PHARMACEUTICAL MICROBIOLOGY and IMMUNOLOGY

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Immunology

OBJECTIVES

- Definition of Immunology
- What is an Antibody (Immunoglobulin)? Basic Antibody Structure, Classes and Functions of Immunoglobulins
- Monoclonal Antibodies
- Cells and Organs of Immune System
- Humoral and Cell-Mediated Immune Responses
- Primary and Secondary Immune Responses
- Immunological Tolerance

Definition of Immunology

Immunology is the study of the body's mechanisms that discriminate between self and non-self and eliminate non-self components such as infectious agents.

What are non-self components?

- These non-self components are antigens.
- **Antigens** are substances that are capable of stimulating an immune response.

- Antigens that are found on the body's own cells are known as '**self-antigens**' and the immune system does not normally attack these.

Definition of Immunology

What are non-self components?

For example; the membrane of each red blood cell contains millions of antigens that are ignored by the immune system. However, when patients receive blood transfusions, their immune system will attack any donor red blood cells that contain antigens that differ from their self antigens. Therefore ensuring that the antigens of transfused red blood cells match those of the patients red blood cell is essential for a safe blood transfusion.

(red blood cell antigens determine your blood group)

Definition of Immunology

What are non-self components?

Microorganisms such as bacteria, viruses, parasites or fungi include antigenic parts.

The immune system consists of a wide array of cells, soluble molecules and tissues distributed all over the body. Surveillance and destruction of substances that are foreign to the body is the primary role of the immune system.

Often these foreign substances are microorganisms such as bacteria, viruses, parasites or fungi.

Immunoglobulin (Antibody)

- An antibody (Ab), also known as an immunoglobulin (Ig), is a large, Y-shaped protein produced mainly by plasma cells that is used by the immune system to identify and neutralize pathogens such as bacteria and viruses.

- Each antibody binds to a specific antigen; an interaction similar to a lock and key.

Immunoglobulin (Antibody)

- Antibodies are specific globular proteins secreted by B-lymphocytes (plasma cells) in response to stimulation by the appropriate antigen.
- They are capable of reacting with the antigen to neutralise a pathogen.
- **B cells differentiate into plasma cells that produce antibody molecules**.

General functions of immunoglobulins

Antigen binding

- Immunoglobulins bind specifically to one or a few closely related antigens. Each immunoglobulin binds to a specific antigenic determinant.

- Antigen binding by antibodies is the primary function of antibodies and can result in protection of the host.

General functions of immunoglobulins

- The valency of antibody refers to the number of antigenic determinants that an individual antibody molecule can bind.
- The valency of all antibodies is at least two and in some instances more.

Effector functions

- **Complement activation** - This results in lysis of cells and release of biologically active molecules.

General functions of immunoglobulins

Effector functions

- **Binding to various cell types** - Phagocytic cells, lymphocytes, platelets, mast cells, and basophils have receptors that bind immunoglobulins. This binding can activate the cells to perform some function.

Immunoglobulins (Antibodies)

All immunoglobulin molecules consist of two identical **light (L) chains** and two identical **heavy (H) chains**, held together as a tetramer (L2H2) by inter-chain **disulfide bonds**.

The arrangement of the four polypeptide chains in an immunoglobulin molecule gives it a “Y” shape.

Each L chain is connected to a H chain and the two H chains are connected to one another by **disulfide bridges**.

Immunoglobulins (Antibodies)

Light and Heavy chains are subdivided into **variable** and **constant regions**.

Each heavy and light chain contains **amino terminal** in variable region, **carboxy terminal** in constant region. The variable regions of both the light and heavy chains are responsible for antigen-binding.

The constant region of the heavy chain is responsible for various biologic functions, e.g., complement activation and binding to cell surface receptors. The complement binding site is in the CH_2 domain. The constant region of the light chain has no known biological function.

Immunoglobulins (Antibodies)

- **Paratope (antigen-binding site)**, is a part of an antibody which recognizes and binds to an antigen.
- The part of the antigen to which the paratope binds is called an **epitope**.

If an antibody molecule is treated with a proteolytic enzyme such as papain, peptide bonds in the "hinge" region are broken, producing two identical **Fab fragments,** and **one Fc fragment.**

Immunoglobulins (Antibodies)

The variable regions are responsible for antigen-binding, whereas the constant regions are responsible for various biologic functions, eg, complement activation and binding to cell surface receptors, placental transfer.

