Lecture: 1

Immune system

The immune system is a network of cells, tissues, and organs that work together to defend the body against attacks by "foreign" invaders such as *bacteria*, *viruses*, *parasites*, and *fungi*.

Immunity:

Immunity can be defined as the way in which the body can protect itself from invasion by pathogenic microorganism and provide a defense against their harmful effect. Immunity is classified in to two major groups:

- Nonspecific immunity (Innate Immunity).
- Specific immunity (Acquired Immunity).

Historical Background of Immunology

There are many scientists who discovered many things in order to understand how the immunity occurs against infectious diseases.

Year	Recipient	Research
1798	Edward Jenner	Initiates smallpox vaccination
1879	Louis Pasteur	Develops an attenuated chicken cholera vaccine
1901	Emil von Behring	Serum antitoxins
1905	Robert Koch	cellular immunity to tuberculosis
1908	Elie Matchenkoff & Paul Ehrlich	Role of phagocytosis (Matchenkoff) and Antitoxins (Ehrlich) in immunity
1913	Charles Ricchet	Anaphylaxis
1919	Jules Bordet	Complement
1930	Karl Landsteiner	Discovery of Human blood groups
1962	Noel Warner & team	Distinguish between cellular and humoral immune response
1972	Rodeny R. Porter	Chemical structure of immunoglobulin
1977	Rosalyn R. Yalow	Development of radioimmunoassay
1980	Jean Dausset	Human MHC
1980	Geroge Snell	MHC for Mouse
1984	Ceaser Milstein	Monoclonal antibody
1984	Nils Jerne	Theory of Immunity
1991	Joseph Murray	Organ transplantation
1996	Peter Doherty	Cellular Immune defense
2011	Bulter & Hoffman	Activation of innate immunity

Immunology:

Immunology is defined as the study of the molecules, cells, organs, and systems responsible for the recognition and disposal of foreign material.

Antigen

A live (e.g., viruses and bacteria) or inactivated substance capable of producing an immune response .

Antibody

Protein molecules (immunoglobulins) produced by B lymphocytes to help eliminate an antigen.

Lecture: 2

Innate Immunity : Non-specific immunity which is the first line of defense against many microorganisms which cause diseases.

It have the following characteristics in common:

1. Are present intrinsically with or without previous stimulation.
2. Have limited specificity for shared microbe and cellular structures (pathogen associated molecular patterns [PAMPs] and damage-associated molecular patterns [DAMPs]) .
3. It involves several defensive barriers:

Anatomical barriers , *Humoral Barriers* and *Cellular Barriers*

Anatomical barriers:

1. Mechanical (*physical*) Factors
2. Chemical Factors
3. Biological Factors

A. Anatomical barriers

1. Mechanical (*physical*) Factors:

Skin is example of the first line of defense against infection. However, other systems act to protect body openings such as lungs, intestines, and the genitourinary tract. In the lungs, ***coughing and sneezing*** mechanically eject pathogens from respiratory tract. ***Tears and urine flow*** also mechanically expels pathogens, while ***mucus membrane*** secreted by the respiratory and gastrointestinal tract serves to trap microorganisms.

2.Chemical factors :

Low pH(3-5) of skin due to fatty acids in sebum which is fungi and bacterial static,

β- defensin **in the** respiratory tract act as antimicrobial. Enzymes like ***lysozyme and phospholipase*** in saliva, tears and breast milk are also antibacterial. In the stomach , ***low pH (1.2-3.0) of gastric juice and proteases*** serve as powerful chemical defenses against ingested pathogens, unless the virulence of these bacteria is too strong that is need another limb of effector defense.

3. Biological Factors :

Within the genitourinary and gastrointestinal tracts, ***normal flora*** is found

A person's normal flora is formed when non-pathogenic bacteria colonize epithelial surfaces. Normal flora protects the host by:

* Competing with pathogenic bacteria for nutrients and attachment sites
* Production of antibacterial substances.

The use of antibiotics can disrupt the normal flora, making pathogenic organisms more likely to cause disease

B. Humoral Barriers (against penetrating pathogens) :

1. Complement System :

It is the major humoral non-specific defense mechanism. Once activated complement can lead to increase vascular permeability , recruitment of phagocytic cells, and lysis and opsonization of bacteria.

2. Coagulation System :

For example

1. ***beta-lysine***, a protein produced by platelet during coagulation can lyse many Gr+ bacteria act as antimicrobial.
2. ***Interferons*** : Are protein that can limit virus replication in cells.
3. ***Lysozyme***: breaks down the cell wall of bacteria.
4. ***Interleukin-1***, IL-4, IL-6 and TNF: induce fever and production of acute phase proteins, some of which are antimicrobial because they can opsonize bacteria.

C. Cellular Barrier to infection :

1. **Neutrophil**: polymorphonuclear cells (PMNs) attack invading organisms and kill them intracellularly.
2. **Macrophages** : Tissue macrophages, intracellular killing of microorganisms.
3. **Natural Killer** (NK) cells: Kill virus infected and tumor cells.
4. **Eosinophils** : Have proteins in granules that are effective in killing certain parasites.
5. **Mast cells, platelets (thrombocytes), and endothelial cells**.

Lecture: 3.

Inflammatory response and phagocytosis(Humoral Barriers)

Inflammation

The body's response to injury of vascularized tissue with a series of events, collectively called inflammation and repair. Ultimate goal is to heal and replace injured tissue.
Some of the many causes of inflammation include:

1. Infection and microbes,
2. Immune reactions between antigen and antibody,
3. trauma, burns, physical or chemical agent and tissue necrosis.
4. Other causes of inflammation include: temperature extremes, oxygen

The duration of inflammation lasts from a few minutes to a few years. Depending on:

- ✓ the extent of the injury
- ✓ the type of injury
- ✓ the vascularity of the tissue

Signs of Inflammation

- ✓ Erythema
- ✓ Heat (fever)
- ✓ Edema
- ✓ Pain
- ✓ Loss of Function

These are signs of inflammation and it caused by:

1. Vasodilation: increase in the diameter of blood vessels (capillary) which is responsible for redness (erythema) and increase temperature (heat).
2. Increase permeability in the walls of blood vessels leading to escape of fluid and cells into space & surrounding tissues (swelling and edema).

Fever (pyrexia): this occurs due to bacterial products such as endotoxins. The functions of fever is :

1. Inhibit multiplication of bacteria.
2. Interfere with nutrition of bacteria.
3. Increase stimulate immune reaction like phagocytosis and specific immune response.

Functions of Inflammation

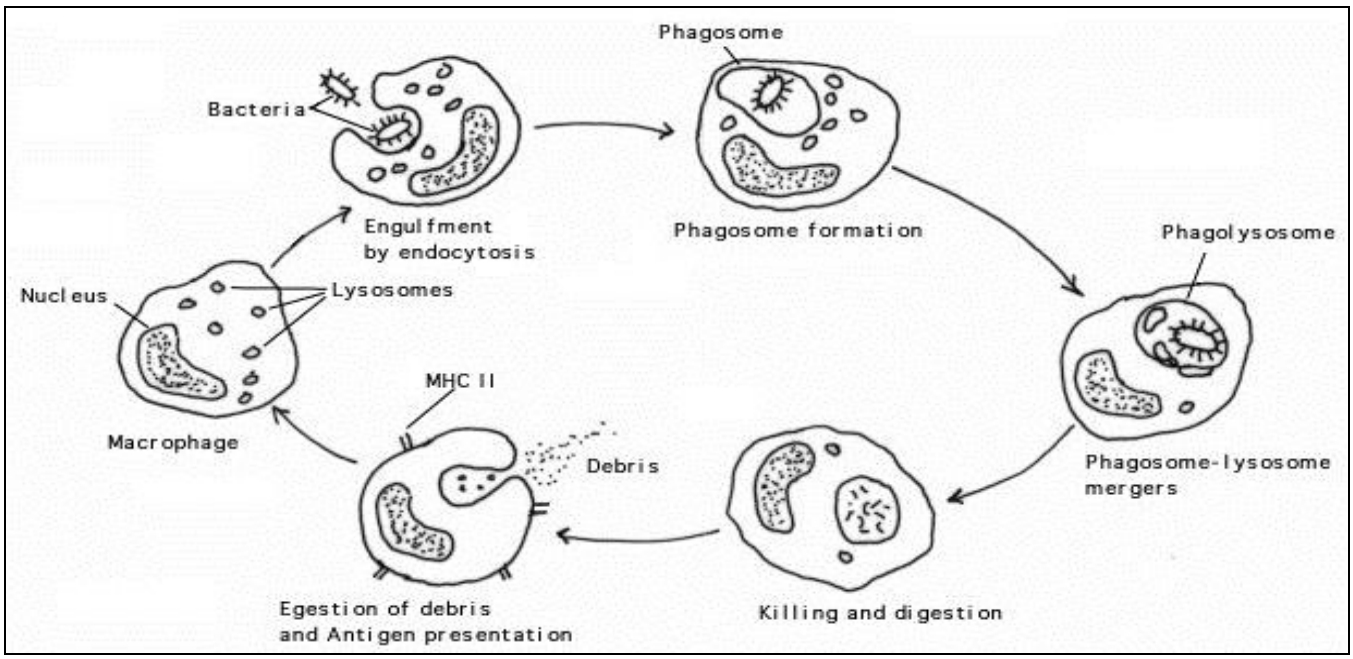
- ✓ Inactivate injurious agent
- ✓ Break down and remove dead tissue
- ✓ Initiate healing of tissue

Phagocytosis:

The primary method used by the body to remove free microorganisms in the blood and tissue fluids , by engulfing, ingestion particles and destroying them enzymatically (lysozyme, myeloperoxidase, Protease) by phagocytic cells like macrophages.

