostic predictor is the presence of serum IgA as well as IgG and IgM. The spectrum of SARS-CoV-2 antibodies is explained, in part, by divergent target antigens. Antibody titres can decrease 7 d after infection. Recent studies have identified SARS-CoV-2-specific antibodies in the saliva. Multiplex SARS-CoV-2 antibody immunoassays were investigated to determine differences between antibody levels in saliva and sera. Antibodies in saliva consistent with those in sera suggest parallel compartmental humoral immune responses. A parallel study developed rapid immunoassay using the BreviTest platform technology for measuring salivary IgA, which correlates with COVID-19 disease severity.

Interestingly, low levels of IgA were seen in individuals with IgG without known exposure to the virus, and suggest that it may represent an indicator of herd immunity. SARS-CoV-2-specific antibody detection, especially that in saliva, may be useful for surveillance. Questions remain as to which antigens are the best candidates for serological testing. While the viral S is perhaps the strongest candidate, what remains unresolved is what part of the S should be developed. Alternatively, multiple isoforms of the S protein, such as those found in variant strains, may be used to ensure assay reproducibility. Time to results can vary from 13 min (Abbott ID NOW) to 45 min (Cepheid Xpert

Xpress). Of the five antibody-based tests available, two are lateral-flow immunoassays (BioMedomics rapid test and SureScreen rapid test cassette), one is a time-resolved fluorescence immunoassay (Goldsite diagnostics kit) and two are colloidal gold immunoassays (Assay Genie rapid PoC kit and VivaDiag COVID-19 IgG–IgM based).

SARS-CoV-2 antigens

A rapid diagnostic assay was also developed to detect the presence of viral antigens expressed by SARS-CoV-2 in samples from the respiratory tract of infected individuals. For this assay, antigen present in the sample binds to antibodies affixed to a paper strip enclosed in a plastic casing. This reaction generates a visually detectable signal within half an hour. The detected antigen(s) are expressed only if the virus is actively replicating; therefore, the tests can be used to identify acute or early infection66. Also, a more common type of rapid diagnostic assay, which detects the presence of antibodies in the blood of infected individuals, has been marketed for COVID-19 by Abbott. Abbott's test can detect the SARS-CoV-2 antibody on ARCHITECT i1000SR and i2000SR laboratory instruments, which can run ~100–200 tests per hour6. Antibodies against SARS-CoV-2 are produced after one week of infection. The strength of any antibody response depends on age, nutritional status, disease severity, comorbid conditions and medications.

PREVENTION FROM COVID-19

Prevention from COVID-19 include several moments:

✓ Get Vaccinated

- Authorized COVID-19 vaccines can help protect from COVID-19.
- To get a COVID-19 vaccine as soon as possible.
- Once person are fully vaccinated, he/she may be able to start doing some things that you had stopped doing because of the pandemic.

✓ Wear a mask

- If you are not fully vaccinated and aged 2 or older, you should wear a mask in indoor public places.
- In areas with high numbers of COVID-19 cases, consider wearing a mask in crowded outdoor settings and for activities with close contact with others who are not fully vaccinated.
- People who have a condition or are taking medications that weaken their immune system may not be fully protected even if they are fully vaccinated. They should continue to take all precautions recommended for unvaccinated people, including wearing a well-fitted mask, until advised otherwise by their healthcare provider.
- If you are fully vaccinated, to maximize protection from the Delta variant and prevent possibly spreading it to others, wear a mask indoors in public if you are in an area of substantial or high transmission.
- If you are fully vaccinated, see When You've Been Fully Vaccinated.

✓ Stay 6 feet away from others

- Inside your home: Avoid close contact with people who are sick.
- If possible, maintain 6 feet between the person who is sick and other household members.
- Outside your home: Put 6 feet of distance between yourself and people who don't live in your household.
- Remember that some people without symptoms may be able to spread virus.
- Stay at least 6 feet (about 2 arm lengths) from other people.

- Keeping distance from others is especially important for people who are at higher risk of getting very sick.
  - ✓ Avoid crowds and poorly ventilated spaces
- Being in crowds like in restaurants, bars, fitness centers, or movie theaters puts you at higher risk for COVID-19.
- Avoid indoor spaces that do not offer fresh air from the outdoors as much as possible.
- If indoors, bring in fresh air by opening windows and doors, if possible.
  - ✓ Wash your hands often
- Wash your hands often with soap and water for at least 20 seconds especially after you have been in a public place, or after blowing your nose, coughing, or sneezing.
- Avoid touching your eyes, nose, and mouth with unwashed hands.
  - ✓ Cover coughs and sneezes
- If you are wearing a mask: You can cough or sneeze into your mask. Put on a new, clean mask as soon as possible and wash your hands.
- If you are not wearing a mask:
- Always cover your mouth and nose with a tissue when you cough or sneeze, or use the inside of your elbow and do not spit.
- Throw used tissues in the trash.
- Immediately wash your hands with soap and water for at least 20 seconds. If soap and water are not readily available, clean your hands with a hand sanitizer that contains at least 60% alcohol.
  - ✓ Clean and disinfect
- Clean high touch surfaces daily. This includes tables, doorknobs, light switches, countertops, handles, desks, phones, keyboards, toilets, faucets, and sinks.
- If someone is sick or has tested positive for COVID-19, disinfect frequently touched surfaces. Use a household disinfectant product from EPA's List N: Disinfectants for Coronavirus (COVID-19) according to manufacturer's labeled directions.
- If surfaces are dirty, clean them using detergent or soap and water prior to disinfection.

Monitor your health daily

- Be alert for symptoms. Watch for fever, cough, shortness of breath, or other symptoms of COVID-19.
- Especially important if you are running essential errands, going into the office or workplace, and in settings where it may be difficult to keep a physical distance of 6 feet.
- Take your temperature if symptoms develop.
- Don't take your temperature within 30 minutes of exercising or after taking medications that could lower your temperature, like acetaminophen.
- Follow CDC guidance if symptoms develop.

Similar to MERS and SARS, no specific treatment has been approved for COVID□19. The first□ line treatment for COVID-19 is supportive treatment, including oxygen therapy, mechanical ventilator support. Similar to MERS and SARS, no specific treatment has been approved for COVID-19. The first line treatment for COVID□19 is supportive treatment, including oxygen therapy, mechanical ventilator support.

IMMUNOTHERAPY OF COVID-19

Similar to MERS and SARS, no specific treatment has been approved for COVID-19. The first-line treatment for COVID-19 is supportive treatment, including oxygen therapy, mechanical ventilator support for patients with respiratory failure, antibiotics for prevention of secondary bacterial infection, and body fluid management. Also, some drugs have shown promising results in the treatment of COVID-19. Since the outbreak of the COVID-19, clinicians have started to assess the antiviral functions of existing drugs on this disease, and multiple preclinical and clinical trials have been launched.