Immunoglobulin G

Ig G is the predominant immunoglobulin in normal human serum (representing approximately 70-75% of serum antibodies)

It is the major immunoglobulin in blood, lymph fluid, cerebrospinal fluid, and peritoneal fluid.

Ig G is the only immunoglobulin able to pass through the placenta before birth (It is the only antibody that can pass from mother to fetus).

Immunoglobulin G

Level of an infant's own Ig G start to rise after birth and reach adult levels at about 2 years of age.

Ig G has two antigen binding sites (Fab fragments). It is bivalent.

It is the second type of antibody synthesized against antigen.

Ig G antibodies play role in precipitation, complement assembly and neutralizing toxins (plays role in the diagnosis of diseases).

Immunoglobulin G

It has a half-life of 23 days, the longest amongst the antibody classes

Ig G is the predominant antibody in the secondary-response and constitutes an important defense against bacteria and viruses (IgM is the main immunoglobulin produced early in the primary response).

Ig G is one of the two immunoglobulins that can activate complement; IgM is the other.

Immunoglobulin M

Ig M constitutes approximately 7-10% of the total serum immunoglobulins

It is a pentamer composed of five H2L2 units. The five units are linked by small polypeptide chain called **J chain**.

It is the first antibody to appear in response to initial exposure to an antigen (first antibodies produced in response to infection) (first antibodies produced in the primary immune response)

Immunoglobulin M

Ig M has a half-life of about 10 days. Short-lived.

High level of immunoglobulin M is indicative of a new contact with the antigen (due to an infection or immunization).

Ig M antibodies do not pass across the human placenta.

It is the first (and only) immunoglobulin synthesized by the fetus (around 20 weeks).

Presence of Ig M antibody in serum of newborn indicate congenital infection (fetus was infected before birth).

Immunoglobulin M

ABO blood group antigens present on red blood cells

The predominant anti-A and anti-B antibodies are Ig M antibodies that do not cross the placenta. (Ig M antibodies present in the serum)

Ig M has the highest molecular weight. Because of its large molecular weight, the majority of Ig M remains intravascular. But due to increased permeability of the capillaries (as a result of inflammation), transition of Ig M gets easier like other plasma proteins.

Immunoglobulin A

Ig A constitutes approximately 10-15% of the total serum immunoglobulins

Ig A has a half-life of 6 days. Short-lived.

In humans, over 80% of serum Ig A exists in a monomeric form. The rest exist as dimers, trimers, or tetramers. In these polymeric forms of Ig A, the monomeric units are linked by disulfide bonds and J chains.

Ig A is present in two forms: one in the serum and the other in various body secretions.

Immunoglobulin A

Ig A is the main immunoglobulin in secretions such as saliva, tears, colostrum and respiratory, intestinal and genital tract secretions. It prevents attachment of microorganisms (e.g. bacteria, viruses) to mucous membranes.

Ig A does not bind complement via the classic pathway but may do so via the alternative pathway.

Secretory Ig A present in breast milk protects newborn during first months of life.

Immunoglobulin A

Secretory Ig A (sIg A) is the predominant immunoglobulin in secretions (saliva, tears, colostrum, bronchial, genitourinary and intestinal secretions).

The sIg A molecule consists of two monomeric units plus J chain and secretory component.

Immunoglobulin A

Secretory Ig A binds to surface antigens of microorganism and prevent its attachment and invasion of the mucosal surfaces of respiratory and digestive tract- immune elimination

Secretory component of sIg A protects the immunoglobulin from being degraded by proteolytic enzymes.

Immunoglobulin D

Ig D constitutes approximately 0.2-1% of the total serum immunoglobulins.

It is a monomer. The structure of Ig D is similar to Ig G.

Ig D has a half-life of 3 days. Short-lived.

Ig D together with IgM is major membrane bound immunoglobulin on unstimulated B lymphocytes. It acts as recognition receptors for antigens.

Immunoglobulin E

The structure of Ig E is similar to Ig G.

It is a monomer. It is mainly located on the surface of mast cells, basophilic and eosinophilic granulocytes.