Stages of Phagocytosis :

1. Recognition and attachment of microbes by phagocytes.
2. Ingestion of microbes and other materials by phagocytes.
3. Enzymatic destruction of microbes and other materials by fusion of lysosome with phagosome creates phagolysosome,
4. Waste material is expelled or assimilated.



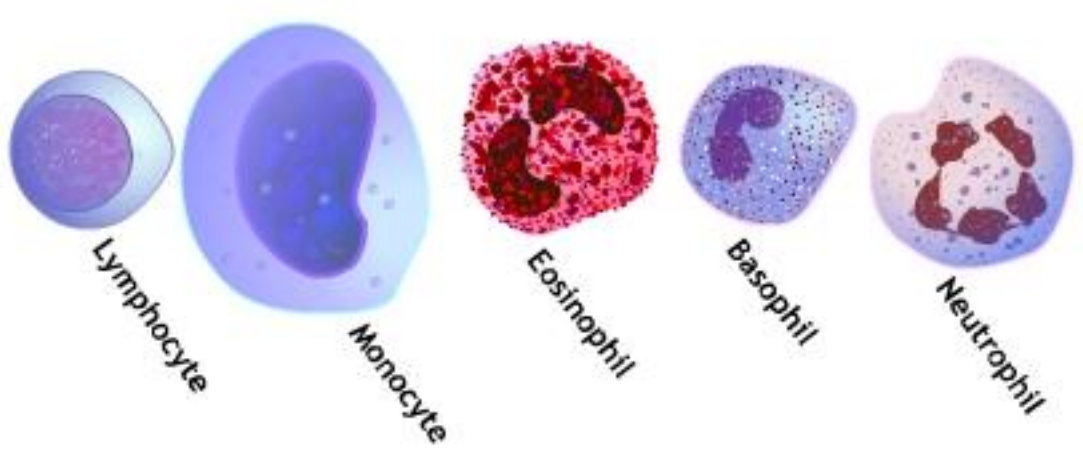
Types of phagocytic cells :

1. **Polymorphonuclear cells (PMNs):** predominate in acute pyogenic infection, short-lived, circulating, granulated contains lysozyme and myeloperoxidase (degradative enzymes) and produce toxic substance (NO) which accelerate destruction of infectious microorganisms.
2. **Macrophages / Monocytes:** present in tissues in different forms **such as** : Alveolar macrophages in lungs.

Kupffer cells in Liver.
Splenic macrophages in white pulp of spleen.
Microglial cells in brain.
Mesangial cells in kidneys.
Osteoclasts in bones.
Langerhans cells in skin and mucous membrane

These cells long-lived also migrate, engulf, and kill the bacteria multiply within the cells, do not possesses myeloperoxidase, secrete plasminogen enzyme.

3. **Eosinophil and Basophil**: release inflammatory mediators.



Lecture: 4

Specific (acquired or adaptive immunity) immunity

Is a defense system that protects the body against pathogenic microorganisms and other type of disease such as cancer and mediated by B lymphocytes (B-cells), or T lymphocytes (T-cells) or both .

adaptive immunity can be subdivided into two major types depending on how immunity was introduced.

- ✓ Naturally acquired immunity occurs through contact with a disease causing agent.
- ✓ Artificially acquired immunity develops for example by vaccination.

Both naturally and artificially acquired immunity can be further subdivided into **active** or **passive**, depending on whether immunity is induced in the host or passively transferred from immune host. and each of these types can in turn be naturally or artificially acquired.

Passive immunity

Passive immunity is protection by products produced by an animal or human and transferred to another human, usually by injection. Passive immunity often provides effective protection, but this protection wanes (disappears) with time, usually within a few weeks or months. They are divided into two:

I. Naturally acquired passive immunity: Refers to antibodies transferred from mother to fetus across the placenta and to the newborn in colostrum and breast milk during the first few months of life.

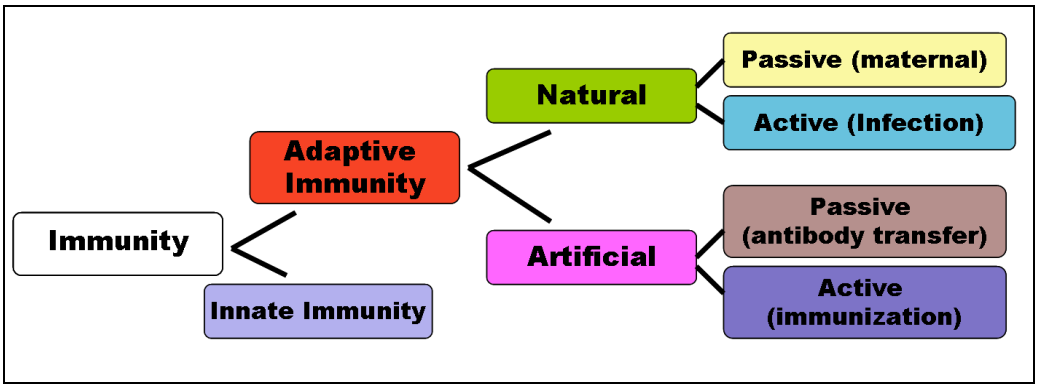
II. Artificially acquired passive immunity: Is introduction of antibodies that are formed by an animal or a human to an individual to prevent or treat infection.

Active immunity

Active immunity is protection that is produced by the person's own immune system. This type of immunity usually lasts for many years, often during a lifetime.

I. Naturally acquired active immunity: Is immunity that comes from infections encountered in daily life.

II. Artificially acquired active immunity: It is stimulated by the use of vaccines to artificially establish a state of immunity.



Characteristics of Acquired Immunity:

B an T cells are responsible for these characteristics :

1. Antigenic specificity.
2. Diversity (antigens are recognized by different lymphocytes).
3. Immunologic memory (Re-exposure to the same antigen induce more rapid and effective response).
4. Self / non-self-recognition.

Comparison between Active and Passive Acquired Immunity:

	Active	Passive
1. Source	Self	other human or lower animal
2. Effectiveness	High	moderate to low
3.Method	infection or vaccination or immunization	through placenta or injection γ-globulin
4.Time to develop	7-10 days	immediately
5. Duration	long time (may be years)	short time (few days or several weeks)
6. Easy of reactivity	easy by booster dose	dangerous (may cause anaphylaxis)

Differences between innate & acquired immunity :

Innate Immunity	Acquired immunity
1. Nonspecific to invader	specific
2. It presented from birth	developed later in life
3. Genetically determination	non
4. Consist of physical chemical barriers & cells	antibody & lymphocytes
5. No memory cells	memory cells

Factors Associated With Immunologic Disease

Many factors appear to be responsible for differences in the immunity of individual's age:

- Age
- Nutrition
- Genetic factors

Lecture: 5

Vaccines

A vaccine{from the Latin Variolae vaccinae (cowpox)} is a biological preparation that improves immunity to a particular disease. It contains certain agents that not only resembles a disease-causing microorganism but it also stimulates body's immune system recognize the foreign agents

Vaccines are dead or inactivated organisms or purified products derived from them.

There are several types of vaccines in use. They are:

- Whole-Organism Vaccines
  - ✓ Killed
  - ✓ Attenuated
- Purified Macromolecules as Vaccines
  - ✓ Toxoids
  - ✓ Capsular polysaccharides
  - ✓ Recombinant microbial antigens/Surface antigens
- Recombinant vaccine
- DNA vaccine
- Multivalent Subunit Vaccines

Properties of ideal vaccine:

- Provide long lasting immunity.
- Should induce both humoral and cellular immunity.
- Should not induce autoimmunity or hypersensitivity.
- Should be inexpensive to produce, easy to store and administer.
- Vaccines must also be perceived to be safe.

Vaccination (Immunization) : is a way to trigger the immune response by giving small doses of an antigen which activate the immune system memory (activated B cells and sensitized T cells). *Immunization may be passive or active, natural or artificial :*

Active Immunization :

Achieved by natural infection with a microorganism or artificially by administration of a vaccine. This type of immunization will give long term protection by formation of memory cells and reactive B & T cells.

Passive Immunization :

Performed by transferred antibodies to recipient, either naturally by transfer of maternal Abs across placenta to the fetus, or by injecting recipient with performed Abs.

Passive immunization is routinely administrated to individuals exposed to tetanus, botulism…… etc, & and it can provide immediate protection to travelers and health-care workers.

Classification of Vaccines:

Killed (Whole Pathogen) Vaccines.

These are preparations of the normal (wild type) infectious, pathogenic microorganisms that have been rendered nonpathogenic, usually by treatment with using heat, formaldehyde or gamma irradiation so that they cannot replicate at all. Such killed vaccines vary greatly in their efficacy.

Example : Cholreae, Rabies, HAV vaccine … etc.

Live Attenuated Vaccine.

These vaccines are composed of live, attenuated microorganisms (viruses) that cause a limited infection in their hosts sufficient to induce an immune response, but insufficient to cause disease. Ex : Bacillus Calmette Guerin (BCG) .

