BT302 – IMMUNOLOGY

MID TERM MOST IMPORTANT QUESTIONS

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1. What are monoclonal antibodies?

The antibodies which are produced from a single antibody producing B-cell are called as monoclonal antibodies. These antibodies also able to bind with single and unique antigen binding sit (epitope). Monoclonal antibodies mainly consist of single subtype of IgG for example IgG1, IgG2 & IgG3.

2. How can we control extracellular pathogens?

Antibodies control the extracellular pathogens by the following three important ways:

- ***Neutralization:*** The pathogens or foreign substance are blocked to bind with their targets as antibodies bind with pathogens. For example, antibodies to bacterial toxins can prevent the binding of the toxin to host cells thereby rendering the toxin ineffective. Similarly, antibody binding to a virus or bacterial pathogen can block the attachment of the pathogen to its target cell thereby preventing infection or colonization.
- ***Opsonization:*** Antibody binding to a pathogen or foreign substance can opsonize the material and facilitate its uptake and destruction by phagocytic cells. The Fc region of the antibody interacts with Fc receptors on phagocytic cells rendering the pathogen more readily phagocytosed.
- ***Complement activation:*** Activation of the complement cascade by antibody can result in lysis of certain bacteria and viruses. In addition, some components of the complement cascade (e.g. C3b) opsonize pathogens and facilitate their uptake via complement receptors on phagocytic cells.

3. Explain Adjuvants and its role in immune system.

Adjuvants:

Substances that can increase the immune response to an immunogen are called adjuvants. The use of adjuvants, however, is often hampered by undesirable side effects such as fever and inflammation.

Role of Adjuvants in Polyclonal Antibodies:

Production Adjuvants are the substances which potentiates immune response against antigens. These have the ability to modulate towards desired immune response. However, with adjuvants there are mostly undesirable effects like cellular toxicity. That's why the selection of adjuvant is being made in order to get maximum immunostimulation. For the production of polyclonal antibodies following adjuvants are used:

1. Freund's Complete Adjuvants
2. Freund's Incomplete Adjuvants

4. Write applications of ELISA.

Applications of ELISA:

Followings are the significant applications of ELISA:

- It is used for determining viral antigens in patient's serum e.g Hepatitis B surface Antigens (HbsAG)
- Also used for the determination of antibodies against viruses e.g Anti Hepatitis C Virus (HCV) antibodies
- Also used for estimating the levels of variou serum cytokine e.g Interleukin-1 (IL-1), Tumor necrotic factor alpha (TNF-a) etc.

5. Differentiate between Direct and Indirect Elisa.

Direct ELISA

In this type of ELISA, unknown antibody can be determined in patient's serum. Principally, known antigen is coated on solid phase which can be a Glass or plastic surface. Patient's serum containing unknown antibody is added for reacting with the specific coated antigen. Afterward, another antibody which is pre-coated with an enzyme e.g horseradish peroxidase is usually used. Then the activity of enzyme is determined by adding its substrate i.e hydrogen peroxide in the form of colour change in the reaction mixture. Moreover, the intensity of color determines the concentration of unknown antibody in the patient's serum indirectly.

Indirect ELISA

In this type of ELISA, unknown antigen can be determined in patient's serum. Principally, known antibody against the unknown antigen is coated on solid phase which can be a Glass or plastic surface. Patient's serum containing unknown antigen is added for reacting with the specific coated antibody. Afterward, another antibody which is pre-coated with an enzyme e.g horseradish peroxidase is usually used, thus the unknown antigen get sandwiched between two antibodies, that's why it is also called as sandwiched ELISA. Then the activity of enzyme is determined by adding its substrate i.e hydrogen peroxide in the form of color change in the reaction mixture. Moreover, the intensity of color determines the concentration of unknown antibody in the patient's serum indirectly.

6. Explain oxygen dependent and independent intracellular killing.

Oxygen Independent:

In this process of intracellular killing of bacteria, there is no need of oxygen. The granules and vesicles of phagocytes secrete various enzymes which are hydrolytic proteins .Those proteins are bacteriocidal in nature according to their modes of action.