Serum Ig E level is low.

Ig E does not activate complement.

Ig E's main functions are immunity to parasites (such as helminths) and it plays role in allergic reactions (anaphylactic type of hypersensitivity).

Immunoglobulin E

Ig E has a half-life of 2 days. Short-lived.

The Fc region of Ig E binds to the surface of mast cells and basophils. Bound Ig E serves as a receptor for antigen (allergen).

When the antigen-binding sites of adjacent Ig Es are cross-linked by allergens, several mediators are released by the cells and immediate (anaphylactic) hypersensitivity reactions occur.

Monoclonal Antibodies

Antibodies produced by a clone of single plasma cells and having specificity against single epitope are called “Monoclonal antibodies”.

Monoclonal antibodies;

- are a single type of antibody that are identical
- are directed against a specific epitope (antigen, antigenic determinant)
- are produced by B-cell clones of a single parent

Monoclonal Antibodies

Monoclonal antibodies are produced by the technique called "**Hybridoma technology**".

To make the hybrid cell with desirable properties, two cells are fused.

- **a cancerous tumor cell (myeloma cells)**, able to multiply indefinitely, is fused to

- **a normal antibody-producing B cell**, which is producing the desired antibody

myeloma cells: cancer of plasma cells / a type of white blood cell normally responsible for producing antibodies

Monoclonal Antibodies

Applications of Monoclonal Antibodies

Diagnostic tests: Monoclonal antibodies are used for the laboratory diagnosis of various diseases.

Analytic and chemical uses: Antibodies can also be used to purify their target compounds from mixtures, using the method of immunoprecipitation.

Monoclonal Antibodies

Applications of Monoclonal Antibodies

Therapeutic treatment: Therapeutic monoclonal antibodies act through multiple mechanisms, such as blocking of targeted molecule functions, inducing apoptosis in cells which express the target, or by modulating signaling pathways.

Cancer treatment: One possible treatment for cancer involves monoclonal antibodies that bind only to cancer cell-specific antigens and induce an immune response against the target cancer cell.

Immunity

Immunity is the ability of an organism to resist the diseases by identifying and destroying foreign substances or organisms.

Immune system is a biological system of structures and processes within an organism that protects against diseases by identifying and killing pathogens and tumour cells.

It detects a wide variety of agents, from viruses to parasitic worms, and needs to distinguish them from the organisms healthy cells and tissues in order to function properly.

Immunity

Humoral Immunity
Managed by B cells (antibodies)

Cell Mediated Immunity
Managed by T cells

Cells and Organs of Immune System

The cells playing role in immunity exist in various tissues and organs in our body. **The first source of all these cells is bone marrow.**

With the improvement of the main cells in different ways, the cells creating immune response occur. **The main cells in bone marrow go to primary lymphoid organs before playing a role in the immune system.**

Cells and Organs of Immune System

The cells which completed their differentiation and improvement in primary lymphoid organs then migrate to secondary lymphoid organs.

These improved cells (B and T lymphocytes) located in secondary lymphoid organs, forms humoral and cellular immune response.

Cells and Organs of Immune System

- Tonsils
- Adenoids
- Thymus
- Lymph nodes and veins
- Spleen
- Peyer patches
- Appendix
- Bone marrow

Cells and Organs of Immune System

Primary Lymphoid Organs

- Bone Marrow
- Thymus

Bone Marrow

The bone marrow is the site of generation of all circulating blood cells in the adult, including immature lymphocytes. The main (root) blood cells matured here migrate to blood circulation and then related organs.

B cells mature in the bone marrow

Cells and Organs of Immune System

In birds, B cells mature in the bursa of Fabricius. Mammals do not have bursa of Fabricius.

In mammals, the bone marrow is responsible for the maturation of B lymphocytes.

B cell development starts in the foetal liver. After birth, development continues in the bone marrow.

Cells and Organs of Immune System

Thymus

Thymus, as a primary lymphoid organ, is the organ where T lymphocytes immigrated from bone marrow are matured and turns into mature T lymphocytes.

The lymphoid cells are located in two areas, the outer (cortex) and the inner (medulla).

Cells and Organs of Immune System

Lymphoid cells are dense in cortex. Immature and proliferating cells which come from bone marrow are located in this area. The cells in medulla are mostly matured.