Live Attenuated *VS* Killed Vaccines

Feature	Live	Killed
Dose	Low	High
No. of dose	Single	Multiple
Need of adjuvant	No	Yes
Duration of Immunity	Many years	Less
Antibody response	IgG	IgG, IgA
CMI (cell mediated immunity)	Good	Poor
Reversion of Virulence	Possible (non safe)	Not possible

Subunit Vaccines:

Subunit vaccines contain purified antigens instead of whole organisms. Subunit vaccines are composed of toxoids, subcellular fragments, or surface antigens. Eg: HA vaccines for Influenza A and B, and HbsAg derived from plasma of carriers. Toxoid like diphtheria, tetanus, botulism. Bacterial Polysaccharides capsule vaccines such as *Streptococcus pneumoniae, Neisseria meningitides* .

Synthetic Peptide Vaccines:

Peptide vaccine consists of those peptides from the microbial antigen that stimulates protective immunity. Synthetic peptides are produced by automated machines rather than by microorganisms. Example: spf66 anti-malarial vaccine

Conjugate Vaccines:

Conjugate vaccines are primarily developed against capsulated bacteria, they stimulate only humoral immunity. **Examples:** *Haemophilus influenzae* HiB polysaccharide is complexed with diphtheria toxoid. Tetramune vaccine, which combines the tetanus and diphtheria toxoids, whole-cell pertussis vaccine, and *H. influenzae* type b conjugate vaccine.

Recombinant Vaccines:

These vaccines are produced using recombinant DNA technology or genetic engineering. Recombinant vaccines are those in which genes for desired antigens of a microbe are inserted into a vector.

Examples:

Hepatitis B surface antigen is produced from a gene transfected into yeast (*Saccharomyces cerevisiae*) cells and purified for injection.

Factors playing a role in immunization :

1- Choice of vaccine type eg: live attenuated is best but disadvantage is possibility of reversion of its pathogenicity.
2- Route is very important eg: Oral or intranasal is more effective in many viral respiratory or gastrointestinal diseases.
3- Time of primary and secondary dose.
4- Adjuvant, the best one is combination of alum with oil gives prolong immune response.

Childhood Immunization Schedule in Iraq

	Types of Vaccines	Age of Vaccination	Number of Doses
1	BCG(Bacillus Calmette Guerin)	Within 72 Hours after birth	Single dose Zero dose First dose
2	DTP (Diphtheria, Tetanus, Pertussis) Oral poliomyelitis Hepatitis B	At 12 months of age	First dose First dose Second dose
3	DTP (Diphtheria, Tetanus, Pertussis) Oral poliomyelitis Hepatitis B	At 4 months of age	Second dose
4	DTP (Diphtheria, Tetanus, Pertussis) Oral poliomyelitis Hepatitis B	At 6 months of age	Third dose
5	Measles	at 9 months of age	Single dose
6	Mixed measles (Measles, Mumps, Rubella	At 15 months of age	First dose
7	DTP (Diphtheria, Tetanus, Pertussis) Oral poliomyelitis	At 18 months of age	First booster dose
8	DTP (Diphtheria, Tetanus, Pertussis) Oral poliomyelitis	From 6-7 years of age	Second booster dose
9	Mixed measles (Measles, Mumps, Rubella	At 6 years	Second dose

Note : BCG vaccine must be repeated if there is no scare appeared after 2 months after vaccination

Lecture: 6

The Structure of The Immune System

Lymphoid Tissue : Specialized connective tissues and organs where the lymphocytes form the major cellular components.

The immune system consist of primary organs (central lymphoid organ) and secondary organs (Peripheral lymphoid organ).

The Primary Lymphoid Organ

The **primary lymphoid organs** are primary lymphoid tissues involved in the production and early selection of lymphocytes and consisting of :

1- **The Thymus :**

Thymus is bilobed organ situated above the heart, each lobe divided into outer part Cortex and inner part Medulla. Thymic function decline with ages, most active during the neonatal and pre-adolescent periods, the growth continue until reaches its maximum size at puberty and then undergo atrophy with aging and replaced by adipose tissue.

Cortex contain immature T-cells, highly dividing, highly dying thymocyte, about 95 % of T-cell will be die, 1-5 % will remain and migrate down to the medulla. There are cortical epithelial cells which also helps the thymocyte in their proliferation and maturation by secreting thymic hormones (Thymopoietin, Thymoline and Thymosine).

Medulla contain mature, less dividing, less dying thymocytes. That is mean thymus deals with proliferation and maturation of T-Lymphocytes and the letter (T) is related to it.

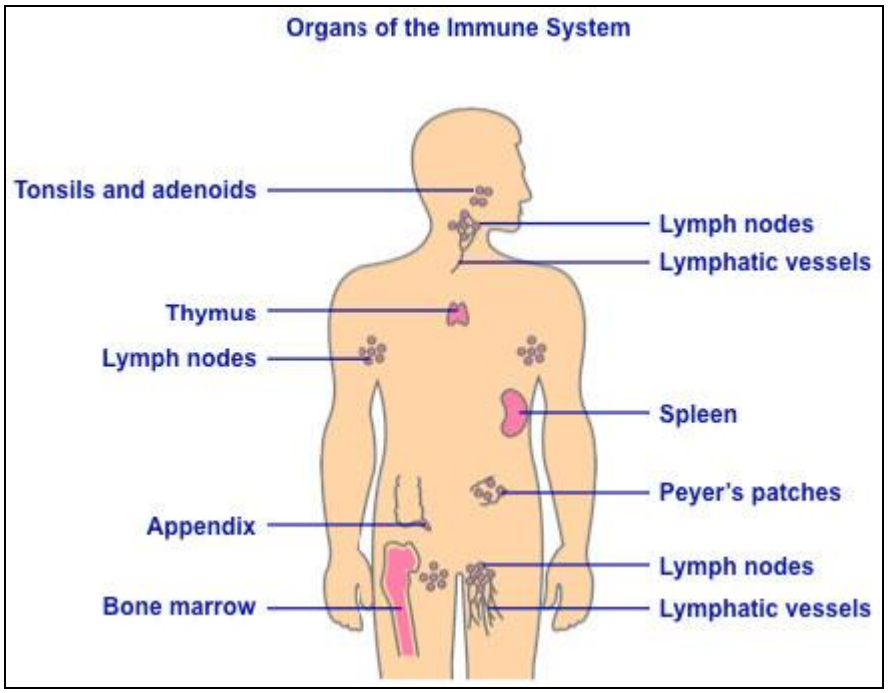
2- **Bone Marrow :**

Is the flexible tissue found in the interior of bones, it is the site of B-cell origin and development in human and mice.

Secondary lymphoid organs : plays a major role in mounting immune response to antigen,

1- Encapsulated organs : include spleen, lymph nodes, appendix, tonsil, skin (Langerhans cells) and liver. These organs act as filters or traps for foreign antigens, and that plays a major roles in mounting immune response to Ags in the blood stream which is come into contact with macrophages and lymphocytes (B & T cells).

2- Uncapsulated organs : called mucosa associated lymphoid tissue (MALT), is found in various body sites which includes Payer's patches (aggregates of cells) in small intestine, and numerous lymphoid follicles in intestine, respiratory tract and genitourinary tract as well.



Primary & Secondary Lymphoid Organs

Features	Primary Lymphoid organ	Secondary Lymphoid Organ
Component	Bone marrow, fetal liver, thymus	Spleen, lymph nodes, and mucosa-associated lymphoid tissue (MALT) including tonsils, adenoids, respiratory, genitourinary, and gastrointestinal tracts
Proliferation and differentiation	Antigen-independent	Antigen-dependent
Product	Immunocompetent cells (B cells and T cells)	Effector cells (antibody-secreting plasma cells for humoral immune response and T helper and T cytotoxic cells for cell-mediated immune response)
Event	Development and maturation of B Cells and T cells	Induction of immune response: encounter of antigens and antigen-presenting cells (APC) with mature B and T cells, generation of effector cells, and memory cells

Cells of Immune system:

1- **Lymphocytes :** produced in the primary lymphoid organs then migrates by the circulation to the secondary lymphoid organs, is a type of white blood cells. The three major types of lymphocytes are T-cell, B-cell and natural killer (NK cells). B-cells and T-cells (bone marrow cells) are major cellular components of the adaptive immune response.

   a- **T- Lymphocytes** : classified according to their function into :

      I- **Cells with $CD4^+$ marker** are called **helper T-cells**, become activated by antigen presenting macrophages and then help in generating T-cytotoxic cells and cooperating with B-cells in production of antibodies.

      II- **Cells with $CD8^+$ marker** are **cytotoxic T-cells** (Tc cells), cytotoxic against tumor cells and host cells infected with intracellular pathogens on the basis of MHC presenting foreign proteins.

      III- **T-suppressor / regulator cells** which suppress T and B-cell responses. T-regulatory are subpopulation of T-helper cells, serve as regulator of T-cells responses by secreting inhibitory cytokines that decrease the activity of both T & B-cells.

   b- **B-lymphocytes:** develop from stem cells in the bone marrow, these cells produce antibodies with specificity for antigen after determination into plasma cells and make it easier for immune cells to destroy the antigens.

2- **Natural Killer (NK) cells**: are population of lymphoid cells characterized by large granulated cytoplasm, they don't have T or B-cell receptors, they attack and destroy tumor cells or cells that have been infected by viruses nonspecifically (they don't need to recognize foreign antigens presented on the target cell). NK cells distinguish tumors and infected cells from normal and uninfected cells by recognizing changes on the surface molecule called MHC class I. they named natural killer because they don't require prior activation in order to kill cells which are missing MHC class I.

3- **Antigen presenting cells (APC):** are found primarily in the skin, lymph nodes, spleen and thy;2mus such as macrophages, dendritic cells and activated B-cells. Main function of APCs is present antigen to antigen sensitive lymphocytes.