Oxygen Dependent:

There is requirement of oxygen for such kind of intracellular killing of bacteria. It is also termed as "Respiratory Burst" as requirement of glucose and oxygen get increased after phagocytosis. As a result, oxygen containing bacteriocidal radicals are produced. This process can be of two types:

1- Oxygen dependent Myeloperoxidase (MPO) dependent
2- Oxygen Dependent Myeloperoxidase (MPO) independent

7. Explain structure of T cell.

Structure of T-Cell Receptor (TCR):

The surface receptor which is present on T-lymphocytes for binding with antigen bounded with major histocompatibility complex (MHC) is called as T-cell receptor (TCR). This receptor is just like immunoglobulin in its structure, all those structures which resemble with immunoglobulin structures are called as immunoglobulin superfamily. That's why TCR is the part of immunoglobulin superfamily. TCR is a heterodimer surface receptor TCR is composed of two following chains of equal length

1. A (α) Chain
2. B (β) Chain

These two chains are anchored through the cell membrane of T- cells through transmembrane region which has hydrophobic amino acids. A short cytoplasmic tail also exist at the end of transmembrane region which is not sufficient for signal transduction. These two chains of TCR also contain disulfide bridge between them. Both of these chains also contain carbohydrate moiety. Structurally, both chains contain constant (C) & variable (V) regions like immunoglobulin molecule (as show in Figure. 9.1). The variable regions of both chains contain hyper variable regions which determine the specificity of TCR. Likewise immunoglobulin, each TCR carry single specificity for a single antigen.

8. What is Variable and Constant region?

Variable & constant regions:

The regions in heavy& light chains which are conserved in nature are called as constant regions and represented as CH & CL respectively. On the other hand, the regions on heavy & light chains which are not conserved and variable in nature are called as variable regions and designated as VH & VL respectively.

9. What is construct validity?

Construct validity concerns the identification of the causes, effects, settings, and participants that are present in a study. For example, a medication might have an effect not because its putative active ingredients are absorbed into the bloodstream but because of its placebo effects.

10. Enlist the specific features of scientific method.

The scientific method is a systematic approach to inquiry that involves a series of steps designed to gather and interpret evidence in a way that minimizes bias and error. While there can be some variation in how the scientific method is described, it generally includes the following key features:

1. Observation:

- The process begins with the observation of a phenomenon or a set of observations related to a particular question.

2. Question/Problem Formulation:

- Based on observations, a question is formulated or a problem is identified that the scientist seeks to address or explain.

3. Hypothesis:

- A hypothesis is a testable and falsifiable statement that proposes a possible explanation for the observed phenomenon. It is often formulated as an "if-then" statement.

4. Prediction:

- The hypothesis leads to specific predictions about what should happen under certain conditions if the hypothesis is correct.

5. Experimentation:

- Controlled experiments are designed and conducted to test the predictions and gather empirical evidence. Variables are manipulated, and the outcomes are carefully observed and measured.

6. Data Collection:

- Systematic and careful collection of relevant data during the experiment is essential. This may involve quantitative measurements, qualitative observations, or a combination of both.

7. Analysis:

- The collected data are analyzed using statistical and other methods to determine if the results support or reject the hypothesis. This step involves drawing conclusions based on the evidence.

8. Conclusion:

- Conclusions are drawn based on the analysis of the data. These conclusions may support or refute the initial hypothesis. It's important to note that the scientific method does not prove hypotheses; rather, it provides evidence to support or reject them.

11. Write importance of immune system.

The importance of the immune system for health is evident by the conditions when individual suffering from various fetal diseases in case of defective immune response. The most important function of immune system is to protect the body from the attack of infectious or invading microbes. If the individual's immune system does not work properly then the chances of acquiring infections also increased for example in case of Acquired immunodeficiency syndrome (AIDS). This indicates the importance of immune system for defending against infections. Similarly, there is also very significant role of immune system in organ transplantation. Immune response act as a barrier for successful organ transplantation as immune system recognizes and responds to tissue graft and newly introduced proteins. Immune system also provides surveillance against tumors and cancers. Moreover, for treatment of cancers immunotherapy also have good implications. The products of immune system like antibodies are highly specific molecules for detecting any kind of molecules. Likewise, antibodies are widely used for developing immunologic approaches for laboratory testing in clinical medicine and research.

12. Differentiate between TCR and BCR.

TCR (T-cell receptor) and BCR (B-cell receptor) are key components of the adaptive immune system. TCR is expressed on the surface of T cells and recognizes antigens presented by major histocompatibility complex (MHC) molecules. TCR facilitates cell-mediated immune responses. In contrast, BCR is found on B cells and binds to antigens directly. BCR initiates humoral immune responses by leading to the production of antibodies. While both receptors play crucial roles in recognizing and responding to antigens, TCR interacts with peptide-MHC complexes, and BCR directly binds to antigens, reflecting the distinct mechanisms through which T cells and B cells contribute to immune surveillance and defense.