Lymphosites differentiate and mature while immigrating to medulla. Matured lymphosites transit to blood circulation through capillaries.

Lymphosites which differentiated and matured in primary lymphoid organs immigrate to secondary lymphoid organs through blood.

Cells and Organs of Immune System

Secondary Lymphoid Organs

- Spleen (encapsulated lymphatic organ)
- Lymph nodes (encapsulated lymphatic organ)
- Mucous membranes in the digestive, respiratory and genital systems (mucosa-associated lymphoid tissue (MALT)) (non-encapsulated lymphoid organ)

  - Tonsils - Adenoids - Peyer's patches - Appendix

* The thymus, spleen and lymph nodes are all completely surrounded by a connective tissue capsule

Cells and Organs of Immune System

Spleen

- It is the largest secondary lymphoid organ
- It is surrounded by a capsule made of connective tissue
- It acts as a filter for blood as part of the immune system
- Approximately 50% of the spleen cells are B lymphocytes, 30-40% are T lymphocytes.

Cells and Organs of Immune System

Functions of Spleen

- Removes old and damaged red blood cells
- Forms red blood cells in fetal life and granulocytes in postnatal life
- Contributes to formation of antibodies through B lymphocytes
- Phagocytosis of pathogens by macrophages
- Erythrocyte storage

Cells and Organs of Immune System

Lymph Nodes

Lymph nodes are oval or kidney-shaped organs of the lymphatic system, present widely throughout the body including the armpit and stomach and linked by lymphatic vessels.

Lymph nodes are important for the proper functioning of the immune system, acting as filters for microorganisms, antigens and cancer cells.

Cells and Organs of Immune System

Each lymph node is surrounded by a fibrous capsule.

Lymph nodes consist of

- cortex
- paracortex
- medulla

Lymph Nodes

Cortex

- Located under the capsule.
- B lymphocytes - small number of T lymphocytes - Follicular dendritic cells are found

Cells and Organs of Immune System

Lymph nodes have two types of follicles, **primary and secondary.** Secondary follicles contain germinal centers (sites of B-cell proliferation).

Lymph Nodes

Medulla

- Medulla contains plasma cells (antibody-secreting cells)
- Dendritic cells (antigen-presenting cells)

Cells and Organs of Immune System

Paracortex

- T lymphocytes are dense in this region
- Paracortex is the area between cortex and medulla and also between follicles
- In this part 2/3 of the lymphocytes are T and 1/3 of the lymphocytes are B.

Afferent lymphatic vessels carry unfiltered lymph into the node. Here waste products, and some of the fluid, are filtered out.

Cells and Organs of Immune System

Efferent lymphatic vessels carry the filtered lymph out of the node to continue its return to the circulatory system.

Lymph nodes kill pathogens and cancer cells. They also remove debris and excess fluid. All the cells (macrophages, B and T lymphocytes) that play role in immune response are found in lymph nodes.

Cells and Organs of Immune System

MALT=Mucosa Associated Lymphoid Tissue

The mucosa of the digestive, respiratory and urinary tracts often contains small aggregations of lymphocytes called lymphoid follicles. These are called **‘Mucosa associated lymphoid tissue’ (MALT)**.

This lymphoid tissue is located at the body parts where microorganisms and other antigens enter to the body.

Cells and Organs of Immune System

MALT=Mucosa Associated Lymphoid Tissue

Tonsils consist of vast amount of lymphatic tissue. They are located at the entrance of respiratory and digestive systems. They create first step immune defense against microorganisms and other antigenic substances.

Peyer's patches are lymphatic tissue plaques surrounding the small intestines. They contain specialized immune cells to protect the digestive system. They keep intestinal lumen pathogens under control.

Appendix is the extension of the large intestine. It is rich in lymphatic tissue.

Cells and Organs of Immune System

- **Reticuloendothelial system** is part of the immune system of human body and consists of phagocytic cells (primarily monocytes and macrophages).
- It is also known as the macrophage system **(mononuclear phagocyte system)**.
- It is a network of connective tissue fibers inhabited by phagocytic cells such as macrophages ready to attack and ingest microorganisms or other antigenic substances (e.g. worn-out blood cells, bacteria, cancer cells).