Cluster of Differentiation (CD):

Unique cell surface molecules, molecules given number designation. The CD describes the cluster of determinants, the number describes the order in which it was discovered, as of March 2010 the list of determinants was to CD 350.

CD specific markers have been useful for determining the function of proteins. Hence, for example CD4 antigens are expressed by T-helper cells while CD8 antigens are expressed by cytotoxic and suppressor T-cells.

Major histocompatibility complex (MHC):

Refers to cluster of gens responsible for immune response. MHC also called human leukocyte antigen in human (HLA). The importance of MHC proteins is that they allow T-cells to distinguish self from non-self. In every cells of our bodies, antigens are constantly broken up and presented to passing T-cells. Without this presentation, other aspects of the immune response cannot occur. MHC determines compatibility of donors for organ transplants as well.

MHC class I molecules: found in all nucleated cell surfaces presents antigens to cytotoxic T-cells ($CD8^{+}$).

MHC class II molecules: found only on B-cells, macrophages and other cells that presents antigens to T-helper cells, that is participate in Ag presentation to T-helper cells ($CD4^{4}$).

Features	B-Cell	T-Cell
Origin	Bone marrow	Bone marrow
Site of maturation	Bone marrow	Thymus
Antigen receptor	B cell receptor (BCR)	T cell receptor (TCR)
Target of binding	Soluble antigens	Biomolecular complex displayed at the surface of APC
Branch of immune response	Antibody-mediated immune response	Cell-mediated and antibody-mediated immune response

Lecture: 7

Complement System

The complement system is a heat-labile series of more than 18 plasma proteins (C1,4,2,3,5,6,7,8, & 9) produced by hepatocytes, macrophages and intestinal epithelial cells. Normally, these proteins are in an inactive form, but specific signal can activate the first protein of the team.

Properties of complement :

1. Inactivated by heating at 56^{o}c for 30 minutes.
2. Activated by microbes.
3. Cause destruction (lysis) of target cell.

Complement Activation:

Three major steps appeared in complement activation :

1. Recognition.
2. Enzyme activation.
3. Cell membrane change.

There are 3 major pathways for complement activation the classical, alternate pathway and lectin pathway.

The Classical path way

The classical pathway is triggered by immune complexes (Ag + Ab IgG or IgM) in the presence of complement components C1, C4, C2, C3, Ca^{+2} and Mg^{+2} cations. The classical pathway is so called because it was discovered first. The complement proteins react in the order C1q, C1r, C1s,C4, C2, C3, C5, C6, C7, C8 and C9. While IgG1, IgG2 and IgG3 (most effective) can activate complement, IgG4 is not able to activate at all.

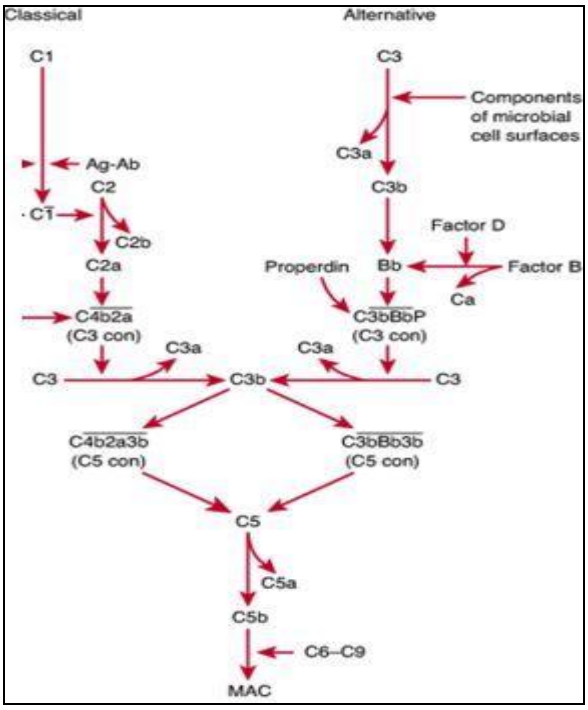
Initiation of this pathway occurs when C1 binds to the FC region of the antibodies that have interacted with antigen (immune complexes) or to certain pathogen surfaces in the absence of antibody. The result is the formation of an activated C1qrs which an enzyme that cleaves C4 into two fragments C4a and C4b.

C4b binds to the Ag-bearing particle or cell membrane while C4a remains a biologically active peptide at the reaction site.

C4b binds C2, and is cleaved into C2a and C2b. C2a remains complexed with C4b whereas C2b is released. C4b2a complex is known as C3 convertase, and in the presence

of Mg^{+2}, cleaves C3 into C3a and C3b. C3b binds to the membrane to form C4b2a3b complex whereas C3a remains in the microenvironment.

C4b2a3b complex functions as C5 convertase, which cleaves C5 into C5a and C5b, C5b binds to C6 and C7 to form a complex that insert into the membrane bilayer. C8 then binds to the C5b/C6/C7 complex followed by the polymerization of up to 16C9 molecules to produce the membrane attack complex that causes cytolysis.



The classical pathway :

1. Initiated by Ag-Ab complex.
2. Include all 9 major complement components.
3. The product C3a, C3b, C5b6789 which cause cell lysis.

Biological activity of the classical pathway products

Component	Biological activity
C3a	Anaphylatoxin : can activate basophils and mast cells to degranulate resulting in increased vascular permeability and contraction of smooth muscle cells which may lead to anaphylaxis.
C3b	Opsonin: promote phagocytosis by binding to complement receptors . Activation of phagocytic cells.

Alternative Pathway

- ✓ Alternate pathway is a non-antibody initiated pathway.
- ✓ This pathway does not require C1, C2 and C4.
- ✓ Activated by bacterial cell wall (lipopolysaccharide), fungi, some viruses, parasites (Trypanosoma) and some other proteins (eg: proteases).
- ✓ It begins with the spontaneous activation of C3 in serum and requires Factors B and D and Mg^{+2}, all present in normal serum.

A C3b-like molecule (C3i) is generated by slow hydrolysis of native C3. C3i binds factor B, which is cleaved by Factor D to produce C3iBb. C3iBb cleaves native C3 into C3a and C3b. C3b binds factor B, which is again cleaved by Factor D to produce C3bBb (now C3 convertase).

***Note** : Alternate pathway is so called because it bypasses the requirement of antigen-antibody complex, C1, C2 and C4 components*

$$C3 \longrightarrow C3i$$
$$C3i + \text{Factor B} \xrightarrow{\text{Factor D}} C3iBb$$
$$C3 \xrightarrow{C3iBb} C3a + C3b$$
$$C3b + \text{Factor B} \longrightarrow C3bB$$
$$C3bB \xrightarrow{\text{Factor D}} C3bBb \text{ (C3 convertase)}$$

The lectin pathway :

Lectin: are proteins that binds to mannose residues (glycoproteins or carbohydrate on the surface of pathogens). The mechanism of it is very similar to classical pathway, but like alternative pathway in its doesn't depend on Ag-Ab complexes in its activation. ***It is initiated by*** the binding of mannose-binding lectin (MBL) to bacterial surfaces with mannose-containing polysaccharides (mannans). Binding of MBL to a pathogen resulting in the association of two serine proteases MASP-1 and MASP-2 (Mannose associated serine protease).

MASP-1 and MASP-2 are similar to C1r and C1s respectively. Because the lectin activates binds to mannose residues, therefore called mannan-binding lectin(MBL) pathway.

The biological activities and regulatory proteins of the lectin pathway are the same as those of the classical pathway.

The Role of Activated Complement in Immune Response

The activation of complement and the product formed during the complement cascade have a variety of physiologic and cellular consequences. Physiologic consequence includes blood vessel dilation and increased vascular permeability. The cellular consequences include the following:

1- **Viral neutralization**: it take place by formation of larger viral aggregated which reduce the net number of infectious viral particles.
2- **Cytotoxic function (lysis):** final stage of complement activation is the formation of MAC which cause a hole in the cell membrane and lead to cell death.
3- **Opsonization**, C3b and C4b are powerful opsonins, which attach to Complement receptor (CR1) on phagocytic cells and promote phagocytosis.
4- **Immune complexes (IC) clearance:** complement system remove IC from circulation & deposit them in the liver and spleen then phagocytosed.
5- **Inflammatory function:** C3a, C5a are all anaphylatoxins which cause basophil / mast cell degranulation and smooth muscle contraction, chemotaxis of neutrophils, basophils and macrophages and cause induction of adhesion molecules on vascular endothelial cells.

Complement deficiencies and diseases

Pathway / Component	Disease	Mechanism
Classical pathway		
C1, C2, C4	SLE	Opsonization of immune complex increased precipitation in tissue and inflammation
Lectin pathway		
MBL	Susceptibility to bacterial infections in infants or immune suppression	Inability to initiate the lectin pathway
Alternative pathway		
C5, C6, C7, C8 and C9	Susceptibility to Gram negative infections	Inability to attack outer membrane of gram negative bacteria

Lecture :8

The Antigen

The Term Antigen is a substance that has the ability to bind to an antibody or a T lymphocyte antigen receptor but may not be able to evoke an immune response initially

An Immunogens: Immunogen is substance which produces an immune response as well as binds to its products i.e., antibodies or sensitized T-cells, when injected into the host.

Note: all immunogens are antigens, but the converse is not true.