13. What are domains of Immunoglobulins?

Immunoglobulins, also known as antibodies, consist of different domains that contribute to their structure and function. The basic structural unit of an immunoglobulin includes two identical heavy chains and two identical light chains. The domains in each chain are:

1. ***Variable (V) Domain:*** Located at the N-terminus of both the heavy and light chains, the V domain is highly variable among different antibodies and is crucial for antigen binding diversity.

2. ***Constant (C) Domain:*** Found at the C-terminus, the C domain determines the antibody's class (IgM, IgD, IgG, IgA, or IgE) and effector functions.

3. ***Hinge Region:*** Connecting the Fab (antigen-binding) and Fc (effector) regions in the heavy chains, the hinge allows flexibility and movement of the antibody fragments.

These domains collectively contribute to the versatility and specificity of antibodies in recognizing and responding to a wide range of antigens.

14. Define phagocytosis. Explain the process of phagocytosis.

Phagocytosis is a vital cellular process by which certain cells engulf and digest solid particles, such as bacteria, cellular debris, and other foreign particles. This mechanism is primarily employed by specialized cells known as phagocytes, which are part of the body's immune system. Phagocytes play a crucial role in protecting the body against infections and maintaining tissue homeostasis.

The process of phagocytosis can be broken down into several stages:

1. ***Chemotaxis:*** Phagocytes are attracted to the site of infection or inflammation by chemical signals released by damaged cells, bacteria, or other foreign substances. This movement toward the source of the signals is known as chemotaxis.

2. ***Attachment:*** The phagocyte adheres to the surface of the foreign particle or microorganism. This is facilitated by specific receptors on the surface of the phagocyte that recognize and bind to molecular patterns present on the target.

3. ***Engulfment:*** The phagocyte extends its cell membrane to surround the particle, forming a pocket-like structure called a phagosome. The engulfed particle is now contained within the phagosome, which is essentially a vesicle formed by the cell membrane.

4. ***Phagosome Formation:*** The phagosome undergoes a series of maturation steps as it moves deeper into the cell. This involves fusion with intracellular vesicles, such as lysosomes, which contain enzymes capable of breaking down the engulfed material.

5. ***Digestion:*** The contents of the phagosome are broken down by enzymes within the lysosomes. This process, known as intracellular digestion, releases nutrients and eliminates potential threats to the organism.

6. ***Exocytosis:*** After digestion, the indigestible material is expelled from the cell through a process called exocytosis. The remaining waste products are released into the extracellular space.

15. Explain briefly pathogen associated molecular patterns.

The antigenic determinant recognized by innate immune system is purely non-specific in nature due to nonspecific attribute of innate immune system. These determinants are present on the surface of the pathogens in a form of various molecular configurations called as pathogen associated molecular patterns (PAMPS). PAMPs include cell membrane proteins, lipopolysaccharide and flagellular proteins. For these antigenic determinants immune cells carry pattern recognition receptors (PRRs) which bind non-specifically with corresponding PAMPs in order to generate immune response.

16. Define lymphocyte recirculation.

Naive (virgin) lymphocytes enter the lymph nodes from the blood via High Endothelial Venules (HEVs). Homing receptors on the lymphocytes direct the cells to the HEVs. It is estimated that about 1-2% lymphocytes recirculate every hour. In the lymph nodes, lymphocytes with the appropriate antigen receptor encounter antigen, which has been transported to the lymph nodes by dendritic cells or macrophages. This process of activation of lymphocytes is called as priming and lymphocytes are called primed lymphocytes. After activation the lymphocytes express new receptors that allow the cells to leave the lymph node and reenter the circulation. Receptors on the activated lymphocytes recognize cell adhesion molecules expressed on endothelial cells near the site of an infection and chemokines produced at the infection site help attract the activated cells.

17. Differentiate between T-dependent and T-independent antigens.

T-cell dependent antigens:

These are antigens which need the help of T-cells for mounting an immune response otherwise these cannot. T-dependent requires the help of T-cells for activation of B-cells. Such kinds of antigens are protein in nature. Moreover, these antigens carry variety of antigenic determinants (epitopes) with few copies.

T-Cell independent antigens:

These antigens are those antigens which can directly activate B-cells without the help of T-cells. Such kinds of antigens are non-protein and usually are polysaccharides in nature. These antigens carry same kind of epitopes in polymeric form (Figure 3.3). Moreover, such kinds of antigens are most resistant to degradation.