Viral targeted inhibitors were among the first studied drugs. Adenosine-analogs such as Remdisivir block the viral RNA synthesis process. Also, Remdesivir has shown promising results in the treatment of COVID-19 patients in the clinical setting. Other nucleoside analogs such as ribavirin and favipiravir are among the antiviral drugs that could be effective in the treatment of COVID-19 patients; however, no reports have been published about the efficacy of these drugs.

Immunotherapy uses the potentials of the patient's immune system to fight diseases. Immunotherapy has shown considerable results in the treatment of many diseases such as cancer and viral infections. Amplification and reinforcement of the immune system using immune-reinforcing material could have benefits in the treatment of COVID-19.

Inadequate antiviral immune response and severe inflammation induced by dysfunctional immune response are the two main challenges in COVID-19. Developing novel immune-based therapeutics that target viral infection and dysfunctional immune response can improve the clinical outcome of patients with COVID-19. This novel approach is named as “immunotherapy of COVID-19,” and is among the novel treatments being developed. Multiple approaches can be administered as immunotherapeutic treatments for COVID-19.

The viral invasion of the target cells implies genomic RNA translation and the synthesis of polyproteins which are further cleaved by two viral proteases, namely 3C-like protease (3CLpro) and Papain Like protease (PLpro), in order to mature the Nsp. Certain protease inhibitors frequently used in the treatment of HIV such as lopinavir/ritonavir have already been tested to verify their ability to block these proteases but showed a debatable efficiency (RECOVERY trial). Viral RNA synthesis involves two stages: genome replication and subgenomic mRNAs transcription, both of which mediated by the replication/transcription complex (RTC), a membrane-bound structure encoded by the virus. Of these two processes, Nsp12-RdRp plays a central role and is targeted by various experimental molecules (favipiravir). The final stages of the viral cycle cover the viral translation of the structural proteins, the assembly of all viral components into the endoplasmic reticulum, the recreation of the virions in the Golgi apparatus and their elimination through exocytosis. Currently, none of these stages if targeted by specific drugs.

TREATMENT STRATEGIES FOR COVID-19

Currently, no specific antiviral agent is available against SARS-CoV-2 infection, with clinically proven efficacy as in case of MERS-CoV and SARS-CoV. To understand SARS-CoV replication, different animal models have been used and they showed severe infection symptoms. On the other hand, no pathogenesis was detected in case of MERS-CoV because of DDP4 receptor noncompatibility in mice. For studying SARS-CoV-2 pathogenicity, SARS-CoV animal models can be used because SARS-CoV-2 is 80% similar to SARS-CoV. SARS-CoV-2 entry into host cells is thought to be similar to SARS-CoV, possibly through ACE2 cell receptor. As per various genomic organization studies and molecular mechanisms of SARS-CoV-2 pathogenesis, there are numerous targets, which

can be used in different ways as therapeutic agents to inhibit the virus replication or to develop an intervention which may be effective against SARS-CoV-2. Various therapeutic (drugs), immunotherapeutic and Vaccination strategies against SARS-CoV-2 are summarized in Table 1 and subsequently discussed below.

Table 1

Strategy	**Methods**	**Mode of action**	**Examples/under trial drugs**
Receptor inhibition	a. ACE2 receptor inhibition b. TMPRSS2 inhibition	Inhibition of viral receptors on host cell by different compounds interfere with Spike or part of Spike refolding	Baricitinib, ruxolitinib, natural hesperidin, nafamostat mesylate, camostat mesylate and other antiviral drugs
Antiviral drugs	a. Antiviral drugs specifically designed for SARS-CoV-2 b. Antiviral drugs already used for other viral infections (such as HIV, hepatitis, influenza etc.) are now under clinical research against novel coronavirus	To identify the viral proteins and stop the replication of virus	Lopinavir/ritonavir, IFN-α, arbidol, favipiravir and darunavir etc., (all these drugs are already approved for other viral infections but now are under clinical trial against SARS-CoV-2). remdesivir, chloroquine and ivermectin are recently US FDA approved drugs for SARS-CoV-2 infection.
Immunotherapy	a. Complement system b. NK cell therapy c. IL-6 inhibitors d. mTOR inhibitors	Suppressing/inhibiting or enhancing the body immune response/s	C3a, C5a inhibitors, CYNK-001, tocilizumab, rapamycin
Vaccination	a. RNA vaccines b. DNA vaccines c. Recombinant protein vaccines d. Live attenuated vaccines e. Killed/inactivated vaccines f. Viral vector-based vaccines	Helps the immune system of the body to identify and to fight against infectious pathogens by providing acquired immunity (In all these vaccines target is S protein except live attenuated and killed vaccine for which the whole virion is the target)	Currently, more than 115 vaccines are developing for example: mRNA-1273 (RNA vaccine by Moderna Inc.), INO-4800 (DNA vaccine by Inovio Pharmaceuticals Inc.), ChAdOx1nCov-19 (killed/inactivated vaccine by Oxford University, AstraZeneca Plc.), Ad5-nCov (live attenuated vaccine by CanSino Biologics Inc.) and NVX-CoV2373 (recombinant protein vaccine by Novavax Inc and by Clover Biopharmaceuticals).
Other therapeutic options	a. Plasma therapy b. Medicinal plants c. Protease inhibitors d. Monoclonal antibody	Inhibit viral replication or to boost body immune response	*Artemisia annua* (medicinal plant), plasma from patient recovered from nCoV infection is used in plasma therapy. Protease inhibitors such as lopinavir, atazanavir and indinavir

NK: Natural killer; SARS-Cov-2: Severe acute respiratory syndrome coronavirus 2.

Topic №5:
Biological therapy in the treatment of organ-specific and systemic autoimmune diseases

1. **Background:**

Actuality: The use of biologic therapies as an adjunct to disease-modifying anti-rheumatic drugs (DMARDs) for the treatment of autoimmune and rheumatologic diseases is rapidly expanding, owing to the good efficacy and safety profiles of these drugs, and the better understanding of the initial targets of altered immune regulation and activity in various diseases.

2. **Aim:**

Academic – students need to know the clinical, instrumental and laboratory diagnosis of organ-specific and systemic autoimmune diseases

Professionally-oriented - students should be able to establish indications for biological therapy of autoimmune diseases on the basis of clinical and laboratory data

Educational - students need to have a sense of responsibility for the timeliness and correctness of professional actions.

3. **Materials:** Equipment to run powerpoint presentation

Main books. Short information due to the topic.