The Epitope (**antigenic determinant or antigenic specificity**): small structure on an antigen which is bound by a particular antibody molecule.
The combining area on the antibody molecules, corresponding to the epitope, is called the **Paratope**

Antigenic molecules may be multivalent, having multiple epitope, or monovalent, having only one epitope. Generally, multivalent antigens produce a stronger immune response than monovalent antigens because wide arrays of antibody molecules are made against the multiple antigens.

Hapten: Hapten refers to a group of substances, usually very small in size, which do not induce an immune response by themselves alone. But if combined with another molecules called carries, the hapten-carrier complex induces an immune response.

An adjuvant is a substance, distinct from antigen, that enhances T cell activation by promoting the accumulation of APCs at a site of antigen exposure and by enhancing the expression of co-stimulators and cytokines by the APCs.
Adjuvants function in one or more of the following ways;

1- By prolonging retention of the immunogen,
2- By increasing the effective size of the immunogen,
3- By stimulating the local influx of macrophages and/or other immune cells to the injection site and promoting their subsequent activities.

The carrier

Carrier is a non-antigenic component and helps in provoking the immune response. Example: Serum Protein such as Albumin or Globulin

Factors Influencing Immunogenicity:

A. Contribution of the Immunogen

1. Foreignness : An antigen must be a foreign substances to elicit an immune response.

2. Molecular weight :

The most active immunogens tend to have a molecular mass of 14,000 to 6,00,000 Da. Examples: tetanus toxoid, egg albumin, thyroglobulin are highly antigenic. Insulin (5700) are either non-antigenic or weakly antigenic.

4. Chemical Composition

In general, the more chemically complex substance is the more immunogenic it will be. Antigens are mainly proteins and some are polysaccharides.

4. Physical Form

In general particulate antigens are more immunogenic than soluble ones. Denatured antigens are more immunogenic than the native form.

B. Contribution of the Biological System

1. Genetic Factors

Some substances are immunogenic in one species but not in another .Similarly, some substances are immunogenic in one individual but not in others (i.e. responders and non-responders).

2. Age

Age can also influence immunogenicity. Usually the very young and the very old have a diminished ability to elicit and immune response in response to an immunogen.

C. Methods of Administration :

1. Dose of the antigen

The dose of administration of an immunogen can influence its immunogenicity. There is a dose of antigen above or below which the immune response will not be optimal.

2. Route of Administration

Generally the subcutaneous route is better than the intravenous or intragastric routes. The route of antigen administration can also alter the nature of the response.

Antigen Specificity

Antigen Specificity depends on the specific active sites on the antigenic molecules (Antigenic determinants).

1. Species Specificity

Tissues of all individuals in a particular species possess, species specific antigen. Human Blood proteins can be differentiated from animal protein by specific antigen-antibody reaction.

2. Organ Specificity

Organ specific antigens are confined to particular organ or tissue. Certain proteins of brain, kidney, thyroglobulin and lens protein of one species share specificity with that of another species.

3. Auto-specificity

The autologous or self-antigens are ordinarily not immunogenic, but under certain circumstances lens protein, thyroglobulin and others may act as ***autoantigens.***

Based on genetic consideration antigens are divided into three types :

1. **Autoantigens:** These are the antigens belonging to host itself.
2. **Alloantigens:** are antigens found in different members of the same species (the red blood cell antigens A and B are examples).

3. **Heteroantigens**: are identical antigens found in the cells of different species. Examples: Forssmann antigen, Cross-reacting microbial antigens, etc.

Endogeneous Antigen

These enters the body from outside i.e external environment. Common examples includes microorganisms, drugs, pollen, pollutants or even food items etc.

Endogenous Antigens : These antigens are produced within the host.

Immunoglobulins (Antibodies)

Antibody: Antibodies are glycoproteins, which are sensitized, and secreted by plasma cells in response to specific antigenic stimulation and it forms about 20% of plasma protein.

Functions of Antibodies :

1. Neutralizing viruses and toxins or toxoids, which neutralize the antigen.
2. Agglutinin: immobilize motile bacteria and aggregate cells forming clumps.
3. Precipitins: which form complexes with soluble antigens forming precipitates.
4. Lysine: antibodies together with complement lyse the antigenic cells.
5. Opsonins: antibodies combine with surface components of microbial and other cells so that they are more readily phagocytized.
6. Activation & fixation of complement: IgM and most IgG subclasses can activate complement system and gives C3b which binds cell and Ag-Ab complex resulting to cell lysis.

Basic Structure of Immunoglobulins

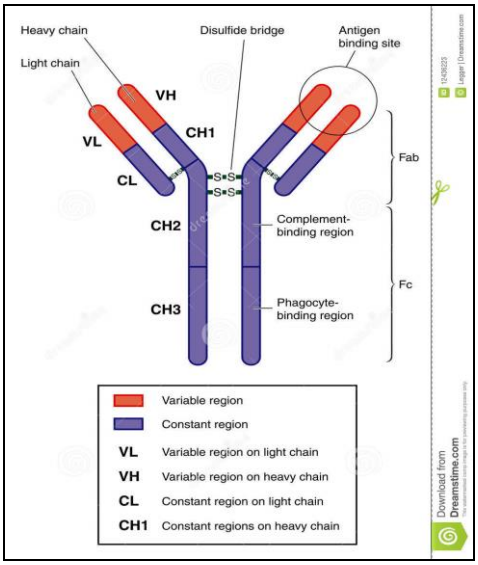
The backbone of immunoglobulin consist of four polypeptides, two heavy (H) chains and two light (L) chains, identical to each other and joined to form Y shaped molecule, The heavy chains have double molecular weight of the light chains (heavy chains have double amino acids found in light chains). These chains linked together by disulphide bond to give monomeric structure, each chain have two terminals :

1. Amino terminal.
2. Carboxyle terminal.

The antibody molecule could be anatomically divided into two fragments: **Fab** fragments (fragment of Ag binding site), and **FC** fragment (fragment of crystalizable). Each Ab contain two Fab fragments and one FC fragment. The light & heavy chain consist of variable (V) and constant (C), the C region of heavy chain divided into CH1 , CH2,CH3 …etc according to type of immunoglobulin .

1. Light chain – V_L (110 amino acids) and C_L (110 amino acids).
2. Heavy chain – V_H (110 amino acids) and C_H (330-440 amino acids).

The constant region of the light chain is determines the mechanisms to destroy antigens. This region of light chain is either Kappa (K) or Lambda (λ) with ratio 2:1.



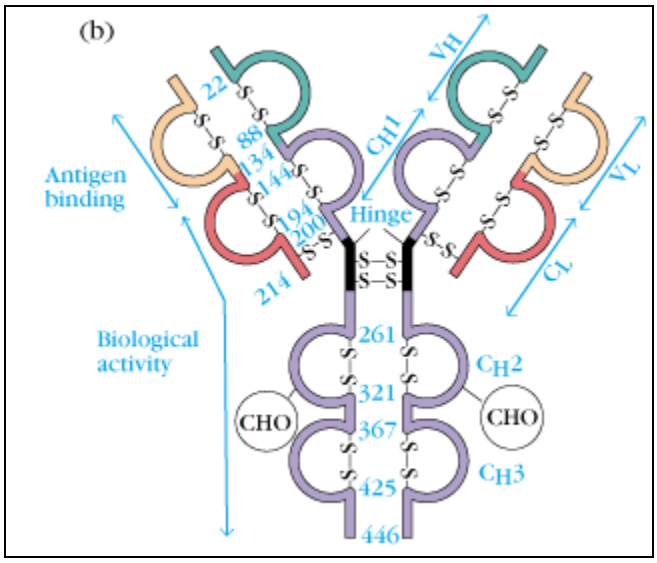
Hinge Region :

This is the region at which the arms of the antibody molecule forms a Y shape. It is called the hinge region because there is some flexibility in the molecule at this point.

Antibodies are divided into 5 classes (isotypes) based on their constant region structure (amino acid sequences) of heavy chain and immune function :

1. **Immunoglobulin Gamma (γ) IgG.**
2. **Immunoglobulin Alpha (α) IgA.**
3. **Immunoglobulin Mu (μ) IgM.**
4. **Immunoglobulin Delta (δ) IgD.**
5. **Immunoglobulin Epsilon (ε) IgE.**

In addition there are subtypes of IgA (IgA1, IgA2), while IgG have 4 subtypes (IgG1-IgG4).



Properties of Human Immunoglobulins

Properties	IgG	IgA	IgM	IgD	IgE
Structure	Monomer	Monomer, Dimer	Pentamer	Monomer	Monomer
Number of binding sites	2	2,4	10	2	2
Biological function	Long term immunity	Secretory Ab on mammary mammalian	Produced on end response to Ag	Receptor on B-cell	Ab of allergy and worm infections
Molecular weight x1000 Da	150	150, 60	900	150	190
Complement fixation	+	0	++++	0	0
Serum concentration approximate (mg/d/)	1000	200	120	3	0.05
Serum half-life (days)	23	6	5	3	2
Placental transfer	+	0	0	0	0
Binding to Fc receptor on	Phagocyte	Phagocyte	B-cell	B-cell	Mast cell and basophil

Lecture : 10

Antigen - Antibody Interactions

Principle of Antigen Antibody Interactions

Known antigen suspension or antiserum is used to detect and measure unknown antibody or microbial antigen.

The reaction between antigen and either antibody or specifically sensitized T-lymphocyte can be divided into 3 levels :

1. Primary antigen – antibody reaction.
2. Secondary antigen – antibody reaction.
3. Tertiary antigen – antibody reaction.