18. Write note on physical barrier.

In immunology, a physical barrier refers to the first line of defense against pathogens provided by the body's anatomical structures. The skin and mucous membranes act as physical barriers to prevent the entry of microorganisms and other harmful substances. The skin serves as a protective outer layer, while mucous membranes line the respiratory, digestive, and urogenital tracts, producing mucus that traps and expels pathogens. These physical barriers play a crucial role in preventing infections and maintaining homeostasis. Additionally, structures like cilia and the mucociliary escalator in the respiratory tract aid in moving trapped particles away from the lungs, reinforcing the body's defense against external threats.

19. Explain basic structure of antibodies.

Basic Structure of Immunoglobulin

Immunoglobulins composed of following important structures:

- ***Heavy & Light chains:*** Typically antibody composed of four chains, Two (02) identical light chains and two (02) identical heavy chains. Light chains are of two kinds: Kappa light Chains and Lambda light chains While heavy chains are of five following types: Gamma Heavy chain, Mu Heavy chain, Delta Heavy Chain, epsilon heavy chain, alpha heavy chain.
- ***Disulfide bonds:*** The heavy & light chains of immunoglobulin have peptide linkage in the form of disulfide bonds, These linkages are of Interchain which are in between light & heavy chain while Intrachain disulfide linkages are inside either heavy or light chains.
- ***Variable & constant regions:*** The regions in heavy& light chains which are conserved in nature are called as constant regions and represented as CH & CL respectively. On the other hand, the regions on heavy & light chains which are not conserved and variable in nature are called as variable regions and designated as VH & VL respectively.
- ***Hinge region:*** This region makes antibodies flexible to change its shape while performing function after binding with its corresponding antigens.
- ***Domains:*** These are folded regions which contain an intra-chain disulfide bond. Usually, heavy chains contain four domains while light chains composed on two domains.
- ***Oligosaccharides:*** These are carbohydrates which are attached on CH2 domain in most of immunoglobulins.

20- Write factors effecting Ag/Ab test.

Factors Effecting on Antigen & Antibody Tests:

The good antigen & antibody reactions are known due to high specific & sensitive nature. Various factors are involved which determine the nature of antigen and antibody reactions which are as follows:

Affinity:

It is the strength of binding between single antigenic determinant & single antibody site. These are the sum of attractive & repulsive forces which make the Ag/Ab reactions stronger. Higher the affinity of Ab for Ag, more stable interaction. Similarly, if the affinity would be weak between Ag & Ab then the reaction would be less stable.

Avidity:

Avidity is the strength of binding of antigen with many antigenic determinant & multivalent antibody. It is also called as overall strength between multivalent antigens & antibodies. In contrast to affinity, avidity is influenced by valency of both Ag & Ab. Multivalent antibodies have more avidity as compare to univalent or oligovalent antibodies. That's why, the Ag/Ab reactions which have more avidity those are more stable & easy to detect.

Antigen & Antibody Ratio:

Ag/Ab ratio also considered as one of the important factor effecting on Ag/Ab reactions. It also determines the complex nature of Ag/Ab complex formation. For good Ag/Ab reaction, there would be the equal ratio between Ag/Ab and that point is also called as equivalence point for Ag/Ab reaction. As a result a complex structure develops which is called as lattice which is easy to detect. However, in the case of unequal ratio between Ag/Ab doesn't form such kind of complex structure.

Physical Form:

Physical form of antigen & antibody also determines the nature of Ag/Ab reaction. Antigen and antibody can exsist in the following two physical forms:

a) Particulate form
b) Soluble form

In case of particulate form i.e the presence of Ag or Ab on the surface of any particle like red blood cells, bacteria or latex particles lead a kind of Ag/Ab reaction called as agglutination reactions. However, in case of soluble form i.e in secretions or serum form precipitate form and these reactions are called as precipitation reaction.

21- How to determine secretory and membrane bound immunoglobulin?

Immunoglobulins (Ig), also known as antibodies, are proteins produced by the immune system to recognize and neutralize foreign substances like bacteria and viruses. There are different classes of immunoglobulins, and they can exist in various forms, including secretory and membrane-bound.

To determine whether an immunoglobulin is secretory or membrane-bound, you typically need to consider the techniques for determination.

Techniques for Determination:

1. Immunohistochemistry (IHC):

- **For membrane-bound Ig:** IHC can be used to detect the presence of membrane-bound immunoglobulins on the surface of cells in tissues.

2. ELISA (Enzyme-Linked Immunosorbent Assay):

- **For secretory Ig:** ELISA can be employed to quantify the concentration of secretory immunoglobulins in body fluids.