4. **Interdiscipline integration**

Subjects	To know	To be able to
Histology and embryology	Lymphocytic infiltrates in tissues	Be able to determine the inflammatory process in the tissue according to the morphological picture
Medical biochemistry	Basic methods of protein isolation and purification	To choose methods of biochemical purification to isolate immunoglobulin preparations from blood plasma
Therapy	Diagnostic criteria for systemic autoimmune diseases	To diagnose rheumatic diseases, establish indications for the use of biological therapy
Endocrinology	Organ-specific autoimmune diseases of the endocrine system	To diagnose organ-specific autoimmune diseases of the endocrine system, establish indications for the use of biological therapy
Pediatrics	Organ-specific and systemic autoimmune diseases in pediatric practice	To determine indications for the use of biological therapy in the treatment of organ-specific and systemic autoimmune diseases in children
Molecular biology	Basic biotechnologies (hybridoma, recombinant)	Using of modern biotechnologies in the manufacture of biological therapy drugs

5. Study questions:

1. Biotechnology is a modern way of creating effective drugs.

2. Approaches to the use of monoclonal antibody drugs in adult and pediatric rheumatology practice.
3. The use of monoclonal antibodies in the treatment of Inflammatory bowel disease (inc. Crohn's disease).
4. Biosimilars: definitions, advantages and disadvantages.
5. Manifestations of immunogenicity of immunobiological drugs.
6. Ways of influence of biosimilars on pathological process at autoimmune diseases.
7. Immunoglobulins for intravenous administration in the treatment of autoimmune diseases.

Main part

Autoimmune diseases are characterized by a pathologic state in which an aberrant immune response directed at a normal bodily constituent leads to inflammation, cell injury, or a functional disturbance with clinical manifestations. The molecular constituent (ie, protein, carbohydrate, nucleic acid) that is targeted in autoimmunity is called a "self"-antigen or an autoantigen; by contrast, a molecule from an infecting organism that stimulates an immune response is called a foreign antigen or "non-self." An autoimmune disease usually involves both a T and B cell response and can be generalized or tissue- or organ-specific and either acute or chronic.

While autoimmune diseases are a pathologic state, autoimmunity nevertheless derives from the same mechanisms that underlie the normal immune response to foreign antigens. Immune responses can be divided into two broad categories: innate and adaptive. The innate immune response is a rapid and nonspecific response to a challenge, whether it arises from infection, trauma, or stress. By contrast, an adaptive immune response is slow (days to weeks) and involves the production of antigen-specific B or T cells to overcome a foreign challenge.

While innate and adaptive immune responses (along with responsible cells and mediators) can be separated for analysis, an adaptive immune response depends upon the presence of an innate immune response to promote the generation of a specific immune response. Importantly, an adaptive immune response can be persistent and show memory. In this framework, an autoimmune disease results from a specific adaptive autoimmune response to an autoantigen; this response is in violation of the normal function of the immune system in which mechanisms of tolerance prevent "hyper-reactive" autoimmune responses to self-antigens.

Epidemiological studies have indicated autoimmune diseases (ADs) to be the 10th most common cause of mortality in developing countries. The incidences of AD, including minor AD, such as thyroiditis, iridocyclitis, etc., are estimated to be around 10%. The treatment strategies and outcome in the majority of ADs have significantly improved in the past two decades. The disease like systemic lupus erythematosus (SLE), which has aggressive course, had a mortality of more than 50% by the end of 5 years two decades ago. At present SLE survival has significantly improved with reported 90% survival beyond 5 years. But, morbidity and mortality in substantial percentage of these treated AD remain unchanged, which suggest that there is a gap in the treatment protocols used currently in the management of ADs.

The current therapeutic strategies in AD, though aim at long-lasting remission, are achieved in only few ADs. Even these ADs which go for remission by their natural history have a tendency to go for spontaneous remission. Thus, in majority of ADs the targets achieved are reduction in symptoms and improving the quality of life. The current strategies are successful in preventing specific organ damage where possible or at least delaying the organ damage. The current understanding of immunobiology of AD has brought forward a large amount of information on disease mechanism and has led to the development of newer drugs and biologicals. In spite of all these advances cure or long-lasting remission still remains elusive in majority of systemic Ads, i.e., rheumatoid arthritis (RA), SLE, vasculitis, etc. In this article we have attempted to review the different models of pharmacological therapy, which are often considered as role models and the relevance of these approaches in the management of ADs as well as in designing the protocols of therapy for ADs. We have attempted to suggest few changes based on current understanding of the management of AD.

The current approach in AD can be broadly categorized into two categories: (1) symptomatic or replacement therapy (a conservative approach) and (2) the immunosuppressive or immune-modulation therapy (aggressive therapy). Autoimmune thyroid disease is the best example, which is predominantly managed either by reducing the thyroxin production at the time of hyperfunctioning or by replacing the hormone once the gland is damaged, whereas in systemic diseases like SLE which targets vital organs such as kidney, etc. the primary treatment is to use immunosuppressive to prevent further organ damage. The response to immunosuppression in AD is seen in 60–70% initially, and subsequently the disease may progress or may stop responding to the drug used. Some of the ADs may go for clinical remission (no demonstrable clinical activity) to relapse after sometime. In a small percentage of patients, the AD does go for long-lasting remission. Significant changes have occurred in the approach to AD with immunosuppressive therapy in last few decades. In the initial years, the immunosuppressive drugs used were nonspecific and were interfering in larger pathways and cells. Currently more target-specific drugs are available which have reduced the toxicity of immunosuppressant drugs on other collateral systems and produces a more profound immunosuppression effect.

The use of biologic therapies as an adjunct to disease-modifying anti-rheumatic drugs (DMARDs) for the treatment of autoimmune and rheumatologic diseases is rapidly expanding, owing to the good efficacy and safety profiles of these drugs, and the better understanding of the initial targets of altered immune regulation and activity in various diseases. Targeted therapies such as these are often well tolerated by patients. However, the inconvenience of intravenous (IV) administration, as well as the high costs and adverse events (AEs) associated with these drugs prevent their wide use as first-line medications. The major targets of most biologic therapies are cytokines, B cells, and co-stimulation molecules. Anti-cytokines include anti-tumor necrosis factor (TNF)-α, anti-interleukin (IL)-1, and anti-IL-6 molecules. B-cell depletion includes use of anti-CD20 antibodies and B cell receptor (BCR) modulation by the B-lymphocyte stimulator (BLyS). Although some of the biologic therapies have been found to be useful in more than one disease, others are specific for a single disease. Research is ongoing to identify other molecular targets.

Below will be the characteristic of several most popular DMART in the treatment of systemic autoimmune disease.