Primary antigen – antibody reaction.

The initial binding between Ag-Ab & and is rarely visible, the assay of this reaction include several techniques :

- ✓ Ammonium sulfate precipitation.
- ✓ Radio immuno assay (RIA).
- ✓ Fluorescent immuno assay (FIA).
- ✓ Enzyme immnuo assay (EIA) or Enzyme Linked Immuno Assay (ELISA).

Secondary antigen – antibody reaction.

This reaction include :

- ✓ Agglutination.
- ✓ Precipitation.
- ✓ Complement fixation.
- ✓ Cytolysis.

Agglutination Reactions

Agglutination is the visible clumping together of bacteria, cells, or particles, by an antigen combining with its specific antibody.

The highest dilution of serum that still causes agglutination, but beyond which no agglutination occurs, is termed the ***titer***.

The tubes with high concentrations of serum, where agglutination does not occur, represent a ***prozone***.

In the prozone, antibodies are present in excess. Agglutination may not occur at high ratio of antibody to antigen because every epitope on one particle may bind only to a single antibody molecule, preventing cross-linking between different particles.

Zeta Potential. The surfaces of certain particulate antigens may possess an electrical charge, as, for example, the net negative charge on the surface of red blood cells caused by the presence of sialic acid. When such charged particles are suspended in saline solution, an electrical potential termed the ***zeta potential*** is created between particles, preventing them from getting very close to each other. This introduces a difficulty in agglutinating charged particles by antibodies, in particular red blood cells by IgG antibodies. The distance between the Fab arms of the IgG molecule, even in its most extended form, is too short to allow effective bridging between two red blood cells across the zeta potential.

Agglutination reaction may be Direct or Indirect :

Direct agglutination:

When the antigen is a natural constituent of a particle, the agglutination reaction is referred to as direct agglutination.

Indirect agglutination:

When the agglutination reaction takes place between antibodies and soluble antigen that had been attached to an insoluble particle, the reaction is referred to as passive agglutination.

Latex particles: these are polystyrene particles that can be coated with either known antigen or specific antibody.

Complement fixation test (C.F.T)

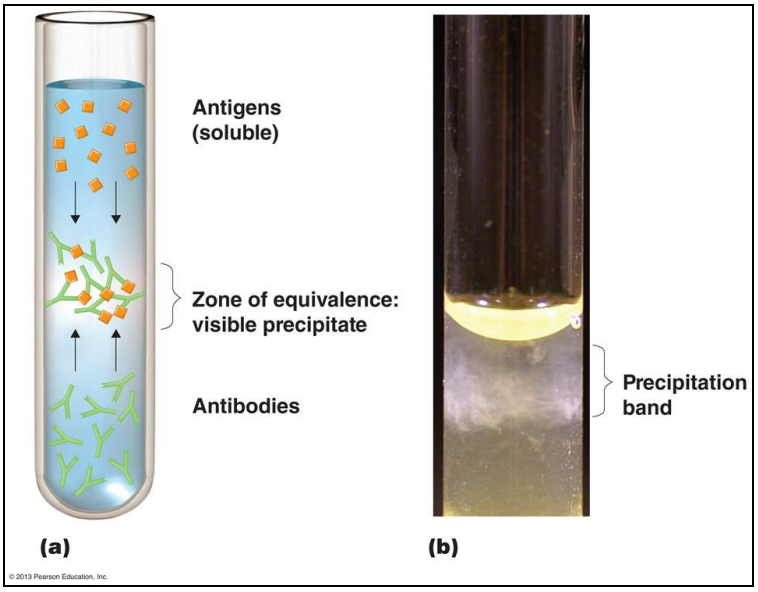
The complement fixation test is an immunological medical test that can be used to detect the presence of either specific antibody or specific antigen in a patient's serum, based on whether complement fixation occurs. It was widely used to diagnose infections, particularly with microbes that are not easily detected by culture methods, and in rheumatic diseases.

Precipitation Reaction

Combination of soluble Ag with specific Ab to give a complex from soluble aggregates, many types of precipitation used for qualitative and quantitative test as the followings :

1. **Ring test :**

   It is the simplest precipitation from placing on fluid (Ag over the Ab in a test tube), the reaction is visible as opaque disc.

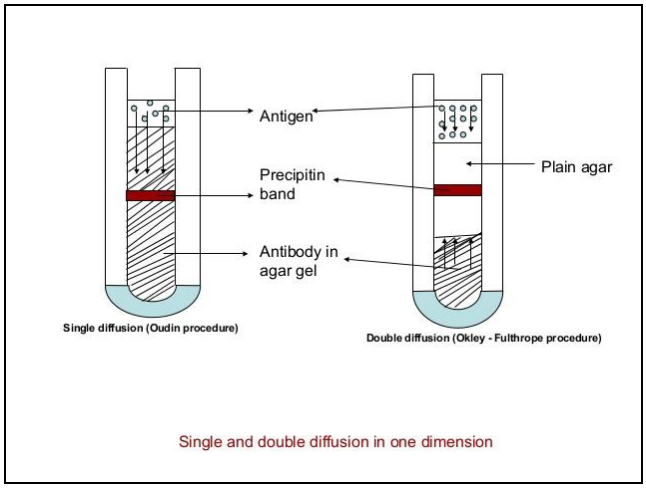


2. **Single diffusion in one dimension:**

   Is incorporation of antisera into agar and layering Ag solution above in the tube and after few hours to few days we observe the precipitation.

3. **Double diffusion in two dimension :**

   Ag & Ab placed in wells in agar gel (agarose) which diffuse forward each to others and precipitation happens to form an opaque line in the region when they meet in optimal proportion.



4. **Single radial immuno diffusion (SRID) (Mansoni test):**
   Where Ag diffuse from wells into agar containing suitable diluted antisera, the Ag concentration and even reach to optimal proportion of Ab concentration and give ring precipitation. The high concentration of Ag is related with the greater diameter of the precipitation.
5. **Immuno electrophoresis :**
   By combination double diffuse method (ouchterlony) with electrophoresis, the diffusion depend on charge and molecular weight.

Tertiary Ag – Ab reaction:

It is the biological expression of Ag – Ab reaction which help the patient such as toxin neutralization and viral neutralization, phagocytes and bacterial agglutination.

Neutralization test:

This use the ability of Ab to block the effect of toxins or infectivity of viruses. They can be used in cell culture (eg: Inhibition of cytopathic effect and plaque reduction assays) or in host animals (eg: mouse protection test).

Lecture : 11

Primary antigen – antibody reaction.

Immunoelectrophoresis (IEOP)

Immunoelectrophoresis is a general name for a number of biochemical methods for separation and characterization of proteins based on electrophoresis and reaction with antibodies. All variants of immunoelectrophoresis require immunoglobulins, also known as antibodies, reacting with the proteins to be separated or characterized.

This technique is also referred to as counter current electrophoresis. Electrophoresis is used to increase the speed with which the antigen and antibody travel in the agar gel.

Principle of Counterimmunoelectrophoresis

In this test, specific antibody is placed in a well at the positive electrode (anode) end of the plate and the unknown antigen in a well at the negative electrode (cathode) end. An electric current is applied and the antibody and antigen move towards each other. Positive samples show a line of precipitation within 30-60 minutes.

Counterimmunoelectrophoresis is used to detect extracellular antigens in cerebrospinal fluid.

Radio Immunoassay (RIA).

RIA is a competitive immunologic procedure for measuring very low concentrations of antigens (or antibodies) by using radioactively labeled antigens as competitors. Radioactive isotopes such as 3H , 14C, 35S, 30P or 125I can be used for labeling. It is a highly sensitive method to detect low concentration of the unknown (unlabeled) antigen and is used to assay: Hormones, Drugs, Enzymes, Microbial antigens e.g. hepatitis B antigen, carcinoembryonic and α- fetoprotein antigen. RIA can also be used for detection of antibody.

RIA technique utilizes three components:-

1. Patient antigen, the specific compound we wish to determine.
2. Labeled antigen, the same compound patient antigen which is attached a radioactive label.
3. Antibody, specific for the sample and labeled antigen.

There are two assay approaches in conventional RIA:

- ✓ Liquid phase Assay
- ✓ Solid phase Assay

Enzyme Linked Immunosorbent Assay ELISA

ELISA is a plate-based assay technique designed for detecting and quantifying peptides, proteins, antibodies and hormones. **In an ELISA**, an antigen must be immobilized to a solid surface and then complexed with an antibody that is linked to an enzyme. Detection is accomplished by assessing the conjugated enzyme activity via incubation with a substrate to produce a measureable product. The most crucial element of the detection strategy is a highly specific antibody-antigen interaction.

ELISA Types

1. Direct ELISA

For direct detection, an antigen coated to a multi-well plate is detected by an antibody that has been directly conjugated to an enzyme. This detection method is a good option if there is no commercially available ELISA kits for your target protein.

2. Indirect ELISA

For indirect detection, the antigen coated to a multi-well plate is detected in two stages or layers. First an unlabeled primary antibody, which is specific for the antigen, is applied. Next, an enzyme-labeled secondary antibody is bound to the first antibody. The secondary antibody is usually an anti-species antibody and is often polyclonal. The indirect assay, the most popular format for ELISA.

