Manual of Medical Microbiology & Immunology

Volume I

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To ***MY FAMILY***

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PREFACE

The aim of this manual is to provide an accurate comprehensive and up to date coverage of medical microbiology and immunology. The book is principally intended for medical students, but house officers and practicing physicians will find it very useful.

The book is presented in two volumes: **Volume I** deals with bacterial structure and physiology, bacterial viruses, bacterial genetics, antibacterial chemotherapy, and host-parasite relationship. Because of the important recent developments in molecular biology a chapter on genetic recombination and its applications in the medical field is included.

Volume I also includes a recent coverage of the fundamentals of immunology in a simplified manner. It includes a review of innate immunity, immunogens, mechanisms of acquired immune response and their participation in protective immunity and immunoprophylaxis, including the most recent immunization schedules. Inappropriate immune responses including; hypersensitivity, autoimmune diseases, graft rejection as well as immunodeficiency diseases are discussed.

Of the laboratory methods, sterilization and antigen antibody reactions are discussed in short. However the laboratory methods used for cultivation, isolation and identification of microorganisms as well as the detailed methods of sterilization and antigen antibody reactions as well as the molecular biology techniques are discussed in details in the **"Manual of Practical Microbiology"** by the same author.

This volume is provided with 44 illustrative diagrams. A list of abbreviations is included at the end.

Note that in this edition, some detailed parts of the text were added, and are written in a very small font size. These are mainly intended for the postgraduate students and for the medical students with high interest in microbiology and immunology.

Volume II of this manual includes systematic and applied medical microbiology.

I would like to thank Prof. Dr. Wafaa Zaghloul, Prof. Dr. Abdel Fattah Attia Prof. Dr. Eman El-Saeidy, and Dr. Abeer Sheneef, for their helpful suggestions. My thanks are extended to Dr. F. Ghobrial and Mr. Y. El-Nabarawi for their valuable assistance in the production of the illustrations.

Abla M. El Mishad **Cairo**
2015

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INTRODUCTION

Microbiology is the science that deals with tiny organisms or microbes that can not be seen by the naked eye. These organisms are widely distributed in nature. Some of them are beneficial to man. For example, some organisms can fix and convert atmospheric nitrogen to useful chemical compounds that fertilize the soil. Others are of use in food and drink industry. In our field of Medical Microbiology, we deal with microbes that are harmful and cause disease to man.

The agents of human infectious diseases belong to 5 major groups of organisms: bacteria, fungi, protozoa, helminthes and viruses. Taken together the helminthes and protozoa are called parasites. Viruses are quite distinct from the other organisms as they are not cells but can replicate only within host cells which provide the machinery for protein synthesis and energy generation.

Bacteria are **prokaryotic** cells. They are more simple and smaller than fungi and protozoa. Their genetic material is a single naked chromosome with no nuclear membrane, surrounded by a rigid cell wall containing peptidoglycan. They contain 70S ribosomes but no organelles and replicate by binary fission.

On the other hand, fungi and protozoa are **eukaryotic** cells (like human and animal cells). They contain a true nucleus with a nuclear membrane, nucleoli, multiple chromosomes and organelles such as mitochondria and lysosomes, and 80S ribosomes. They are bound by a flexible cell membrane or in case of fungi a rigid cell wall containing chitin. They multiply by budding or mitosis. The cytoplasmic membrane of eukaryotes contains sterols which is not present in prokaryotes except in mycoplasma.

Bacteria are classified into orders, each containing many families under which are included a variety of genera. Each genus comprises some species which may be further divided into types. Individual organisms are recognized by their generic and species names e.g. *Bacillus anthracis* or *Mycobacterium tuberculosis; Mycobacterium* is the genus and *tuberculosis* is the species name.

Characteristics of Prokaryotic and Eukaryotic Cells

Characteristic	Prokaryotic Bacterial Cells	Eukaryotic Human Cells
DNA within a nuclear membrane	No	Yes
Mitotic division	No	Yes
DNA associated with histones	No	Yes
Chromosome number	One	More than one
Membrane-bound organelles, such as mitochondria and lysosomes	No	Yes
Size of ribosome	70S	80S
Cell wall containing peptidoglycan	Yes	No

CHAPTER 1 STRUCTURE OF THE BACTERIAL CELL

Bacteria are small prokaryotic unicellular organisms that multiply by binary fission.

Size, Shape and Arrangement of Bacteria:

Bacteria range in **size** from about 0.2 to 1.2 pm in width and 0.4-14 pm in length. There are three basic **shapes;** cocci, bacilli and spirals. Some bacteria are variable in shape and are said to be pleomorphic. The shape of a bacterium is determined by its rigid cell wall.

In addition to their characteristic shapes, the **arrangement** of bacteria is important. For example, certain cocci occur in pairs (pneumococci), some in chains (streptococci) and others in grape-like clusters (staphylococci). These arrangements are determined by the orientation and degree of attachment of the bacteria at the time of cell division.

The microscopic appearance of a bacterium is one of the most important criteria used in its identification. Bacteria can be easily seen under the microscope when **stained** by simple stains, e.g. methylene blue or by differential stains, e.g. gram stain. The **gram stain** is the most important differential stain it divides bacteria into gram positive (violet staining) and gram negative (red-staining) bacteria. The Ziehl Neelsen stain is used to stain a special group of bacteria called acid-fast bacteria, e.g. *M. tuberculosis.*

The Bacterial Cell is Composed of the Following Structures (Fig. 1):

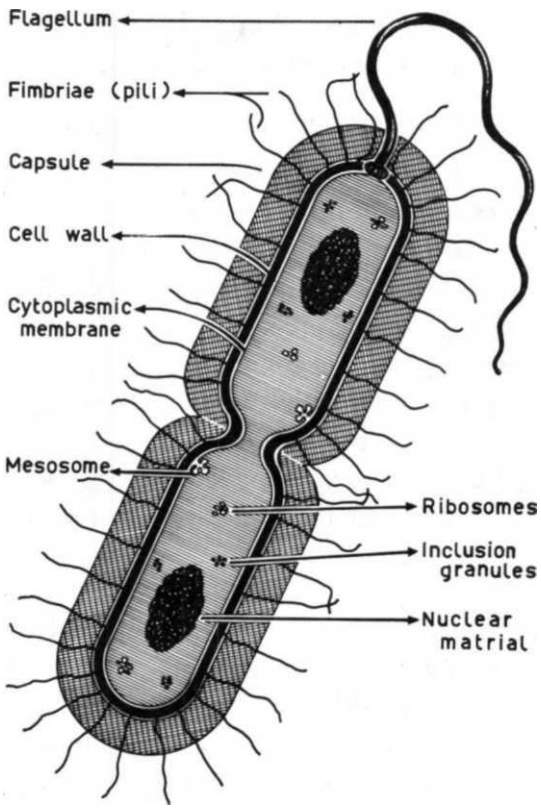
Bacterial Cell Wall (Fig. 2):

The cell wall is the outermost component of all bacteria. It is a multilayered structure located external to the cytoplasmic membrane. It is composed of a layer of peptidoglycan to which the cell owes its rigidity.

In gram positive bacteria, a thick **"peptidoglycan"*** layer (40 sheets) forms about 50% of the cell wall material. It is responsible for the rigidity of the cell wall and for maintaining the shape of the bacterial cell. **Teichoic acid**** also forms a major surface component; it may be on both sides of the peptidoglycan layer and is highly immunogenic. It induces the production of tumour necrosis factor (TNF-a) and interleukin 1 (IL-1) by macrophages and participates in toxic shock caused by some gram positive bacteria. It may mediate adherence of some organisms to mucosal cells.

* **Peptidoglycan** is an open 3-D net composed of a backbone of N-acetyl-glucosamine and N-acetyl muramic acid. Attached to each of the muramic acid molecules is a tetrapeptide consisting of both D- and L- amino acids of which **diaminopimelic acid** is unique to bacterial cell wall.

It is a polymer of glycerol or ribitol phosphate. Some of the polymers penetrate the peptidoglycan layer and are linked to the lipid in the cytoplasmic membrane to form **lipoteichoic acid.