Cells and Organs of Immune System

- The **monocyte** is formed in the bone marrow and transported by the blood; it migrates into the tissues, where it transforms into a **histiocytes or a macrophage**.

- **The mononuclear phagocytic system consists of monocytes circulating in the blood and macrophages in the tissue.**

- Macrophages are cells produced by the differentiation of monocytes in tissues.

Cells and Organs of Immune System

Most of the macrophages remain constant, some of them move by amoeboid movements toward infected areas.

Macrophages contain Fc receptors against various class of immunoglobulins, receptors against the C3b, C3d and C5a fragments of the complement, receptors against various lymphokines involved in the activation of macrophages.

Cells and Organs of Immune System

Role of macrophages in immune response:

1. Phagocytosis: They are specialized phagocytic cells that attack foreign substances and infectious microorganisms through destruction and ingestion.

2. Presenting antigen to lymphocytes: Macrophages are antigen presenting cells. They stimulate lymphocytes and other immune cells to respond to pathogens. Antigen presenting cells (such as B cells and macrophages) Express major histocompatibility complex class II (MHC-Class-2) molecules.

Cells and Organs of Immune System

T Lymphocytes

- T lymphocytes mature in the thymus.
- Mature T cells contain either the **CD4** protein or the **CD8** protein on their surfaces. These proteins play role in interactions with antigen-presenting cells.

CD4 interact with **MHC Class II**

CD8 interact with **MHC Class I**

Cells and Organs of Immune System

T Lymphocytes

The **T-cell receptor (TCR)** is a molecule found on the surface of T lymphocytes that is responsible for recognizing fragments of antigen as peptides bound to major histocompatibility complex (MHC) molecules.

Unlike B lymphocytes, T lymphocytes don’t have immunoglobulins on their surfaces.

Cells and Organs of Immune System

Types of T Cells

1. Regulatory T Lymphocytes

T Helper (TH) Cells

- They express the **CD4** protein on their surface.
- Helper T cells become activated when they are presented with peptide antigens by **MHC Class II** molecules that are expressed on the surface of Antigen Presenting Cells.
- Once activated, they divide rapidly and secrete small proteins called cytokines that regulate or assist in the active immune response.
- They help B cells to be transformed to plasma cells and CD8 T cells to become activated cytotoxic T cells.

Cells and Organs of Immune System

Types Of T Cells

1. Regulatory T Lymphocytes

TS (Supressor T Cells)

- Supressor T cells regulate the immune response.
- They also control reactions against self-antigens (auto-immune response)
- **TS cells contain CD8** proteins on their surfaces. They interact with **MHC Class I** molecules.

Cells and Organs of Immune System

Types Of T Cells

2. Activator T Lymphocytes

TC (Cytotoxic T Cells)

- TC cells contain **CD8** proteins on their surfaces. They interact with **MHC Class I** molecules.
- MHC Class I molecules are found on the surface of all nucleated body cells.

- Cytotoxic T cells kill cancer cells, cells that are infected (particularly with viruses), or cells that are damaged in other ways.

Cells and Organs of Immune System

B Lymphocytes

- B cells mature in the bone marrow.
- B lymphocytes have immunoglobulins on their surfaces. The first antigen receptors expressed by B cells are Ig M and Ig D.
- MHC Class II molecules are found on the surface of B lymphocytes.

B cells express **MHC Class II** to present antigen to helper T cells. CD4 protein (T helper cells) interact with MHC Class II molecules.

Cells and Organs of Immune System

B Lymphocytes

B cells differentiate into plasma cells that produce antibody molecules. They also produce memory cells that can trigger the production of antibodies if reinfection occurs.

B lymphocytes have two other important functions in addition to producing antibodies:

- Presenting antigen to T lymphocytes
- Influence other immunological cells with lymphokines they release

Cells and Organs of Immune System

Natural Killer (NK) Cells

- The NK cell is a nonspecific effector cell that can kill tumor cells and virus-infected cells.
- Unlike T cytotoxic cells, they do not need to recognize a specific antigen before being activated.

Cells and Organs of Immune System

Mast Cells

Mast cells are present in most tissues characteristically surrounding blood vessels and nerves, and are especially prominent near the boundaries between the outside world and the internal milieu, such as the skin, mucosa of the lungs, and digestive tract, as well as the mouth, conjunctiva, and nose.