Tocilizumab

Mechanism. Tocilizumab (TCZ; trade names Actemra, Roactemra) is a recombinant monoclonal IgG1 anti-human IL-6 receptor (IL-6R) antibody. IL-6 binds to either membrane-bound or soluble IL-6R, and this complex in turn binds to the 130 gp signal transducer. This process enhances the inflammatory cascade, inducing angiogenesis and amplifying the activity of adhesion molecules and the activation of osteoclasts. IL-6 is also responsible for activating both T and B helper cells, and is involved in B-cell differentiation, thus by blocking IL-6, the inflammatory response is decreased

In patients with RA, a high level of IL-6 is present in the blood and in the synovium of involved joints. In an animal study, injecting TCZ into the inflamed joints reduced the swelling and the inflammatory response.

Indications and dosage

The recommended dose of TCZ is 8 mg/kg every 4 weeks.

Efficacy

Monotherapy with TCZ for 52 weeks resulted in significantly reduced radiographic change (total Sharp score) compared with DMARDs. In a 24-week study comparing TCZ and methotrexate (MTX), TCZ was found to be non-inferior to MTX in the first week and superior to MTX in the second week in the intention-to-treat group, as measured by ACR20

Rituximab

Mechanism

Rituximab (trade names Rituxan, Mabthera) is a chimeric human monoclonal antibody against the CD20 protein found on naive, mature, and memory B cells. Rituximab depletes the B-cell population via apoptosis, cellular cytotoxicity, and complement activation. In a number of studies measuring markers for immature B cells, memory B cells, and pre-B cell colony-enhancing factor (visfatin), B-cell depletion occures after treatment with rituximab. In addition, rituximab affects the interferon (IFN) I response genes. In patients with RA responding to rituximab treatment, expression of IFN response genes (*RSAD2*,*IFNI44L*, *HERC5*, *LY6E*, *Mx1*) increased, whereas the non-responding patients had limited or no IFN gene-expression activity

Indications and dosage

The most popular protocol for RA is IV infusion of 1000 mg/m2 on days 1 and 15 in combination with MTX. Subsequent courses may be administered every 24 weeks (based on clinical evaluation), and if necessary, may be repeated, but no sooner than every 16 weeks. For patients with RA, pre-medication with IV methylprednisolone 100 mg (or equivalent) is recommended, 30 minutes before each dose of rituximab. For granulomatosis with polyangiitis (GP) (previously Wegener's granulomatosis), the protocol is different: IV infusion with 375 mg/m2 once weekly for four doses (in combination with IV methylprednisolone for 1 to 3 days followed by daily prednisone). The protocol for microscopic polyangiitis (MPA) is similar to that of GP

Adverse effects and safety

One of the AEs associated with rituximab is an infusion reaction, characterized by fever, chills, rash, swelling (of hands, feet, and face), bronchospasm, and hypotension. In most cases, the reaction is immediate (30 minutes to 2 hours), usually during the first infusion, but is less severe with subsequent infusions. Pretreatment with acetaminophen and an anti-histamine is recommended to prevent this infusion reaction.

Abatacept

Mechanism

T cells play a major role in the pathogenesis of RA. Co-activation of CD28 with the antigen-presenting cell (APC) protein CD80/86 results in release of inflammatory cytokines. Cytotoxic T lymphocyte-associated (CTLA)-4 is a protein with a high affinity to CD80/86, which inhibits T-cell activation by blocking the CD28 binding. Abatacept (trade name Orencia) is a CTLA-4 IgG1 that binds to CD80/86 on APCs, inhibiting the co-stimulation of CD28 on T cells.

Indications and dosage

Abatacept is approved by the FDA for the treatment of RA that is non-responsive to DMARDs and anti-TNF-alpha blockers, and for JIA. For RA treatment, the dose is based on body weight, with a dose of approximately 10 mg/kg (patients weighing less than 60 kg receive 500 mg; those weighing 60 to 100 kg received 750 mg, and those weighing more than 100 kg receive 1000 mg). The initial IV dose can be repeated by additional doses after 2 and 4 weeks, with further doses every 4 weeks after that. Another treatment option after the initial IV dose is to administer a subcutaneous (SC) injection of 125 mg at 24 hours after the first infusion, repeated by weekly SC injections of 125 mg

Efficacy
In a long-term study comparing abatacept with placebo (with background therapy for both groups of a steady dose of MTX), the response to abatacept was superior and was maintained for 3 years including physical function scores. Evaluation of radiographic changes identified a reduction in bone erosion scores (Genant-modified Sharp scoring method) every year within the 3-year follow-up, and 40% of patients had no radiographic progression after 3 years
Adverse effects and safety
The main AEs caused by abatacept are infections, URT symptoms, nausea, headache, infusion reaction, fever, hypertension, and back and limb pain.

Certolizumab pegol

Mechanism

Certolizumab (trade name Cimzia) is a pegylated Fab fragment of a humanized TNF-alpha monoclonal antibody, which binds to and inhibits TNF-alpha. The pegylation extends the half-life of the antibody, and the missing Fc fragment reduces the risk of cytotoxicity.

Indications and dosage

Certolizumab is approved by the FDA for the treatment of active RA disease (Table 1), given as SC injection of 400 mg every 2 weeks for three consecutive cycles, followed by maintenance therapy of 200 mg SC every 2 weeks.
Efficacy
In a study of patients diagnosed with RA for no less than 6 months and no more than 15 years, who had not received any biologic therapy for 6 months before the beginning of the study, but had responded to anti-TNF-alpha blockers in the past, were enrolled. The first group was treated with MTX plus placebo and the second with MTX plus certolizumab. ACR20 response, physical improvement, and reduction in radiographic progression were achieved more rapidly in the certolizumab group compared with the placebo group over a 1-year period.
In a different study of patients with RA who had experienced treatment failure with DMARDs, a significant ACR20 response of up to 50% was accomplished with certolizumab monotherapy. Similar improvements were also encountered for disease activity, physical function, and arthritic pain

Golimumab

Mechanism

Golimumab (trade name Simponi) is a fully human monoclonal IgG1 antibody, acting on both soluble and membrane-bound TNF-α.

Indications and dosage
Golimumab is approved by the FDA for RA, psoriatic arthritis (PsA), and ankylosing spondylitis (AS). The indicated dose for all three diseases is 50 mg monthly by SC injection. For RA, golimumab is administered in combination with MTX; for PsA it may be administered alone or with MTX; and for AS, it may be administered alone.
In a study comparing treatment groups given doses of 50 or 100 mg golimumab SC, no significant difference occurred between the two groups. The lower dosage is recommended by the FDA
In a study of patients with RA who were not responsive to MTX, (GO-FORWARD study) the efficacy of MTX plus placebo, MTX plus golimumab 50 mg, MTX plus golilumab 100 mg, or golimumab 100 mg plus placebo, were compared. The most significant results were seen in the MTX plus golimumab groups (both doses) compared with MTX alone. However, a higher incidence of AEs was noted in the golilumab 100 mg group.