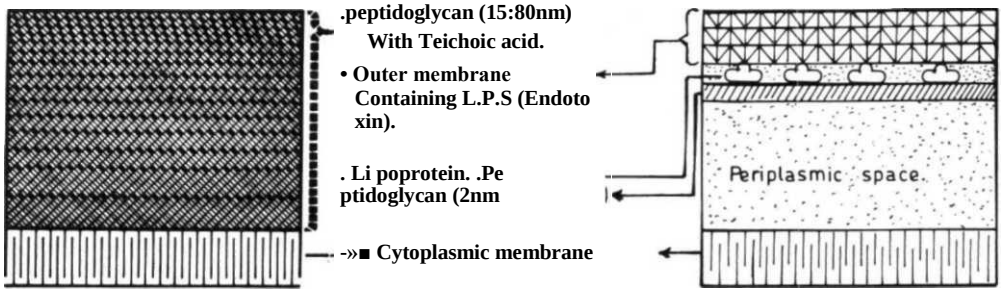
(Fig. 1): A **bacterial cell during replication by binary fission.**

In gram negative bacteria, there is an outer layer of **lipopolysaccharides (LPS)** which is extremely toxic to the human body and is called the **endotoxin.** It is released only when the bacterial cells are lysed, and is responsible for the fever, hypotension and shock caused by gram negative organisms. The toxicity is associated with the lipid fraction which is called lipid A. The polysaccharide represents a major surface antigen called somatic (O) antigen which is immunogenic.

A thin layer of **peptidoglycan** (2 sheets) which forms only 5-10% of the cell wall material is located inside the outer layer of LPS. **Lipoprotein** molecules cross link the outer membrane and peptidoglycan layers.

The space between the outer and inner layer (cytoplasmic membrane) is called the **periplasmic space** and is filled with a gel-like solution of proteins. It is the site, in some species, of the enzymes called beta lactamases that degrade penicillins and other P-lactam drugs.

Since peptidoglycan is present in bacteria but not in human cells, it is a good target for antibacterial drugs. Several of these drugs, such as, the penicillins and cephalosporins, inhibit its synthesis.



(Fig. 2): Diagram showing the structure of the cell wall of gram positive and gram negative bacteria.

Functions of the Cell Wall

1- It is a rigid structure that maintains the shape of bacteria.

2- It is osmotically insensitive; it protects the cytoplasmic membrane from bursting in hypotonic solutions.

3- The cell wall composition plays an important role in determining the cell's reaction to gram stain. Gram negative cells can not retain the dye-iodine complex in the face of the decolourizing agent. These have a more porous cell wall than the gram positive cells which resist decolourization.

4- It plays a role in cell division.

Cell Wall Deficient Bacteria

Mycoplasma is the only bacterial species deficient in cell wall. Hence, they are pleomorphic, are not destroyed by penicillin which acts on the bacterial cell wall, and can not be stained with gram stain.

Protoplasts, Spheroplasts and **L-Forms:** Bacterial cell wall may be lost under the effect of certain environmental conditions e.g. treatment with penicillins and lysozyme. If such treated cells are placed in osmotically protective media, they liberate **protoplasts** from gram positive cells and **spheroplasts** from gram negative cells.

If such cells are able to grow and divide they are called **"L-forms".** Unlike mycoplasma, L-forms can revert to the parental form upon removal of the cell wall inhibitor. However, some are stable and never revert.

L-forms may be formed during active infection under the effect of antibiotics, since they are resistant to cell wall inhibiting antibiotics e.g., penicillins, they can cause chronic infection e.g. urinary tract infection. Their reversion to the parent form can cause relapses of the overt infection.

Cytoplasmic Membrane:

It is a semi-permeable double-layered structure, composed of phospholipid and protein. Its main function is to maintain a constant environment within the interior of the cell by controlled transport mechanisms including:

1- Selective permeability to different molecules.

2- Active transport of ions (H^+, Na^+, K^+, etc..) and nutrients to achieve osmotic balance and a pool of nutrients.

3- It also supplies the cell with energy through electron transport and oxidative phosphorylation, i.e. site of respiration.

4- Excretion of hydrolytic exoenzymes which degrade the different nutrients into subunits small enough to penetrate the cytoplasmic membrane.

5- Excretion of pathogenicity proteins e.g. IgA protease and some exotoxins.

6- It provides enzymes and carrier molecules that function in DNA, cell wall, and lipid membrane synthesis.

7- It bears receptors and other proteins of the chemotactic and other sensory transduction systems.

Mesosomes are invaginations of the cytoplasmic membrane and play a role in respiration and cell division. They function as the origin of the transverse septum that divides the cell in half and as the binding site of the cell DNA which duplicates to provide the genetic material of each daughter cell.

Intracytoplasmic Structures:

1- The nucleoid is the area of the cytoplasm in which DNA is located. It is a single, circular packed bundle of double stranded DNA molecule (chromosome). There is no nuclear membrane or nucleolus. The DNA carries the genetic information to daughter cells and it is duplicated before cell division. Linear DNA was found in some species e.g. *Borrelia burgdorferi.*

2- **Ribosomes:** These are tightly packed spherical particles present in the cytoplasm. Clusters of ribosomes are called polysomes. They are composed of 40% protein and 60% RNA. Bacterial ribosomes are 70S in size, with 30S and 50S subunits. They are the site of protein synthesis.

3- Intracytoplasmic inclusions: The cytoplasm contains granules which represent accumulation of food reserve. They may be rich in lipids or carbohydrates. The volutin granules rich in metaphosphate are found in the genus *Corynebacterium.*

4- **Plasmids:** These are extrachromosomal double stranded circular DNA molecules capable of replicating independent of the bacterial chromosome (see chapter 4).

Structures Outside the Cell Wall

1- Capsule: It is a gelatinous layer outside the cell wall of some species of bacteria. It is composed of polysaccharide, except in the anthrax bacillus which has a polypeptide capsule. The capsule is best formed inside the animal body i.e. *in vivo*. Capsules are not usually stained by gram stain and so they appear as unstained halos around the organism.

Variation in the sugar components of the polysaccharide is responsible for existence of different serologic types within the species. For example, there are 91 types of *Strept. pneumoniae* different in the polysaccharide capsule. Identification of an organism can be made by adding anti-capsular antibodies to the organism, if the antibody is specific, the capsule swells and can be seen by the microscope, this is called the quellung reaction.

The capsule is an important virulence factor, it protects the bacteria from phagocytosis (antiphagocytic). This may be due to the slimy capsule that makes it difficult for phagocytes to hold firmly on bacterial surface, or the capsule may mask bacterial cell wall components e.g. complement receptors. The capsule may play a role in adherence of bacteria to human tissues.

The capsular polysaccharides are used as immunogens in certain vaccines, as they are capable of inducing protective antibodies e.g. the pneumococcal, the meningococcal and *H. influenzae* type b vaccines.

Glycocalyx (slime layer) is a loose polysaccharide meshwork of fibrils extending outward from the cell of some bacteria. It allows the bacteria to adhere firmly to various structures e.g. skin, heart valves, and catheters. It mediates adherence of *Strept. mutans* to the surface of the teeth leading to the formation of dental plaque which is the precursor of dental caries.

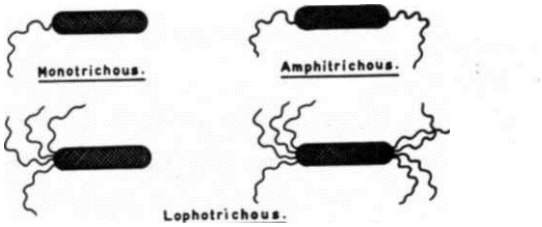
2- Flagella: These are the organs of motility in all motile bacteria. They move the bacteria towards nutrients and other attractants (chemotaxis). Flagella are long thin filamentous appendages that are attached to the cell wall and cytoplasmic membrane by a basal body.

They are composed of a single protein; "flagellin", which is antigenic and differs in different bacterial species. Specific antisera can be prepared, against flagellar "H" antigens, and are used to determine the serologic type of a strain in a certain flagellated species e.g. serotyping of *Salmonellae.*

The distribution and number of flagella are constant in any species (Fig.3). Peritrichous and monotrichous arrangements are the most frequent in pathogenic species. The flagella can be seen by electron microscopy.