3. Western Blotting:

- **For both types:** Western blotting can be utilized to detect and analyze the presence of immunoglobulins in different samples.

22- Explain physical response in immune system.

The physical response in the immune system involves various mechanisms and components that work together to defend the body against harmful pathogens such as bacteria, viruses, and other foreign invaders. This response is a crucial part of the overall immune system, which is responsible for maintaining the body's health and preventing infections.

The physical response in the immune system can be categorized into two main components: the innate immune system and the adaptive immune system.

1. Innate Immune System:

- **Physical Barriers:** The first line of defense includes physical barriers like the skin and mucous membranes, which act as protective layers preventing pathogens from entering the body.
- **Phagocytosis:** Specialized cells such as macrophages and neutrophils engulf and digest pathogens through a process called phagocytosis. These cells are part of the innate immune system and provide a rapid response to infections.

2. Adaptive Immune System:

- **Cellular Response:** T cells, a type of white blood cell, play a central role in the cellular response. They can directly attack and destroy infected cells.
- **Humoral Response:** B cells, another type of white blood cell, are involved in the production of antibodies. Antibodies are proteins that circulate in the blood and other bodily fluids, binding to pathogens and marking them for destruction or neutralization by other components of the immune system.

- **Memory Cells:** Both T cells and B cells have memory cells that "remember" specific pathogens. If the same pathogen enters the body in the future, the immune system can mount a faster and more targeted response.

3. Inflammatory Response:

- When tissues are damaged or infected, the immune system triggers an inflammatory response. This involves the release of chemical signals (such as cytokines) that attract immune cells to the site of infection or injury.
- Inflammation helps contain and eliminate the pathogen, as well as promote tissue repair.

4. Complement System:

- The complement system is a group of proteins that enhance the immune response. These proteins can directly attack pathogens, promote phagocytosis, and contribute to the inflammatory response.

23- Write general characteristics of antibodies.

General Characteristics of Antibody Response:

Antibody formation process is well controlled mechanisms with following significant characteristics

1) Self/Non-Self Discrimination
2) Memory
3) Specificity

Self/Non-Self Discrimination:

The antibodies which are generated during antibody formation process would be reactive only against nonself (foreign) antigens. If there is any reactivity against self-antigen then that is very important clinical condition which is called as autoimmunity meaning destruction of self-tissues by antibodies.

Memory:

Remembering the nature of antigen is another important significant characteristic of antibody formation process. There is an illicit memory response against same antigen in case of again and again exposure. Memory response is also called as anamnestic response. This memory response facilitate the robust way of clearing non-self (foreign) antigen. There are specific cells for exhibiting the memory which are called as Blymphocytes memory cells.

Specificity:

The antibodies have high degree of specificity against specific antigens. This form of specificity lessens the chances of cross reactivity with irrelevant antigens. Moreover, this characteristic of specificity is the characteristic of adaptive immune response.

24- What is Humoral response?

The humoral response is a crucial aspect of the adaptive immune system, involving B cells. When these white blood cells encounter specific antigens on pathogens, they undergo activation and differentiation. This process gives rise to plasma cells that produce antibodies—proteins capable of recognizing and binding to antigens. Antibodies circulate in the bloodstream, targeting and neutralizing pathogens, marking them for destruction or facilitating their removal by other immune cells. Additionally, memory B cells are generated to provide a rapid and heightened response upon subsequent encounters with the same antigen, contributing to the immune system's ability to mount targeted and efficient defenses against infections.

25- What is MHC, MHC-I and MHC-II?

MHC stands for Major Histocompatibility Complex, which is a group of genes that code for proteins found on the surfaces of cells. These proteins play a crucial role in the immune system by presenting antigens to T cells, which are a type of white blood cell. The MHC is also known as the human leukocyte antigen (HLA) system in humans.

There are two main classes of MHC molecules: MHC class I and MHC class II.

1. MHC Class I:

- Found on the surface of almost all nucleated cells in the body.
- Presents antigens derived from intracellular pathogens, such as viruses and certain bacteria.
- It plays a crucial role in the detection of infected or abnormal cells by cytotoxic T cells (CD8+ T cells).
- MHC class I molecules present endogenous antigens, meaning they come from within the cell.

2. MHC Class II:

- Found on the surface of antigen-presenting cells (APCs) such as macrophages, dendritic cells, and B cells.
- Presents antigens derived from extracellular pathogens, like bacteria and parasites.
- It is involved in the activation of helper T cells (CD4+ T cells).
- MHC class II molecules present exogenous antigens, meaning they come from outside the cell and have been engulfed by the cell.

26-