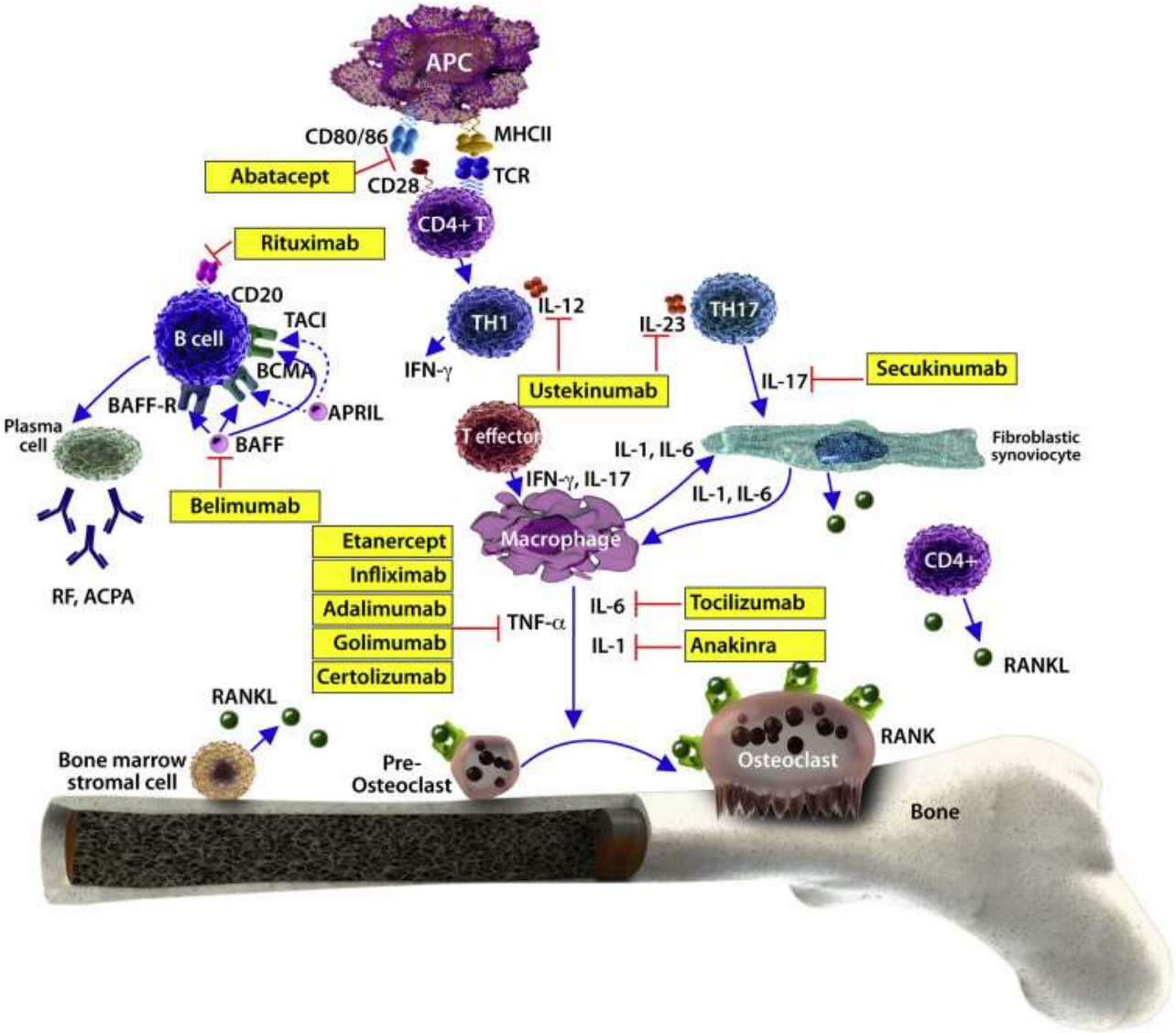

Figure 1. Mechanism of action of DMARDs

Biosimilars are a growing drug class designed to be used interchangeably with biologics. Biologics are created in living cells and are typically large, complex proteins that may have a variety of uses. Within the field of gastroenterology alone, biologics are used to treat inflammatory bowel diseases, cancers, and endocrine disorders. While biologics have proven to be effective in treating or managing many diseases, patient access is often limited by high costs. The development of biosimilars is an attempt to reduce treatment costs. Biosimilars must be nearly identical to their reference biologics in terms of efficacy, side effect risk profile, and immunogenicity. Although the manufacturing process still involves production within living cells, biosimilars undergo fewer clinical trials than do their reference biologics. This ultimately reduces the cost of production and the cost of the biosimilar drug compared to its reference biologic. Currently, seven biosimilars have been approved by the United States Food and Drug Administration (FDA) for use in Crohn's disease, ulcerative colitis, and colorectal cancer. There are other biologics involved in treating gastroenterologic diseases for which there are no FDA approved biosimilars. Although biosimilars have the potential to reduce healthcare costs in chronic disease management, they face challenges in establishing a significant market share. Physician comfort in prescribing reference biologics instead of biosimilars and patient reluctance to switch from a biologic to a biosimilar are two common contributing factors to biosimilars' slow

increase in use. More time will be needed for biosimilars to establish a larger and more consistent market share compared to their reference biologics. Additional data confirming the safety and efficacy of biosimilars, increased number of available biosimilars, and further cost reduction of biosimilars will all be necessary to improve physician confidence in biosimilars and patient comfort with biosimilars.

Therapeutic proteins, also known as biologics, are pharmaceutical agents created in a laboratory setting to mimic the structure of naturally produced proteins in the body. They may either mimic the natural protein's function or antagonize the function of the natural protein. These drugs are produced in living cellular systems, and they have proven to be effective treatment for many diseases including rheumatoid arthritis, ankylosing spondylitis, and inflammatory bowel diseases.