Spirochetes move by using a flagellum-like structure i.e. **axial filament** which wraps around the spiral-shaped cell to produce an undulating motion.

(Fig. 3):
Various distributions of flagella.



3- **Pili (Fimbriae):** These are hair-like filaments that extend outwards from the cell surface. They are shorter and thinner than flagella and are composed of subunits of a protein; pilin. They are found mainly on gram negative organisms. Two types can be distinguished:

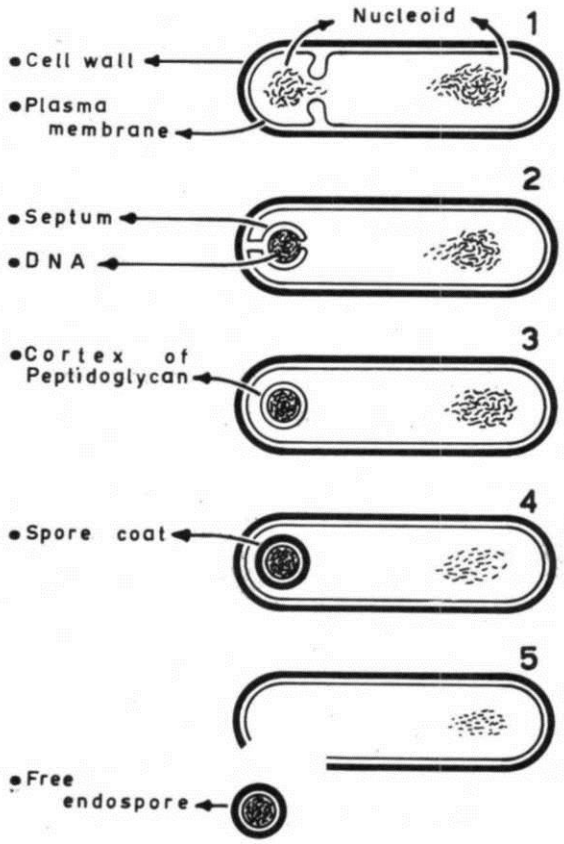
a-Ordinary pili: Also known as "colonization antigens", these mediate adherence of bacteria to specific receptors on human cell surface which is a necessary step in the initiation of infection for some organisms. Mutants of *N. gonorrhoeae* that do not have pili are non-pathogenic. Pili are also antiphagocytic, so they are virulence factors in some species of bacteria.

b- Sex pili: These are responsible for attachment between the male (F* donor) and the female (F" recipient) bacteria during conjugation (p. 23).

Bacterial Endospores:

These are highly resistant resting forms of some gram positive bacteria. They are formed upon exposure to unfavourable conditions, e.g. depletion of nutrients, heat, dryness, etc... Spore formation is a characteristic feature of the gram positive rods in the aerobic genus *Bacillus*, e.g. *B. anthracis* and the anaerobic genus *Clostridium*, e.g. *CI. tetani.*

Sporulation (Fig. 4) occurs by the development of an ingrowth of the cytoplasmic membrane cutting off a portion of the cell's cytoplasm and including the nuclear material, ribosomes, glycolytic enzymes and little water (spore core). Then a thick cortex of peptidoglycan and a tough keratin-like spore coat are formed around. Once formed, the spore has no metabolic activity and can remain dormant for years. Upon exposure to water and appropriate nutrients, **germination** to vegetative form occurs. Spores are formed only *in vitro;* they do not develop in the animal tissues where nutrients are adequate. The **position** of the spore in relation to the body of the bacillus varies and is characteristic for the species (Fig. 5). They are **stained** by special stains. They appear as unstained areas by gram stain.



(Fig. 4): Spore formation.

The medical importance of spores lies in their extraordinary resistance to heat and chemicals. Mere boiling does not destroy spores and sterilization by autoclaving at 121°C for 20-30 minutes is required to free instruments and products, for medical use, from spores.

The marked resistance of spores is due to:

a) The thick spore cortex and tough spore coat.

b) The large amounts of **calcium dipicolinate** which is found only in the core of spores.

c) Their dehydrated state.

d) Their very low metabolic and enzymatic activity.

(Fig. 5): Position of spores.

CHAPTER 2 BACTERIAL GROWTH and METABOLISM

Bacterial Reproduction:

Bacteria reproduce by **"binary fission",** i.e. one cell divides into two equal daughter cells similar in genetic character to the mother cell (Fig. 1). The process starts by elongation of the bacterial cell and duplication of the chromosome. The two sister chromosomes become attached to the cytoplasmic membrane (at the mesosome) across the bacterial cell. These are separated by the in-growing newly synthesized cell membrane and cell wall, forming a septum that divides the cell into two. The new daughter cells may remain temporarily attached before complete separation giving certain characteristic arrangement, e.g. clusters, pairs, chains, etc...

The doubling (generation) time, which is the time required by the bacteria to double its number, varies from one species to another. It may range from, as little as 20 minutes for the rapid growing *E. coli,* to more than 24 hours for the slow growing *M. tuberculosis.*

Bacterial Growth Curve (Growth phases):** (Fig. 6)

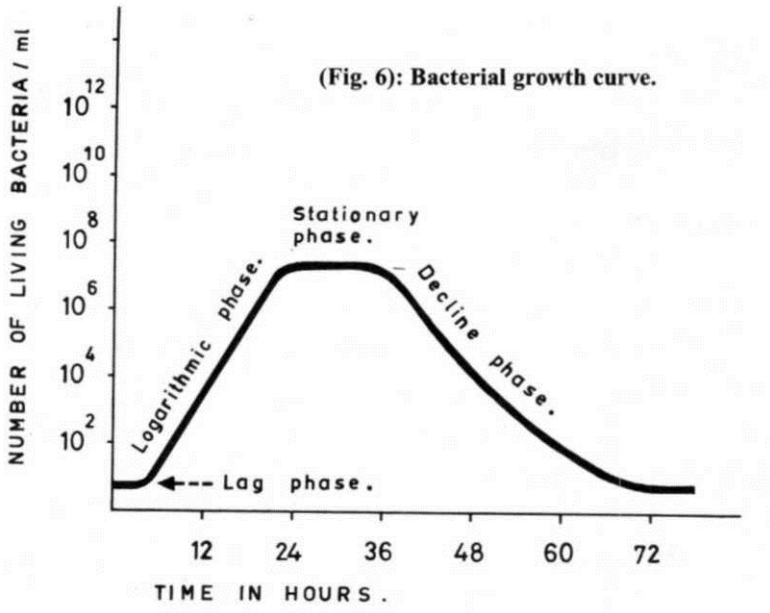
If a small number of bacteria are inoculated into a liquid nutrient medium and the bacteria are counted at frequent intervals and the results plotted, a characteristic growth curve with four phases is obtained:

1- **Lag phase:** It is the first phase during which no cell division occurs. The bacteria adapt to the new environment by formation of new enzymes and macromolecules needed for replication.

2- **Logarithmic phase:** During this phase, rapid cell division occurs and the number of bacterial cells increases steadily by time. Many antibiotics are effective during this phase, e.g. 13-lactam drugs, such as penicillin, which act when the cell is making peptidoglycan.

3- **Stationary phase:** As the nutrients in the medium are exhausted and toxic products accumulate, the rate of growth decreases. The number of dying cells equals that of newly formed cells. The number of living bacteria remains constant.

4- **Decline phase:** As the exhaustion of nutrients and accumulation of toxic products continue, the death rate exceeds the multiplication rate and the number of living bacteria decreases steadily.



(Fig. 6): Bacterial growth curve.

Bacterial Metabolism:

Many bacteria secrete enzymes e.g. lipases, nucleases, proteinases, and other hydrolytic enzymes. These enzymes breakdown extracellular nutritive material into simpler molecules that are actively transported across the cytoplasmic membrane into the bacterial cell. These molecules are then oxidized by bacteria to yield energy; and the degradation products are used to build up structural components and essential macromolecules for cell metabolism (anabolism).