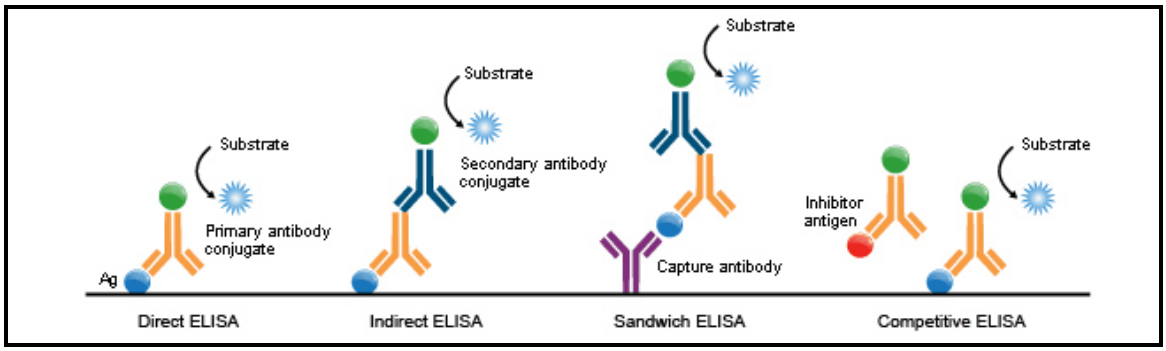
3.Sandwich ELISA:

Sandwich ELISAs typically require the use of matched antibody pairs, where each antibody is specific for a different, non-overlapping part (epitope) of the antigen molecule. A first antibody (known as capture antibody) is coated to the wells. The sample solution is then added to the well. A second antibody (known as detection antibody) follows this step in order to measure the concentration of the sample.

4. Competitive ELISA

The key event of competitive ELISA (also known as inhibition ELISA) is the process of competitive reaction between the sample antigen and antigen bound to the wells of a microtiter plate with the primary antibody. First, the primary antibody is incubated with the sample antigen and the resulting antibody–antigen complexes are added to wells that have been coated with the same antigen. After an incubation period, any unbound antibody is washed off. The more antigen in the sample, the more primary antibody will be bound to the sample antigen. Therefore, there will be a smaller amount of primary antibody available to bind to the antigen coated on the well, resulting in a signal

reduction. The main advantage of this type of ELISA arises from its high sensitivity to compositional differences in complex antigen mixtures, even when the specific detecting antibody is present in relatively small amounts.



Membrane-Based Cassette Assays

Membrane-based cassette assays are a relatively new type of enzyme immunoassay. They are rapid, easy to perform, and give reproducible results.

Typically these are designed as single-use, disposable assays in a plastic cartridge. The membrane is usually nitrocellulose, which is able to easily immobilize proteins and nucleic acids. Either antigen or antibody can be coupled to the membrane, and the reaction is read by looking for the presence of a colored reaction product

Another type of rapid assay, called ***immunochromatography***, The analyte is applied at one end of the strip and migrates toward the distal end, where there is an absorbent pad to maintain a constant capillary flow rate. The labeling and detection zones are set between the two ends.

As the sample is loaded, it reconstitutes the labeled antigen or antibody, and the two form a complex that migrates toward the detection zone. An antigen or antibody immobilized in the detection zone captures the immune complex and forms a colored line for a positive test. This type of test device has been used to identify microorganisms such as *Streptococcus pyogenes* and *Streptococcus agalactiae* and has been used to test for pregnancy, for troponin in a heart attack, and for hepatitis B surface antigen.

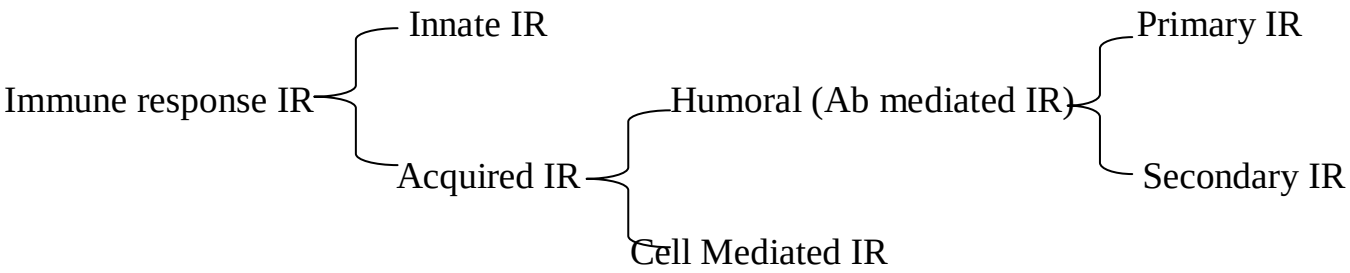
Lecture : 12

Immune Response

Immune response (IR): a protective response of the body's immune system to an antigen, especially a microorganism or that causes disease. The immune response is how your body recognizes and defends itself against bacteria, viruses and substances that appear foreign and harmful.

Mechanisms of IR:

1. Recognize, processed and presentation of antigen.
2. Cooperation of cells.
3. Destruction of specific Ag.



Humoral Immune Response :

Due to activation of B-cells and needs binding of antigen to specific surface IgM on B-cell leads to their proliferation and differentiation into effective plasma cells and memory cells. Plasma cells produce and secrets antibody specific for the antigen.

Functions:

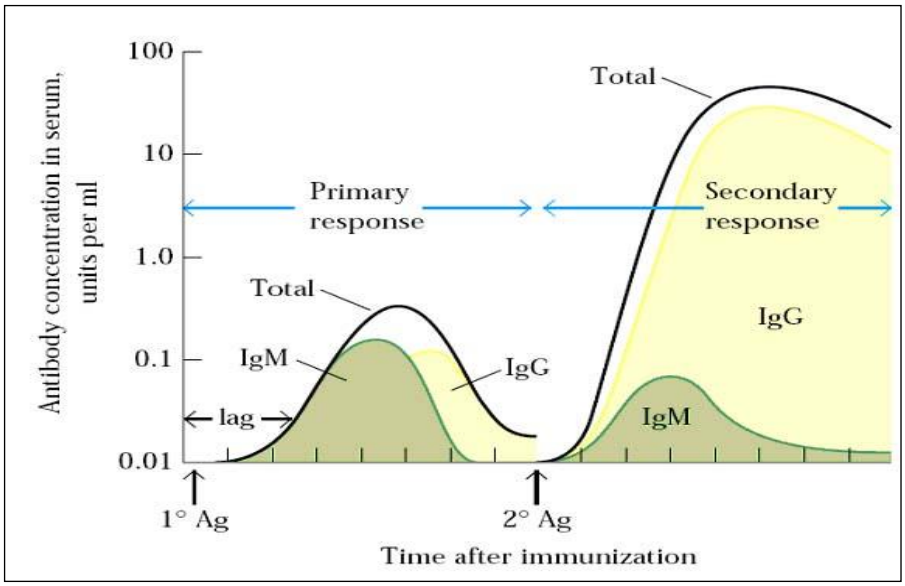
Humoral or Ab mediated immunity develops mainly against extracellular pathogens such as staphylococci, streptococci, encapsulated pathogens (eg: Pneumococcus, Neisseria) and against pathogens that secrets exotoxins (eg: Corynebacterium diphtheria). Antibodies neutralize exotoxins and some viruses(eg: rabies, Hepatitis A and B). IgM and IgG can activate the complement system which can opsonize pathogens leading to ingestion by phagocytic cells, or can cause lysis of the organisms.

Exposure to an Ag by an individual for the first time is called primary IR and re-exposure to the same antigen is called secondary IR which develops due to immunologic memory.

There are two types of humoral IR:

1. **Primary humoral IR: which characterized by :-**

- ✓ Latent phase (lag phase): in the first few days (7-10 days), is a period for T and B cells to bind Ag then activated and differentiated. Ab levels in the serum is zero (no Ab production).
- ✓ Exponential phase (log phase): increased of serum Ab level logarethmatically and reaches a peak.
- ✓ Steady phase (plateaus phase): production and degradation of Abs are balanced.
- ✓ Decline phase: the IR begins to shut down, and Abs concentration in the serum decreases rapidly due to combination with Ags.



2. **Secondary IR (anamnestic or memory IR):**

In the second exposure to same Ag, there is a rapid Ab response (the lag phase is only 3-5 days) this is related to memory cells produced after the first contact.

Comparison between Primary & Secondary IR:

Primary IR	Secondary IR
The lag phase is often longer, weeks or months	Very short (3-4days) due to memory presence cells
IgM predominant with low IgG	IgG predominant with low IgM
Antibody titer is low	Antibody titer is high
Cell (native B cell)	Memory B cell
Ab level decline rapidly	Ab level tend to remain high for longer

Cell-mediated immunity

The main defense is against intracellular pathogens like Mycobacterium, viruses, fungi and protozoa. It may be involved in tumor immunity. Immune response in this case is due to activation of T-cells leads to production of cell-mediated immunity by a direct attack on foreign cells.

Two different types of T-cells are generated, T-helper ($CD+^4$) and cytotoxic T-cells ($CD+^8$), their killing are specific.

Helper T-cells are killed the target cells specifically by producing lymphokines, while cytotoxic T-cells are responsible for direct usually of virally-infected cells.

Lecture 13.

Tolerance and Autoimmunity

When Ag introduced into the body there will be an immune response (IR), but sometimes there is no IR or unresponsiveness (tolerance).

Tolerance : unresponsiveness towards specific antigen in the immune competent host.

Generally, antigens that are present during embryonic life are considered as (self) and don't stimulate an IR. On the other hand, antigens that are present during the process of maturation are considered as non-self and usually stimulate an IR. Antigens that induce tolerance are called toleragens rather than immunogens, tolerance is either:

1. Natural tolerance: Antigens present during the process of maturation is considered as self and usually not stimulate IR.
2. Specific acquired tolerance.