Below we will consider in more detail the use of biosimilars in the treatment of ***inflammatory bowel disease.***

The anti-tumor necrosis factor alpha (TNF-α) biologic infliximab is an effective treatment for inflammatory bowel diseases. The PLANETAS study, a phase I study, established biosimilar infliximab, CT-P13, as having equivalent pharmacokinetics with comparable safety and efficacy profiles to its reference infliximab while the PLANETRA study, a phase III study, found that CT-P13 had equivalent efficacy to reference infliximab after 30 wk of treatment. The patient populations in these studies, however, were patients with ankylosing spondylitis and rheumatoid arthritis. The PROSIT-BIO cohort study specifically investigated the safety and efficacy of CT-P13 in patients with ulcerative colitis and Crohn's disease. The data showed comparable results to those of similar studies with reference infliximab, but the study did not directly compare the biosimilar with its reference biologic. A prospective study of 210 patients also found that CT-P13 is effective in inducing clinical remission in Crohn's disease and ulcerative colitis but noted decreased response to treatment and increased risk of allergic reactions in those previously treated with reference infliximab. A study of 96 patients comparing the efficacy of infliximab compared to biosimilar CT-P13 in maintaining remission in inflammatory bowel diseases found similar long-term outcomes and safety between the two treatment groups. Additionally, a study on CT-P13 in pediatric Crohn's disease reported remission after three doses in 24 of 36 patients and clinical response in 31 of 36. CT-P13 is currently marketed as Remsima™ and Inflectra™.

A double-blind, parallel-group study comparing another infliximab biosimilar, SB2, with reference infliximab in 584 patients with rheumatoid arthritis demonstrated similar safety, efficacy, immunogenicity, and pharmacokinetics at weeks 30 and 54. SB2 is currently marketed as Flixabi® and approved for treatment of multiple chronic inflammatory diseases including the treatment of Crohn's disease and ulcerative colitis in patients between the ages of 6 and 17.

A 2016 study examined survey responses of inflammatory bowel disease specialists regarding biosimilars. Out of 118 responses, only 19.5% were not confident with using biosimilars, and 44.4% believed the biosimilar to be interchangeable with the reference biologic. The primary perceived benefit reported was cost red–uction, and the main concern was immunogenicity. A prospective multicenter study done in 2015 similarly elucidates a positive response profile of biosimilars, and further illustrates safety regarding immunogenicity. The overall positive outcomes when comparing biosimilar infliximab to its reference biologic have improved physicians' attitudes towards biosimilars in the context of treating inflammatory bowel disease.

Immunogenicity can be a significant problem in the treatment of patients with therapeutic biologicals and this is addressed in the 'Guideline on Immunogenicity Assessment of Biotechnology-Derived Therapeutic Proteins' by the CHMP (adopted April 2008, referred to henceforth as 'the general guideline'), which in principle is applicable to monoclonal antibodies (mAbs). While many aspects of immunogenicity of mAbs are not different from those for other therapeutic proteins, there are several aspects that require more specific considerations. MAbs are not expected to induce antibodies that cross-react and neutralize an endogenous counterpart (as can

occur with EPO) and are not used as replacement therapies. Often mAbs are used as therapeutic or diagnostic agents where alternative treatments or diagnostics may exist. However, some specific aspects of immunogenicity are exclusively or primarily relevant for mAbs or novel mAb derivatives (e.g. Fab fragments, scFv, nanobodies, minibodies) and these are addressed in this guideline.

MAbs comprise a large important class of therapeutic biologicals. The range of clinical indications with potential for treatment with mAbs is very wide. Many mAb products are known to be associated with unwanted immunogenicity and in some cases the immunogenicity causes impaired clinical responses or rare serious adverse reactions which require clinical intervention. The wide range of mAbs in development, and approved for different clinical indications precludes specific guidelines that are pertinent to all situations.

Topic 6
Therapeutic use of vaccines in allergology, oncology, reproductive medicine

1. **Background:**

Actuality: Today, it is especially important for students to understand the importance of using vaccines for therapeutic purposes.

2. **Aim:**

Academic - students need to know the changes in immune factors after the use of vaccines in allergology and oncology for therapeutic purposes
Professionally-oriented - students must be able on the basis of clinical and laboratory data to diagnose indications for therapeutic use of vaccines, evaluate the effectiveness of such treatment
Educational - students must have a sense of responsibility for the timeliness and correctness of professional actions.

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

Subjects	To know	To be able to
1	2	3
Histology and embryology	The structure of eggs and sperm. Meiosis.	Be able to determine the state of maturity and functional activity of gametes
Therapy	Diagnostic criteria for allergic diseases	Differentiate IgE-dependent and IgE-independent allergic diseases by clinical and laboratory features
Endocrinology	The main organs of the endocrine system: hypothalamus, pituitary gland, pineal gland	Diagnose fertility disorders associated with their function
Obstetrics and gynecology	The main immune-dependent diseases and conditions in obstetric and gynecological practice associated with the formation of anti-ovarian antibodies	Diagnose these diseases, prescribe treatment
Pediatrics	Approaches to anti-infective vaccination in adolescence in boys	To establish the probability of cross-reaction of anti-infective post-vaccine antibodies with antigens on sperm

1	2	3
Urology	Relationship of chronic inflammatory diseases of the genitourinary system in men, varicocele, with the formation of antisperm antibodies	Establish indications for therapeutic treatment of patients with antisperm antibodies and infertility.
Oncology	Types of tumors, diagnosis of tumor antigens	Establish indications for the treatment of cancer patients with antitumor vaccines
Virology	Features of hepatitis B viruses and papillomavirus groups	Be able to appoint a laboratory test for hepatitis B virus infection and the main pathogenic strains of papillomavirus, know the indications for antiviral prophylactic

5. Study questions:

1. Positive and negative immunological memory.
2. Prophylactic and therapeutic effect of vaccines
3. Allergic IgE-dependent diseases: modern approaches to their treatment
4. Immune system and carcinogenesis. Basic antitumor immune mechanisms
5. Basic principles of laboratory diagnosis of allergy and cancer patients before and after vaccine treatment
6. Classical and modern views on immune-dependent infertility, which is formed after the use of contraceptive vaccines

Main part

The concept of vaccination has now expanded beyond interventions that prevent disease, and towards approaches that target disease-specific antigens to treat or ameliorate ongoing pathology. These therapeutic vaccines stem from the realization that in addition to eliciting new immune responses in naïve individuals, vaccines are capable of enhancing pre-existing immunity and modulate its type to better tackle the targeted disease (e.g., systemic vs. mucosal; Th1 vs. Th2). Antigen specific immunization has the potential to alter not only the course of acute and chronic infectious illness, but autoimmunity, graft rejection, and cancer. However, in contrast to the success of prophylactic vaccines against hepatitis B virus (HBV) and human papillomavirus (HPV) in preventing liver and cervical cancer, most of the clinically tested cancer therapeutic vaccines have shown at best a modest efficacy. One of the reasons for this is that many immunogens trialled in these vaccines have been non-mutated self-antigens to which natural tolerance has been induced and therefore only weak anti-tumour responses are achieved. Conversely, viral antigens and mutated self (neo) antigens that are not subject to thymus-induced tolerance can now be identified through genomics and proteomics practices, offering a diverse range of personalized tumour specific-antigens with the potential to overcome the problems of innate or tumour induced tolerance. In addition to the comprehensive definition of relevant antigens, improvements in vaccine delivery technologies, including more powerful adjuvants and novel antigen expression systems, have returned antigen-based therapies to the spotlight. Importantly, the past 10 years of cancer treatment and management has dramatically improved with the discovery and adoption of immune checkpoint inhibitor monoclonal antibodies (ICIs), which in combination with tumour-antigen specific vaccines are being trialled to treat some of the most devastating cancer types, and are currently showing promising results.