The oxidation processes involve a series of reactions in which hydrogen ions (electrons) are released in the reaction and are transferred to a hydrogen acceptor. The hydrogen acceptor is molecular oxygen in aerobic respiration or an inorganic compound, (e.g. nitrate) in anaerobic respiration. The whole process is catalysed by a set of enzymes and coenzymes similar to the cytochrome system. The energy that results from these reactions is stored as high energy bonds, e.g. ATP to be used in anabolic processes.

Fermentation refers to the breakdown of a sugar to pyruvic acid and then, usually, to lactic acid. Fermentation is also called the glycolytic cycle, and this is the process by which facultative bacteria generate ATP in the absence of oxygen. If oxygen is present, the pyruvate produced by fermentation enters the Krebs cycle (oxidation cycle, tricarboxylic acid cycle) and is metabolized to two final products; $C0_2$ and H_20. The Krebs cycle generates much more ATP than the glycolytic cycle; therefore, facultative bacteria grow faster in the presence of oxygen. Facultative and anaerobic

bacteria ferment but aerobes, which can only grow in presence of oxygen, do not.

In the clinical laboratory, identification of several important human pathogens is based on the fermentation of certain sugars. The pyruvate and lactate produced turn the medium acidic and are detected by a pH indicator.

Bacterial Nutrition:

Bacteria, like all cells, require nutrients for maintenance of their metabolism and for cell division. They differ widely in their nutritional requirements. Two nutritional groups can be distinguished:

I- Autotrophs: These are bacteria which can utilize simple inorganic substances, e.g. CO_2 as a source of carbon and ammonium salts as a source of nitrogen; from which they synthesize organic substances, e.g. proteins, carbohydrates, etc...

The energy needed for their metabolism is obtained from light or from oxidation of inorganic substances. These are free living, non-parasitic organisms of no direct medical importance.

II- Heterotrophs: These bacteria require complex preformed organic substances, e.g. sugars, proteins etc., which are derived from plant or animal sources. All bacteria of medical importance are heterotrophs. They live in or on the animal body and are called parasitic bacteria. Many of the heterotrophs can grow on simple media containing peptone or meat extract as in broth and nutrient agar. Others require complex organic material, e.g. blood or serum. Media containing such substances are called enriched media.

Growth Factors or Essential Metabolites:

Many pathogenic species of bacteria require for growth essential substances, in small amounts, which they can not synthesize, e.g. vitamins, amino acids, purines and pyrimidines. These have to be added to culture media. However, these substances are present in host tissues.

Fastidious organisms are those which require unusually complex nutrients added to the growth media.

Gaseous Requirement of Bacteria:

A- **Oxygen:** According to their oxygen requirement, bacteria are classified into 5 groups:

1- **Obligate aerobes:** Some bacteria will only grow in the presence of oxygen because their energy generating system is dependent on oxygen as the hydrogen acceptor, e.g. ***M. tuberculosis*** and ***Pseudomonas aeruginosa.***

2- **Facultative anaerobes:** These bacteria can grow in the presence or absence of 0_2. They use 0_2 to generate energy by aerobic respiration if it is present. However, they can use anaerobic respiration to generate energy in the absence of 0_2.

3- Obligate anaerobes: These bacteria grow only in the complete absence of 0_2 and die in its presence, e.g. *CI. tetani.*

In presence of 0_2, two toxic molecules are produced; hydrogen peroxide and superoxide ion. Anaerobic bacteria lack the enzymes catalase and superoxide dismutase that breakdown these molecules. For these organisms, the final hydrogen acceptors in energy metabolism are organic or inorganic molecules but not 0_2.

4- **Micro-aerophilic bacteria:** These require for growth a low oxygen tension; lower than that present in the atmosphere; e.g. *Campylobacter jejuni*.

5- Aerotolerant anaerobes; these have a fermentative (anaerobic) pattern of metabolism but can tolerate the presence of oxygen because they possess superoxide dismutase e.g. *CI. perfringens.*

B- **Carbon dioxide ($C0_2$)** in minute quantities as that present in air is required by most bacteria. Certain species of bacteria, e.g. *Brucella abortus* and the pathogenic neisseria require higher concentrations (5-20%), which should be included in the atmosphere surrounding the culture.

Temperature for Growth:

Bacteria differ as regards the optimal temperature for their growth. Most medically important species grow at a range of temperature between 25-40°C. Optimum growth occurs at 37°C, i.e. the normal body temperature.

Non-pathogenic bacteria may grow at temperatures lower than 20°C or higher than 55°C.

Hydrogen Ion Concentration (pH):

Most pathogenic species of bacteria can grow at a narrow range of pH 7.2-7.6. However, **few** species, **e.g. *V. cholerae*** grow at an alkaline pH 8-9 and lactobacilli prefer an acidic pH 4.

For information about "Culture media " and "Methods of cultivation of bacteria ", refer to the* "Manual of Practical Microbiology" *by the same author.

CHAPTER 3

BACTERIAL VIRUSES
BACTERIOPHAGES

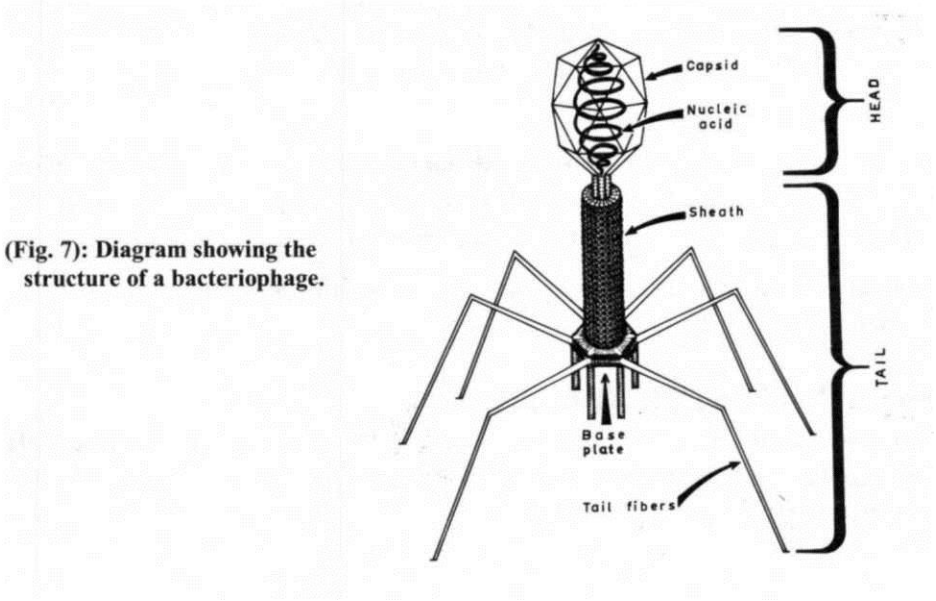
Viruses are obligate intracellular parasites, i.e. they only grow on living cells. Replication of viruses depends upon the metabolic energy and the macromolecular synthetic machinery of the host cell.

Bacteriophages are viruses that parasitise bacterial cells. Different types of bacteria serve as hosts for one or more phages.

Morphology of a Bacteriophage: (Fig. 7)

A typical bacteriophage consists of a head and a tail. The head is composed of a protein coat (capsid) containing the nucleic acid which is usually DNA and less commonly RNA. The head may take different shapes, it is usually hexagonal.

The tail* consists of a hollow core surrounded by a contractile sheath and a terminal base plate to which are attached tail fibers. The phage tail is the organ of attachment to host cells.



(Fig. 7): Diagram showing the structure of a bacteriophage.

This is the most complex tail structure of the T2 phage that infects *E. coli*, other forms exist.

Replication of Bacteriophage: (Fig. 8) There are

two cycles of replication:

I- Lytic or vegetative cycle

This cycle ends by lysis of the host cell and the release of many copies of the phage. The stages of replication are:

1- **Adsorption:** As the phage and bacteria come in contact, adsorption occurs between attachment sites on the phage (tail fibers) and specific receptor sites on bacteria. This step is very specific and it determines the difference in sensitivity of bacteria to different phages.

2- **Penetration:** The tail sheath will contract and inject the DNA into the cell like a hypodermic syringe leaving the empty head and tail outside the cell.