Factors playing a role in inducing tolerance:

1. Time of introduction of toleragen: it mean either in perinatal or adult period.
2. Nature (structure), high dose of toleragen, e.g: very simple molecule induce tolerance more rapidly than complex, and very high or very low doses may result in tolerance of an IR.
3. Persistence of toleragen in host, e.g: in cancer, there will be continuous shedding of antigens.

Cellular involvement of tolerance:

Tolerance can be exist in T and B cells or both of them:

1. T-cell which is tolerated quickly (within 2-5 days) and remains tolerant longer than B-cells.
2. B-cell which is tolerated within 1-2 weeks because these cells cannot make Abs to most Ags without help of T-cells.

Autoimmune reaction:

Breakdown in self-tolerance lead to immune cells react with self-tissues antigens which result in tissue injury and leading to autoimmune diseases (AID).

Factors playing a role in induction of (AID):

1. Release of sequestered antigens and certain tissue e.g: eye lens and CNS are sequestered (hidden) so that their antigens are not exposed to immune system. When such Ags enter circulation accidentally during injury or trauma this will lead to stimulate humoral and cellular IR as in cataract operation lead to uveitis.
2. Genetic factors, many autoimmune diseases exhibit familial incidence (means genetic predisposition to these diseases). There is a strong association of some diseases with HLA especially class-II genes. For e.g: RA occurs predominantly in individuals carrying HLA-DR4 gene, and HLA-B27 gene in ankylosing spondylitis, while HLA-DR2 gene in systemic lupus erythematosus SLE disease.
3. T-cell bypass: means not giving help and doing the ordinary job such as :
    a. Modification (alteration) of autoantigen, e.g: using chemicals (drugs), physical (radiation) or environmental (infection) factors.
    b. Cross reaction (some microorganisms have antigenic determinant area) like self-antigenic determinant, so when a body form Abs to microorganisms will attack the self-antigens also.
4. Polyclonal activation of B-cells.
5. Hormonal factors: female more prone to autoimmune diseases than male due to sex hormones.
6. In appropriate expression of class-II HLA Ag as in thyroiditis, sometimes cells acquired HLA-II and become as APC and present self Ag to immune system.
7. Environmental factors.
8. Thymic defect: especially in old age, thymus evaluated and T-cell not works properly.

Lecture : 14.

Autoimmune diseases

Autoimmune diseases are divided into :

1. Organ specific means injury occurs in one organ eg: gravies disease.
2. Non organ specific (Systemic) : means injury occurs in tissues of many organs eg: RA or any connective tissue disease.

An example of autoimmune diseases:

1. Organ specific autoimmune diseases:

- ✓ **autoimmune hemolytic anemia** : autoantibodies to RBCs surface antigens, triggering complement – mediated lysis or Ab – mediated opsonization and phagocytosis of RBCs. Such anemia is drug induced like using penicillin or methyldopa which interact with RBCs and diagnosed by coombs test.
- ✓ **Insulin dependent diabetes mellitus (IDDM):** autoantibodies attack pancreas (islet – cell, enzymes) such as glutamine acid decarboxylase (GAD), insulin autoantibodies (IAA), insulinoma autoantibodies (IA2), these autoantibodies destroys islet – cells and resulting in increased levels of blood glucose.
- ✓ **Myasthenia gravis:** autoimmune disease mediated by blocking antibodies by producing autoantibodies that bind acetyl choline receptor (AChR) on the motor end plates of muscles and also inducing complement – mediated lysis of the cell, leading to weakened of skeletal muscles.

2. Non organic (systemic) autoimmune diseases:

Systemic lupus erythematosus (SLE): in this disease there is autoantibodies against DNA, nucleoproteins and other component of the nucleus. Symptoms vary between people and may be mild to severe. Common symptoms include painful and swollen joints, fever, chest pain, hair loss, mouth ulcers, swollen lymph nodes, feeling tired, and a red rash which is most commonly on the face. Rates of disease vary between countries from 20 to 70 per 100,000. Women of childbearing

age are affected about nine times more often than men. Diagnosis can be difficult and is based on a combination of symptoms and laboratory tests

Tests used to diagnose SLE may include:

1. Antinuclear antibody (ANA).
2. CBC with differential.
3. Chest x-ray.
4. Serum creatinine.
5. Urinalysis.

Goodpasture syndrome:

autoantibodies are formed against collagen in basement membrane of the kidneys and lungs. This disease primarily affect young men. Subsequent complement activation leads to direct cellular damage (glomerular and alveolar membrane) leading to kidney damage and lung hemorrhage. Death may occur in several months. Diagnosis by immunofluorescent – labeled anti – IgG.

Rheumatoid arthritis:

Sever chronic systemic inflammatory autoimmune disease of unknown etiology, characterized by inflammation of synovial membrane, affecting peripheral joints in a symmetric fashion, leads to cartilage destruction, bone erosion and joint deformities, extra – articular manifestations like vasculitis and subcutaneous nodules also can occur.

Symptoms are:

- ✓ Joint pain, tenderness, swelling or stiffness for six weeks or longer
- ✓ Morning stiffness for 30 minutes or longer
- ✓ More than one joint is affected
- ✓ Small joints (wrists, certain joints of the hands and feet) are affected
- ✓ The same joints on both sides of the body are affected.

Laboratory diagnosis :

Blood Tests

The blood tests will measure inflammation levels and look for biomarkers such as antibodies (blood proteins) linked with RA.

Inflammation

Erythrocyte sedimentation rate (ESR, or "sed rate") and C-reactive protein (CRP) level are markers of inflammation. A high ESR or CRP is not specific to RA, but when combined with other clues, such as antibodies, helps make the RA diagnosis.

Antibodies

Rheumatoid factor (RF) is an antibody found in about 80 percent of people with RA during the course of their disease. Because RF can occur in other inflammatory diseases, it's not a sure sign of having RA. But a different antibody – anti-cyclic citrullinated peptide (anti-CCP) – occurs primarily in patients with RA. That makes a positive anti-CCP test a stronger clue to RA. But anti-CCP antibodies are found in only 60 to 70 percent of people with RA and can exist even before symptoms start.

Lecture : 15.

Immune Responses to Viruses

Immune response against viruses can be achieved by cytotoxic cells, interferons and antibodies.

Via cytotoxic cells

When a virus infects a person (host), it invades the cells of its host in order to survive and replicate. Once inside, the cells of the immune system cannot **'see'** the virus and therefore do not know that the host cell is infected. So, cells employ a system that allows them to show other cells what is inside them – they use or **MHC class I** to display pieces of protein from inside the cell upon the cell surface. If the cell is infected with a virus, these pieces of peptide will include fragments of proteins made by the virus to activate **cytotoxic T cell**

Cytotoxic T cells have specialized proteins on their surface that help them to recognize virally-infected cells. These proteins are called **T cell receptors** (**TCRs**) that can specifically recognize a particular antigenic peptide bound to an MHC molecule. If the T cell receptor detects a peptide from a virus, it warns its T cell of an infection. The T cell releases **cytotoxic factors** to kill the infected cell and, therefore, prevent survival of the invading virus

Some viruses stop MHC molecules from getting to the cell surface to display viral peptides. If this happens, the T cell doesn't know there's a virus inside the infected cell.

However, another immune cell specializes in killing cells that have a reduced number of MHC class I molecules on their surface – this cell is a **NK cell**. When the NK cell finds a

cell displaying fewer than normal MHC molecules it releases toxic substances, which kill the virally-infected cell.

Mechanisms of cytotoxic IR against Virus

1. **Apoptosis :**
   Once inside the target cell, they initiate a process known as programmed cell death or **apoptosis,** causing the target cell to die.
2. Cytokines include **tumor necrosis factor** that transfer a signal from the T cell to the infected, or other neighboring cells, to enhance the killing mechanisms.

Cytotoxic factors (granules) of Cytotoxic cells are :

1. **Perforin**, a protein that can make pores in cell membranes; these pores allow entry of other factors into a target cell to facilitate destruction of the cell.
2. **Granulysin** is a substance released by cytotoxic T cells (CD8) and NK cell. It functions to create holes in the target cell membrane and destroy it. Granulysin is able to induce apoptosis in target cells and also has antimicrobial action.
3. Enzymes called **granzymes** are also stored in cytotoxic cells, and released from, the granules. Granzymes enter target cells through the holes made by perforin.

Via interferons

Virally infected cells produce and release small proteins called **interferons**, which

1. Prevent replication of viruses, by directly interfering with their ability to replicate within an infected cell.
2. They also act as signaling molecules that allow infected cells to warn nearby cells of a viral presence - this signal makes neighboring cells increase the numbers of MHC class I molecules upon their surfaces, so that T cells

surveying the area can identify and eliminate the viral infection as described above.

Via antibodies

1. Firstly, the antibodies **neutralize** the virus, meaning that it is no longer capable of infecting the host cell.
2. Secondly, many antibodies can work together, causing virus particles to stick together in a process called **agglutination**. Agglutinated viruses make an easier target for immune cells than single viral particles.
3. The activation of phagocytes. A virus-bound antibody binds to receptors, called Fc receptors, on the surface of phagocytic cells and triggers **phagocytosis**, by which the cell engulfs and destroys the virus.
4. Finally, antibodies can also activate the complement system, which opsonizes and promotes phagocytosis of viruses. Complement can also damage the envelope (phospholipid bilayer) that is present on some types of virus.