A **therapeutic vaccine** is a vaccine which is administered after a disease or infection has already occurred. A therapeutic vaccine works by activating the immune system of a patient to fight an infection. A therapeutic vaccine differs from a prophylactic vaccine in that prophylactic vaccines are ad-

ministered to individuals as a precautionary measure to avoid the infection or disease while therapeutic vaccines are administered after the individual is already affected by the disease or infection. A therapeutic vaccine fights an existing infection in the body rather than immunizing the body for protection against future diseases and infections. Therapeutic vaccines are mostly used against viral infections. Patients affected with chronic viral infections are administered with therapeutic vaccines, as their immune system is not able to produce enough efficient antibodies.

Allergic diseases represent a complex innate and adoptive immune response to natural environmental allergens with Th2-type T cells and allergenspecific IgE predominance. ***Allergen-specific immunotherapy*** is the most effective therapeutic approach for disregulated immune response towards allergens by enhancing immune tolerance mechanisms. The main aim of immunotherapy is the generation of allergen nonresponsive or tolerant T cells in sensitized patients and downregulation of predominant T cell- and IgE-mediated immune responses. During allergen-specific immunotherapy, T regulatory cells are generated, which secrete IL-10 and induce allergen-specific B cells for the production of IgG4 antibodies. These mechanisms induce tolerance to antigens that reduces allergic symptoms. Although current knowledge highlights the role of T regulatory cell-mediated immunetolerance, definite mechanisms that lead to a successful clinical outcomes of allergen-specific immunotherapy still remains an open area of research.

MECHANISMS OF ALLERGEN-SPECIFIC IMMUNOTHERAPY

The primary purpose of allergen-SIT is the induction of peripheral T cell tolerance to allergens. Once peripheral T cell tolerance is triggered, allergen-specific Treg cells produce elaveted levels of IL-10 and TGF-β which are anti-inflammatory cytokines. The cytokines secreted from Treg cells mostly depend on the type of organ they dwell and the path in which they are stimulated. Experimental and clinical data revealed that Treg cells may secrete only IL-10, IL-10, and IFN-γ or IL-10 and TGF-β.Treg cells do not only suppress Th2 immune response and peripheral tolerance is achieved with multiple mechanism to overcome and suppress allergic inflammation. The other roles of Treg cells are suppression of dendritic cells and by this, enhance the generation of effector or induction of dendritic cells that support the generation of Treg cells, suppression of Th1 and Th2 cells, suppression of allergen-specific IgE and induction of IgG4 and/or IgA, suppression of mast cells, basophils and eosinophils, interaction with resident cells and remodelling. In SCIT both circulating and mucosal Tregs form and these Tregs may induce B cells to produce protective IgG4 antibodies and IgA2 antibodies. Proliferative response of T cell clones are also inhibited by IgG4 antibodies through prevention of IgE-faciliated allergen binding to B cells and subsequent presentation to allergen-specific T cell clones (**Figure 2**). Mucosal immunotherapy to house dust mite, birch polen or food antigens leads to induction of Tr1 cells, which secrete IL10 and TGF-β. Treg cells stimulated with toll-like receptors produce IL-10 and IFN-γ. After venom allergen-SIT, there is a induction of Tr1 cells producing only IL-10. Overall, it is obvious that allergen-SIT has a modulatory effect on allergen-specific T cells. In the mechanism of succesful allergen-SIT, shift in Th2 immunity to Th1 immune response is observed in peripheral blood, allergic rinitis and cutaneous late phase responses. One of the studies carried on patients with allergic rihinitis revealed that after grass pollen immunotherapy, Foxp3+ CD25+ and Foxp3+ CD4+ cells numbers were found to be increased in the nasal mucosa. Also after this allergen-SIT, IL 10-producing Tr1 cells increased with supporting the role of Treg cells in the induction of allergen-specific tolerance in the humans. A very recent study showed that SIT with grass pollen extract leads to an increase in Foxp3+ cells in the sublingual epithelium.

bodies through prevention of IgE-faciliated allergen binding to creased in the nasal mucosa.[111] Also after this allergen-SIT, IL-