3- **Eclipse:** For a variable period (minutes to hours) following infection, no phage components are detected in the cell. During this period, the viral DNA directs the host cell metabolism to synthesize new enzymes and new proteins for phage synthesis, e.g. phage DNA polymerase.

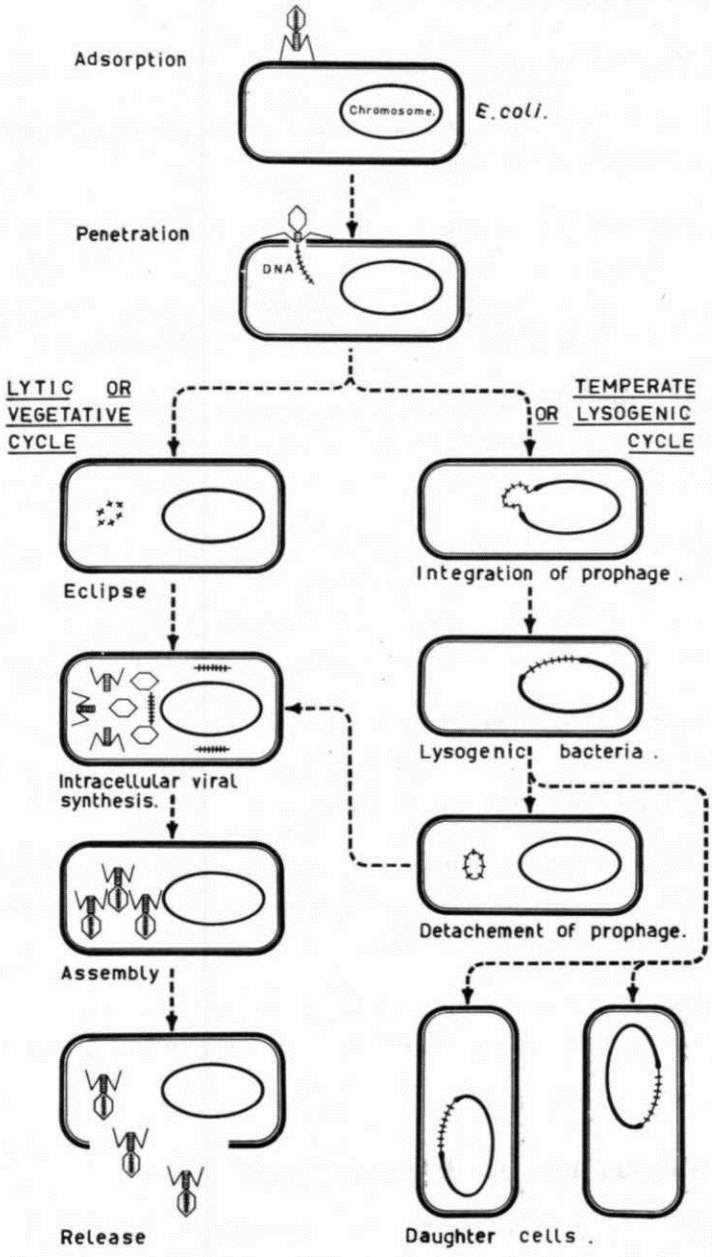
4- **Intracellular synthesis:** The host cell machinery is directed by the genetic information provided by the phage nucleic acid, to synthesize phage coats and phage nucleic acids.

5- **Assembly:** Protein subunits of the phage head and tail aggregate spontaneously. Then each capsid acquires a nucleic acid molecule to become a mature phage particle.

6- **Release:** After maturation and accumulation of a huge number of phages, the cell bursts. Phage particles are released and infect new cells.

II- Temperate phage cycle "lysogenic cycle"

Some phages "temperate phages" fail to replicate or lyse the cell they infect. Their DNA is integrated with the bacterial chromosome and it passes to daughter cells as the bacteria divide. The part of the phage DNA integrated in the bacterial chromosome is called the **"prophage"** and the bacteria carrying the prophage are called " **lysogenic"** bacteria.



(Fig. 8): The lytic (vegetative) and temperate (lysogenic) cycles of bacteriophage.

The continuous presence of the prophage in the lysogenic bacterium gives it certain characters:

1- It becomes immune to infection by another phage.

2- It may acquire new properties, e.g. production of exotoxin by diphtheria bacilli, *CI. botulinum, Strept. pyogenes* erythrogenic toxin and *Staph, aureus* enterotoxin. If the organism loses the prophage, it will become non-toxigenic. Acquisition of new characters coded for by prophage DNA is called **"lysogenic conversion".**

Outcome of the Temperate Cycle:

1- The cell may continue carrying the prophage indefinitely passing it to daughter cells.

2- The prophage may be induced (by UV, cold, or alkylating agents) to detach from the bacterial chromosome and start a lytic cycle.

3- Rarely as the prophage is induced, an excisional error occurs, and it may carry with it part of the adjacent genetic material of the bacterial chromosome. As it infects another bacterium, it will transmit to it new characters. This is called **specialized transduction** (see chapter 4).

Practical Uses of Bacteriophages:

1- Bacteriophages are used as **cloning vectors** in recombinant DNA technology. A fragment of DNA (foreign gene) is carried on the phage DNA. As the phage infects a bacterial cell, e.g. *E. coli,* its DNA carrying the foreign gene is incorporated into the bacterial chromosome and the gene is replicated in each cell division (see chapter **5).**

2- They are used in **phage typing:** Since bacteria differ in their sensitivity to different phages, phages are used to identify and type bacteria according to the pattern of lysis. This is very useful in epidemiologic tracing of sources of outbreaks of infections, e.g. wound infection or food poisoning.

4- Bacteriophages are used as research elements in several genetic and biologic studies.

5- Phage in therapy: The therapeutic use of bacteriophages to treat bacterial infections in animals, plants and humans is under trial. When used to treat humans the term biocontrol is used. They are used to treat bacterial infections that do not respond to conventional antibiotics mainly those in which bacterial biofilms are involved. Phages are more specific in their action on bacteria than many drugs, and are expected to have minimal or no side effects on the host.

CHAPTER 4

BACTERIAL GENETICS

Genetics is the study of inheritance of the different characters from parents to offsprings, who usually have the same characters as the parents, except when variations rarely occur. The same occurs with bacteria where the daughter cells inherit the same characters as the parent forms.

Genes are the units of heredity. They are segments on the chromosome or DNA that carry the information for a specific character or a specific structure. Some genes coding for certain characters which are not essential for growth are carried on plasmids, transposable genetic elements and prophages.

Bacterial Chromosome:

It is a double stranded DNA molecule *(E. coli* chromosome is about 4000 kbp* and roughly 1 mm long) which is circular and wound on itself to form the nuclear mass. Chemically, it is composed of a backbone of phosphate and sugar (deoxyribose) alternating with one another; to which are attached the purine and pyrimidine bases; guanine, adenine, cytosine and thymine. The two strands are held together by hydrogen bonds between neighbouring bases. The bonds are formed only between adenine and thymine (A-T)** or guanine and cytosine (G-C). Thus, a sequence of bases along one strand must be matched on the opposite strand by a complementary sequence.

The chromosome is functionally subdivided into segments or genes (more than 1000 genes can be mapped on *E. coli* chromosome). The sequence of nucleotides in a gene detenriines the amino acid sequence and hence, the structure of a discrete protein, e.g. an enzyme, a cell wall component, etc...