- Mature mast cells have prominent cytoplasmic granules that contain histamine, and other chemical mediators.
- At the same time they contain surface receptors that bind the Fc portion of Ig E with high affinity.

Cells and Organs of Immune System

Mast Cells

- Mast cells mediate inflammatory responses such as hypersensitivity and allergic reactions.
- Mast cells store a number of different chemical mediators (including histamine, interleukins, proteoglycans (e.g., heparin), and various enzymes) in coarse granules found throughout the cytoplasm of the cell.
- Upon stimulation by an allergen, the mast cells release the contents of their granules into the surrounding tissues.

Cells and Organs of Immune System

Mast Cells

- The chemical mediators produce local responses characteristic of an allergic reaction, such as increased permeability of blood vessels (i.e., inflammation and swelling), contraction of smooth muscles (e.g., bronchial muscles), and increased mucus production.
- Mast cells are activated in response to infection by pathogenic parasites, such as certain helminths and protozoa, through Ig E signaling.
- Mast cells are also stimulated by activated complement components (C3a, C5a) which may cause anaphylactic reactions.

Cells and Organs of Immune System

Neutrophils

- Neutrophils are actively phagocytic; they engulf bacteria and other microorganisms and microscopic particles.
- The granules of the neutrophil are microscopic packets of potent enzymes capable of digesting many types of cellular materials.
- Neutrophils play role in tissue damage.
- Neutrophils migrate to areas of infection or tissue injury.

Cells and Organs of Immune System

Neutrophils

- Neutrophils migrate from blood vessels to the infected tissue via chemotaxis, where they remove pathogens through phagocytosis and degranulation.

- Neutrophils are predominant phagocytic cells in acute inflammation. Acute inflammation is a rapid response to injury or microorganisms and other foreign substances that is designed to deliver leukocytes and plasma proteins to sites of injury.

Cells and Organs of Immune System

Basophils are responsible for inflammatory reactions during immune response, as well as in the formation of acute and chronic allergic diseases, including anaphylaxis, asthma, atopic dermatitis.

They can perform phagocytosis, produce histamine and serotonin that induce inflammation, and heparin that prevents blood clotting.

Cells and Organs of Immune System

Eosinophils play a role in protecting us from some parasitic infections.

Type 1 hypersensitivity reactions may be accompanied by an increase in eosinophils.

Thrombocytes (Platelets) release serotonin. Serotonin plays a role in hypersensitivity. Thrombocytes bind to each other with the effect of antigen-antibody complexes and form thrombus and affect histamine release. Thrombocytes contain epinephrine, adenosine, clotting factors.

Immune Response

There are three possibilities for the antigens that enter the body.

1. Digestion of substances outside the cell **(does not lead to any immunity)**
2. Antigenic substances taken by phagocytes are destroyed by enzymatic digestion in the cell **(does not lead to any immunity)**
3. When antigens (foreign substances that invade the body) are detected, several types of cells work together to recognize them and respond **(immunity occurs)**

Immune Response

The immune response is essentially related with three major cell types. a) Macrophage b) T lymphocyte c) B lymphocyte These cells carry out their relations with each other either directly or through cytokines.

Antigens are classified as either **T-cell dependent** or **T-cell independent.**

- **T cell dependent antigens** require T cells to activate immune system.
- **T-cell independent antigens** can activate B cells on their own.

Primary and Secondary Immune Responses

Primary Immune Response:

When B cells first encounter an antigen, the antigen attaches to a receptor, stimulating the B cells. Some B cells change into memory cells, which remember that specific antigen, and others change into plasma cells. Helper T cells help B cells in this process. Plasma cells produce antibodies that are specific to the antigen that stimulated their production. After the first encounter with an antigen, production of enough of the specific antibody takes several days. Thus, the primary immune response is slow.

Primary and Secondary Immune Responses

Secondary Immune Response:

Whenever B cells encounter the antigen again, memory B cells very rapidly recognize the antigen, multiply, change into plasma cells, and produce antibodies. This response is quick and very effective.

Immunological Tolerance

Immunological tolerance is a state of unresponsiveness of the immune system to substances or tissue that have the capacity to elicit an immune response in given organism.