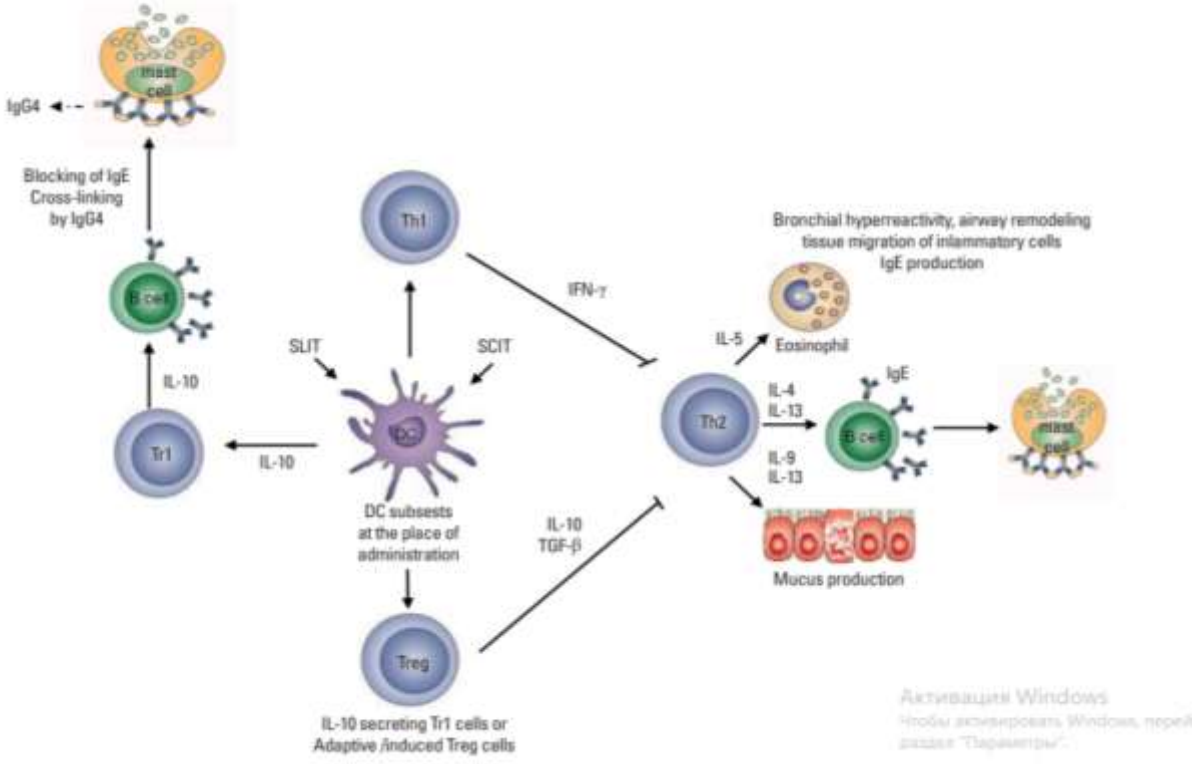

Figure 2. Mechanisms of allergen-specific immunotherapy. Both subcutaneous and sublingual SITs first affect the regional antigen-presenting cell, namely the local dendritic cell subset in the place of administration and draining lymph nodes. Although in vivo mechanisms are not clearly known, these dendritic cells induce Treg (CD4+CD25+FoxP3+) cells and Tr1 cells (IL-10+). Treg cells and regulatory cytokines (such as interleukin-10 (IL-10) and transforming growth factor-β, TGFβ) may contribute to the control of allergen-induced immune responses in several different ways. TReg cells utilize multiple suppressor factors to regulate the immune response. IL-10 and TGF-β suppress IgE production and IL-10 induces inflammatory immunoglobulin isotype, IgG4. These two cytokines directly suppress allergic inflammation induced by effector cells such as mast cells, basophils and eosinophils. TReg cells influence the generation of dendritic cells and promote the development of IL-10- producing dendritic cells. In addition, TReg cells inhibit Th2 cells, which can no longer provide cytokines such as IL-3, IL-4, IL-5, and IL-9. These cytokines are required for the differentiation, survival and activity of mast cells, basophils, eosinophils and mucus producing cells, as well as for the tissue homing of Th2 cells. SIT, specific immunotherapy; SLIT, sublingual immunotheraphy; SCIT, subcutaneous immunotheraphy; Treg, T regulatory cells.

Therapeutic cancer vaccines

Cancer is a common and potentially deadly disease. Some of the cancers may be difficult to treat by conventional means such as surgery, radiation, and chemotherapy, but may be controlled by the stimulation of the immune response of the body with the help of cancer vaccines. The use of vaccines for preventing infections by oncogenic viruses such as hepatitis B virus and human papilloma virus has been extremely successful in reducing the incidence of cancers resulting from these infections. The use of vaccines for treating cancers that are not due to viral infections and that are already established is currently the object of numerous clinical trials. Several types of cancer vaccines are being tried. These include antigen vaccines, tumor cell vaccines, dendritic vaccines, deoxyribonucleic acid vaccines, and viral vector vaccines. The development of these therapeutic vaccines is proving difficult with only 1 recent success. However, there is significant enthusiasm and optimism regarding the development of effective therapeutic vaccines stemming from the fact that

our understanding regarding the cancer immunology is considerably enhanced in recent years. This expanded knowledge regarding the mechanisms that cancers use to escape the immune system is likely to open new avenues in modulating the immune response to cancer, thus enhancing the effectiveness of therapeutic cancer vaccines.

Therapeutic cancer vaccines have undergone a resurgence in the past decade. A better understanding of the breadth of tumour-associated antigens, the native immune response and development of novel technologies for antigen delivery has facilitated improved vaccine design. The goal of therapeutic cancer vaccines is to induce tumour regression, eradicate minimal residual disease, establish lasting antitumour memory and avoid non-specific or adverse reactions. However, tumour-induced immunosuppression and immunoresistance pose significant challenges to achieving this goal. In this Review, we deliberate on how to improve and expand the antigen repertoire for vaccines, consider developments in vaccine platforms and explore antigen-agnostic in situ vaccines. Furthermore, we summarize the reasons for failure of cancer vaccines in the past and provide an overview of various mechanisms of resistance posed by the tumour. Finally, we propose strategies for combining suitable vaccine platforms with novel immunomodulatory approaches and standard-of-care treatments for overcoming tumour resistance and enhancing clinical efficacy.

Targeted Antigens

In the context of antitumor vaccine improvement, significant efforts have been focused on the choice of tumor antigen to target. Although numerous cancer vaccine strategies have been studied, targeted antigens remain at the heart of the discussions. They are classified into two main categories.

Tumor-associated antigens (TAAs) are expressed by tumor cells and also by normal cells such as overexpressed antigens (Her2/neu, survivin, MUC-1 …), cancer testis antigens (MAGE-3, NY-ESO-1 …), or differentiation antigens (Mart1, PSA, PAP …). Although TAAs are expressed at a certain level by normal cells, their immunogenicity induces specific T-cell responses. However, a certain degree of self-tolerance can be applied to TAAs.
In the case of tumor-specific antigens (TSAs), such as oncogenic viral proteins in virally induced cancers or neoantigens generated by non-silent somatic mutations of normal proteins, such central thymic tolerance is bypassed, being regarded as foreign antigens by the immune system. Neoantigen-based vaccine strategies have shown specific anti-tumor immunity in numerous preclinical models and have been tested in early (Phase I) human clinical trials with very promising results.

Although therapeutic cancer vaccines have been associated with past failures, the era of combinatorial strategies in the treatment of cancer prompts their reconsideration. Strategies have been optimized and immunologic enhancement due to vaccines is now accepted. The overwhelming immunosuppressive tumor microenvironment that reduces the clinical efficacy of vaccines can now be modified by different approaches. Combinations of cancer vaccines and antiangiogenic therapies or ICB have emerged and shown promising results. To date, very impressive results for those combinations described in mice have not yet been recapitulated in humans. However, studies in mice have mainly used sub-cutaneous tumor grafts growing rapidly and representing an early stage of the disease. Conversely, clinical trials mainly concern patients with advanced cancers, i.e., at a late phase of the disease when immunosuppressive mechanisms are induced. Consequently, we currently lack clinical data showing any breakthrough, a better understanding of the tumor microenvironment will allow us to consider new combinations. Questions remain concerning the timing of treatments, adjuvants, immunization routes, optimal immunogenic vaccines, and tumor remodeling. There is also a need to set up clinical trials in patients at early disease stages. Combinations including newly developed ICB or costimulatory pathways as well as other antiangiogenic strategies such as vaccines directly targeting angiogenic compounds could also bring new hope and lead to clinical success. Finally, in the near future, multiple therapies involving distinct but complementary aspects of antitumor responses may be considered as the combination of vaccines, antiangiogenic therapies and ICB.

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