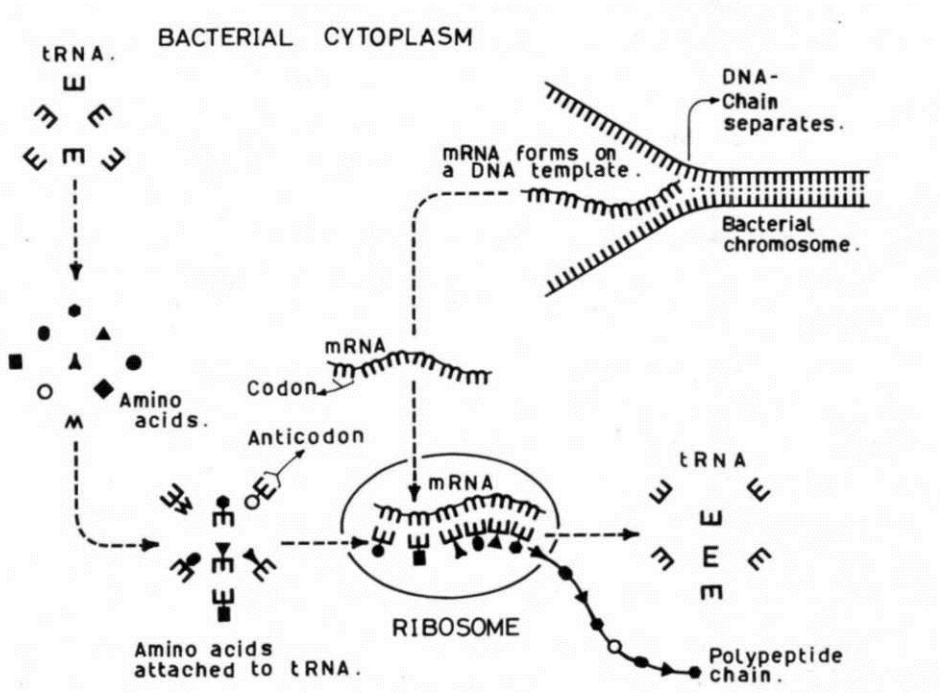
Genes essential for bacterial growth are carried on the chromosome. Few genes associated with specialized functions are carried on plasmids.

Chromosomal Replication:

Before cell division the 2 strands of the chromosome separate, each strand is attached to a mesosome and acts as a template on which a complementary strand is formed by the action of DNA polymerase enzyme. Cell division follows chromosomal replication by formation of a transverse septum between the attachment sites. Each daughter cell will contain an identical copy of the parent chromosome.

The length of a DNA molecule is usually expressed in thousands of base pairs or kilobase pairs (kbp). In RNA adenine bonds with uracil (A-U).

Gene Expression (protein synthesis): It is the process by which the sequence of nucleotides in a gene determines the sequence of amino acids in a protein, i.e. protein synthesis occurs as follows: (Fig. 9)



(Fig. 9): Diagram showing the process of protein synthesis.

1- **Transcription:** The 2 strands of the chromosome are separated and one acts as a template for the synthesis of a complementary strand of RNA called messenger RNA (mRNA) by the RNA polymerase enzyme. The mRNA attaches to a ribosome where the specific protein will be constructed.

The amino acids are found disseminated in the cytoplasm. These are enzymatically activated and transferred to specific adapter molecules of RNA called transfer RNA (tRNA). For each amino acid, there is a specific tRNA, which attaches to the amino acid at one end, where at its other end, it has a triplet of bases (anti-codon) complementary to the triplet of bases on mRNA. The triplet of bases on mRNA is called the codon for that amino acid.

2- **Translation:** mRNA and tRNA come together on the surface of the ribosome. Each tRNA finds its complementary nucleotide triplet on mRNA and the amino acid is linked with the adjacent one to form a polypeptide chain. The entire mRNA is **translated** into a corresponding sequence of amino acids linked to form polypeptide chains and proteins are formed.

Plasmids: (Fig. 10)

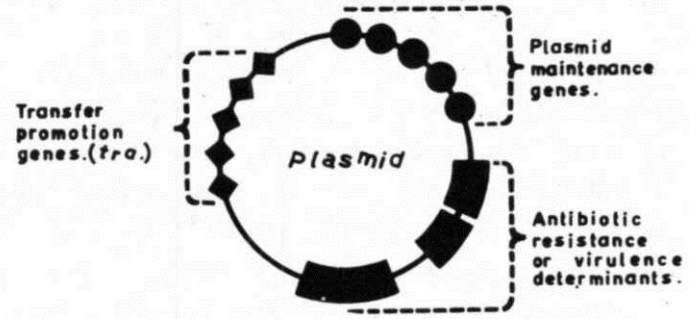
Some genetic properties in the bacterial cell are carried on plasmids. Plasmids are dispensable and the genetic properties they carry are not essential for growth. This is proved by the fact that experimental kicking off of the plasmids (plasmid curing) by chemical or physical agents or their spontaneous loss does not affect bacterial replication.

Plasmids are extra-chromosomal double stranded, circular DNA molecules smaller than the chromosome. They replicate autonomously, i.e. they multiply independent of the host cell chromosome. Multiple copies of the same plasmid may be present in each bacterial cell. Different plasmids may co-exist within the same bacterium. They are inherited by daughter cells. There are two types of plasmids:

1- Conjugative (transmissible) plasmids. These can transfer to other bacteria of the same or different species. Transfer takes place normally by conjugation. The ability to transfer is mediated by the *tra* or transfer promotion genes. They are large in size since they contain about a dozen genes responsible for synthesis of the sex pilus and for the enzymes required for transfer. They are present in a few (1-3) copies^ per cell (stringent plasmid). They are common in gram negative bacteria.

2- Non-conjugative (non-transmissible) plasmids. These do not contain the *tra* genes. They are small in size and are frequently present in many, 10-60 copies / cell (relaxed plasmid). They are common in gram positive bacteria.

Since plasmids can transfer from cell to cell, they are widely used as vectors for cloning DNA, (i.e. implanting certain genes) in yeast and bacteria.



(Fig. 10): Diagram showing the structure of a plasmid that codes for antibiotic resistance in bacteria.

Conjugative and Non-conjugative plasmids

	Conjugative plasmid	Non-conjugative plasmid
Size	Large	Usually small
Copy number	1-2 (stringent)	10-60 (relaxed)
F factor	Present	Absent
Contain *tra* gene	Yes	No
Sex pilus formation	Yes	No
Transfer among bacteria	By conjugation	By the help of a conjugative plasmid
Host bacteria	Common in gram negative bacilli	Common in gram positive cocci

Bacterial Properties Carried on Plasmids include:

1- Sex factor plasmids carry the fertility F factor which can mediate gene transfer by conjugation (conjugative plasmids).

2- Drug resistance: Genes coding for drug resistance of many bacteria are carried on plasmids. They are called R-factor plasmids. These are usually conjugative plasmids that can be transferred among bacteria by conjugation resulting in rapid spread of drug resistance.

3- Virulence: Some plasmids carry genes whose products are the virulence factors of some pathogenic organisms, e.g. neurotoxin production by *CI. tetani;* invasiveness in *Yersinia enterocolitica* or adhesive colonization antigens (pili) in enteropathogenic *E. coli.*

4- Production of antimicrobial agents by bacteria, e.g. antibiotic production by *Streptomyces* is coded for by plasmid genes.

5- Production of bacteriocins e.g. colicins produced byii. *coli* are controlled by plasmids which are called Col factors. Bacteriocins are bactericidal substances produced by certain strains of bacteria, active against some other strains of the same or closely related species.

Transposable Genetic Elements are **transposons, insertion sequences, integrons** and **pathogenicity islands.** These are non-replicating DNA segments that are capable of inserting themselves into other DNA molecules. They are also capable of mediating their own transfer from one location to another on the same chromosome or between chromosomes and plasmids.

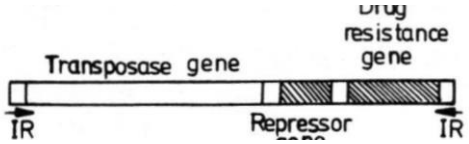
Transposition relies on the ability of the transposable elements to synthesize their own specific recombination enzymes (transposases or integrases). They are valuable tools for gene manipulation. Insertion of such elements in a gene usually leads to inactivation of that gene. They are utilized for insertional inactivation and mapping of genes.

Transposons "jumping genes": (Fig. 11)

These are non-reptirating pieces of DNA that move readily from one site to another either within or between the DNAs of bacteria, plasmids, and bacteriophages. Due to their unusual ability to move, they are called "jumping genes". Transposons typically have four identifiable domains: (1) A short DNA sequence of inverted repeats (IR), on each end, which are involved in the integration of the transposon into the recipient DNA. (2) The gene for the transposase, which is the enzyme that mediates the excision and integration processes. (3) A repressor gene that regulates the synthesis of both die transposase and the gene product of the fourth domain. (4) It is in many cases, an enzyme mediating antibiotic resistance or toxin production gene.

In contrast to plasmids or bacteriophages, transposons are not capable of autonomous replication and they do not exist in the free state; hence they replicate as part of the recipient DNA. More than one transposon can be located in the DNA; for example, a plasmid can contain several transposons carrying drug resistance genes.

Insertion sequences are simple types of transposons that have fewer bases (800-1500 base pair). They may carry the genetic information that codes for their integration and can cause mutation at their site of insertion. They can be found in multiple copies at the ends of larger transnnsnns



Integrons are a specific class of transposons having genes of a site specific recombination system, capable of capturing and mobilizing genes contained in mobile elements called "gene cassettes". They have a gene coding for integrase enzyme that catalyzes the deletion or insertion of resistance genes, a recombination site and a strong promoter that causes vigorous expression of these genes. Five or more resistance genes may be contained in a single integron. This is a mechanism of dissemination of drug resistance genes among gram negative bacteria.

Pathogenicity islands (PAIs) are DNA segments containing mobile genetic elements inserted within the bacterial chromosome. The genes that encode many virulence factors in bacteria are clustered in these PAIs. For example in many bacteria, the genes encoding adhesins, invasins and exotoxins are adjacent to each other on PAIs. It appears that these large regions of the bacterial genome are inherited as a block *via* conjugation or transduction. They are usually unstable and loss of PAIs results in loss of virulence of the organism. PAIs are found in many gram negative rods e.g. *E. coli* and *H. pylori* and in gram positive cocci e.g. *Staph, aureus* (see p.4 and 57 Vol. II).

Bacterial Variation

Bacterial variation may be phenotypic or genotypic variation. A **genotype** is the set of genetic determinants within the cell. A **phenotype** is the observable structural and physiologic properties of the cell.

Phenotypic Variations are changes in bacterial characters under the influence of the environment with no underlying genetic changes. It is reversible when the environmental cause is removed. It is not heritable. For example, variation in colony appearance from smooth to rough when grown on certain media, or increased pigment production by *Staph, aureus* when grown at room temperature, L-forms of bacteria, etc..

Genotypic Variation is a heritable irreversible variation due to changes in genetic

1- Mutation. **2- Gene transfer.**

Phenotypic and Genotypic Variations

Phenotypic Variations	**Genotypic Variations**
Changes in bacterial characters under the influence of the environment with no change in genetic constitution	Change in bacterial characters due to underlying genetic changes.
Reversible (transient)	Irreversible (permanent)
Not-heritable	Heritable
Examples: 1 - Change in colony morphology from smooth to rough. 2- Loss of cell wall in L-forms of bacteria. 3- Increased pigment production by staphylococci at room temperature.	Examples: 1- Mutation 2- Gene transfer a- Transformation, b- Conjugation, c- Transduction.

constitution. Genetic variation occurs through:

I- Mutation:

This is due to change in the sequence of bases in the DNA double helix. Mutation may be due to **substitution** of one base pair for another, **deletion** of bases or **insertion** of new bases. This causes alteration of the genetic code (codon) and hence a different amino acid results, thus changing the protein product of the gene affected. This results in a mutant (variant) with a new character which is heritable and usually irreversible.

Mutation occurs **spontaneously** at a rate varying between 1 in 10^7 and 1 in 10^{10} replication error. It can be **induced** at a higher rate by mutagenic agents such as x-rays, ultraviolet light and alkylating agents.

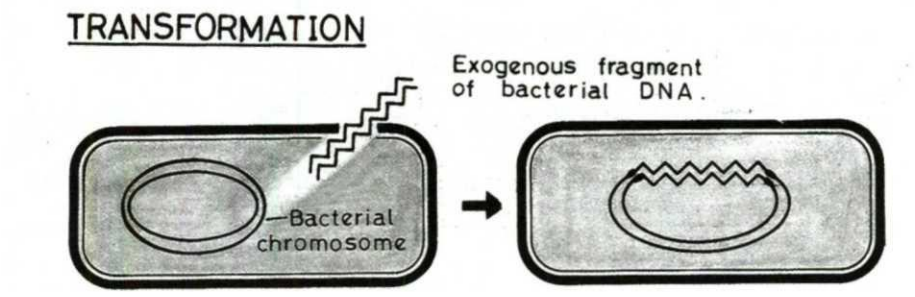
II- Gene Transfer:

There are 3 types of gene transfer from one bacterium to the other leading to bacterial variation:

1- **Transformation** (Fig. 12): This occurs when a recipient cell takes up a fragment of bacterial DNA, present free in the surrounding medium. This DNA fragment recombines with the bacterial chromosome which is transformed and the new genes are expressed leading to changes in characters of recipient cells. In transformation with plasmid DNA, it becomes re-established in the recipient cell and autonomously replicates.

Natural occurrence of transformation between bacteria is unusual. However, some species can readily undergo natural transformation and these are very useful in genetic engineering because of the ease with which they incorporate foreign DNA into their chromosome. Transformation can be enhanced in the laboratory by treatment of cells with calcium chloride and heat shock.

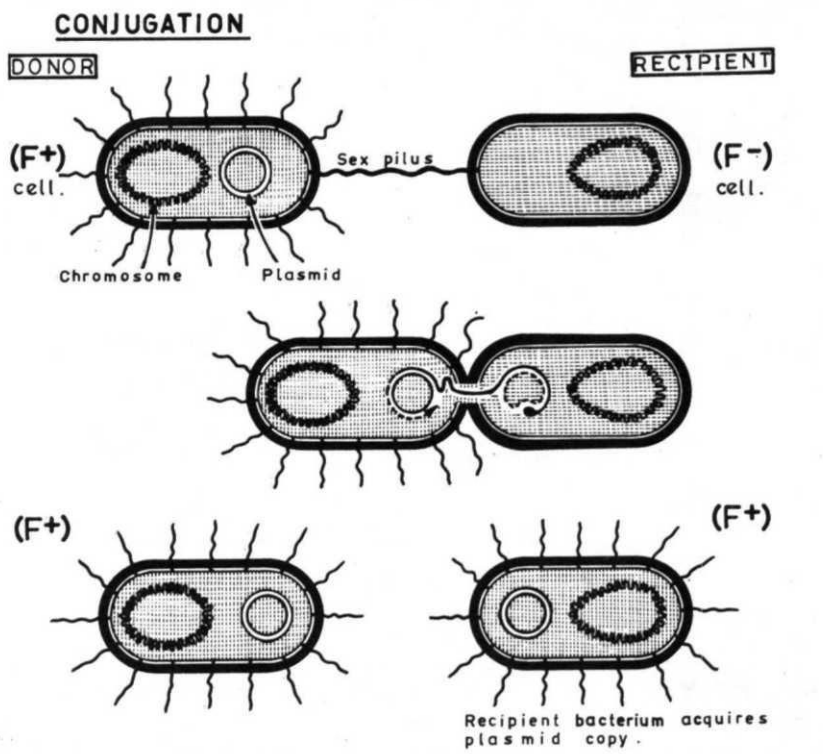
2- **Conjugation** (Fig. 13)



(Fig. 12): Transformation; gene transfer by the uptake and subsequent recombination of a fragment of exogenous bacterial DNA.

It is the mating of 2 bacterial cells, of the same or different species e.g. *E. coli* and *Ps. aeruginosa,* during which DNA is transferred from the donor to the recipient cell. The process is controlled by an F (fertility) plasmid which carries the genes *(tra* region) that code for formation of sex pilus which bridges between the 2 cells. As the 2 come in contact, one strand of the plasmid separates and passes from F* donor cell to the F" recipient cell (does not contain F plasmid) through the conjugation tube formed by the sex pilus. At the same time, a complementary strand is formed by both cells. The process ends with an F^{l_n} recipient cell that acquires a plasmid copy.

High frequency recombination (Hfr): When F^+ cells have their F plasmid integrated into the bacterial DNA, these cells acquire the capability of fransferring the chromosome into another cell. The cells are called Hfr cells. During this transfer, the single strand of DNA that enters the recipient F" cell contains a piece of the F factor at the leading end followed by the bacterial chromosome and then by the remainder of the F factor (that includes the *tra* gene). Most matings result in the transfer of only a portion of the donor chromosome, because the attachment between the two cells usually breaks. The bacterial genes adjacent



(Fig. 13): Gene transfer by conjugation.

to the leading piece of the F factor are the first and most frequently transferred. Note that in this case the recipient F'cell does not get the *tra* region and so it does not become Hfr cell. The newly acquired DNA can recombine into the recipient's DNA and become a stable component.

3- Transduction (Fig. 14)

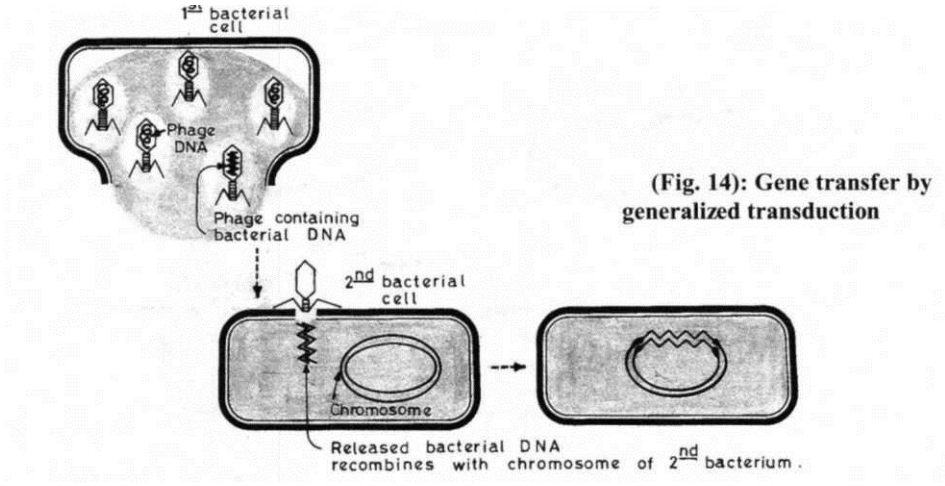
Fragments of chromosomal DNA can be transferred or transduced into a second bacterium by bacteriophage. There are 2 types of transduction:

a- Generalized transduction: During the lytic cycle of phage replication, a piece of bacterial DNA becomes, by accident, enclosed within a phage particle instead of the normal phage DNA. When this phage infects a second bacterium, the DNA from the first bacterium is released and recombines with the chromosome of the second bacterium.

Plasmid DNA can also be transferred to the second bacterium by transductionwhere itwill replicate autonomously. Beta lactamase production in *Staph, aureus,* is plasmid mediated and the responsible plasmid transfers between staphylococcal strains by transduction.

b- Specialized transduction occurs as a **prophage** is induced i.e. released from the lysogenic bacteria to infect another cell. It may carry with it a fragment of the adjacent genetic material of the bacterial chromosome, and pass it to another bacterium (see chapter 3).

TRANSDUCTION



(Fig. 14): Gene transfer by generalized transduction

Generalized and Specialized Transduction

	Generalized	Specialized
Types of phage	Lytic (virulent) phage	Temperate (lysogenic) phage
Replication cycle	Lytic cycle	Lysogenic cycle
Mechanism	Error in assembly	Error in excision
What genes may be transferred?	Any genes . (chromosomal or Plasmid)	Only chromosomal genes next to the insertion site of prophage

N.B. Transduction is more reproducible than transformation because the DNA is protected from damage by the surrounding phage coat.

Recombination:

When the DNA is transferred from the donor to the recipient cell by transformation, conjugation or transduction, it can integrate into the host cell chromosome by recombination. There are two types of recombination:-

1- Homologous recombination, in which two pieces of DNA that have extensive homologous regions pair up and exchange pieces by the processes of breakage and reunion. The process requires several enzymes/factors coded for by the recombination genes, mainly *recA* gene.

2- Non-homologous or site specific recombination, which is the integration of a DNA molecule into a DNA with which it has no homology except for a small site on each DNA (called an attachment, integration or insertion site). The process requires restriction endonucleases and restriction endonuclease site on each DNA (often called integration site or insertion sequences). It results in a molecule which is the sum of the original two molecules. Three major roles of site specific integration are:-

a- Integration of a fertility factor to make an Hfr cell.

b- Integration of a temperate phage DNA into a bacterial chromosome to create a prophage c- Movement and insertion of transposons, a process called transposition.

CHAPTER 5

GENETIC RECOMBINATION

The development of methods to isolate genes (DNA segments) coding for certain proteins from different species, (e.g. bacteria, viruses, animal cells, etc...), and the ability to join them together to form new combinations is a major scientific advance. This science which is called genetic recombination, recombinant DNA technology, gene cloning or genetic engineering, has very important applications in the medical as well as other fields.

The technique requires separation of DNA fragment (gene) by restriction endonucleases. The DNA fragment is carried on a vector which is introduced into a host cell by transformation where it is amplified.

Restriction Endonucleases: These are enzymes, derived from bacteria and fungi, that can recognize and cut DNA molecules at specific sites, (i.e. at specific base sequence).They also allow separation of fragments containing specific genes. They are named according to their source, e.g. EcoRI enzyme is derived from *E. coli* and recognizes the sequence GAATTC.

The bacterial own DNA is methylated in a specific manner to be protected from cleaving by the restriction endonucleases it produces. The activity of the enzymes is, thus, restricted to unmodified foreign DNA, hence they are called restriction enzymes.

Molecular Cloning Vectors: These are vehicles used to carry and introduce foreign DNA fragments into a host cell. An ideal vector must;

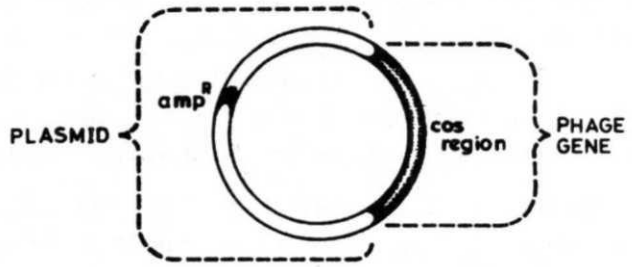
1- be as small as possible **2-** be well characterized regarding gene location, restriction endonuclease cleavage sites, etc... **3-** be capable of autonomous replication within the host **4-** possess non-essential regions within which target DNA can be inserted **5-** carry a selectable marker (e.g. antibiotic resistance gene) so that cells transformed with the vector can be distinguished from non-transformed cells **6-** have an additional marker that can be inactivated by the insertion of target DNA **7-** possess a single cleavage site for at least one restriction endonuclease **8-** display a limited host range in order to reduce the biohazards associated with the recombinant molecules. The best cloning vectors are:-

1- Plasmids: These are very useful vectors as they fulfill most of the above characters plus the fact that they can easily transfer from one cell to another.

2- Bacteriophages: The DNA of the lambda *(X)* phage is used to carry foreign genes into *E. coli* by transduction (p. 15).

3- Cosmids: (Fig. 15) These are circular double-stranded DNA molecules which are artificially constructed from plasmid DNA and the cohesive end (COS) of lambda phage DNA. Cosmids can efficiently carry large genes that can not be carried by either plasmid or phage alone.

4- Viruses e.g. retroviruses, adenoviruses, vaccinia virus and others, are used as vectors to clone DNA in mammalian cells (see gene therapy, p. 28).



(Fig. 15):
Structure of a cosmid.

Recombinant DNA Technique: (Fig. 16)

1- Chromosomal DNA from any desired source is extracted and cleaved by a specific restriction endonuclease which cuts at specific sites to separate the required fragment containing the gene in question, which is called the "insert".

2- The vector, e.g. plasmid is cleaved by the same endonuclease, used in step 1, which will cut at the same sequence. It should also cut the plasmid at a single site.

3- The "insert" and the plasmid are mixed under conditions, (e.g. heating and slowly cooling) that promote annealing (hydrogen bonding) of their complementary ends. The DNA ligase enzyme is added to seal the gaps. This results in the recombinant plasmid (plasmid + insert).

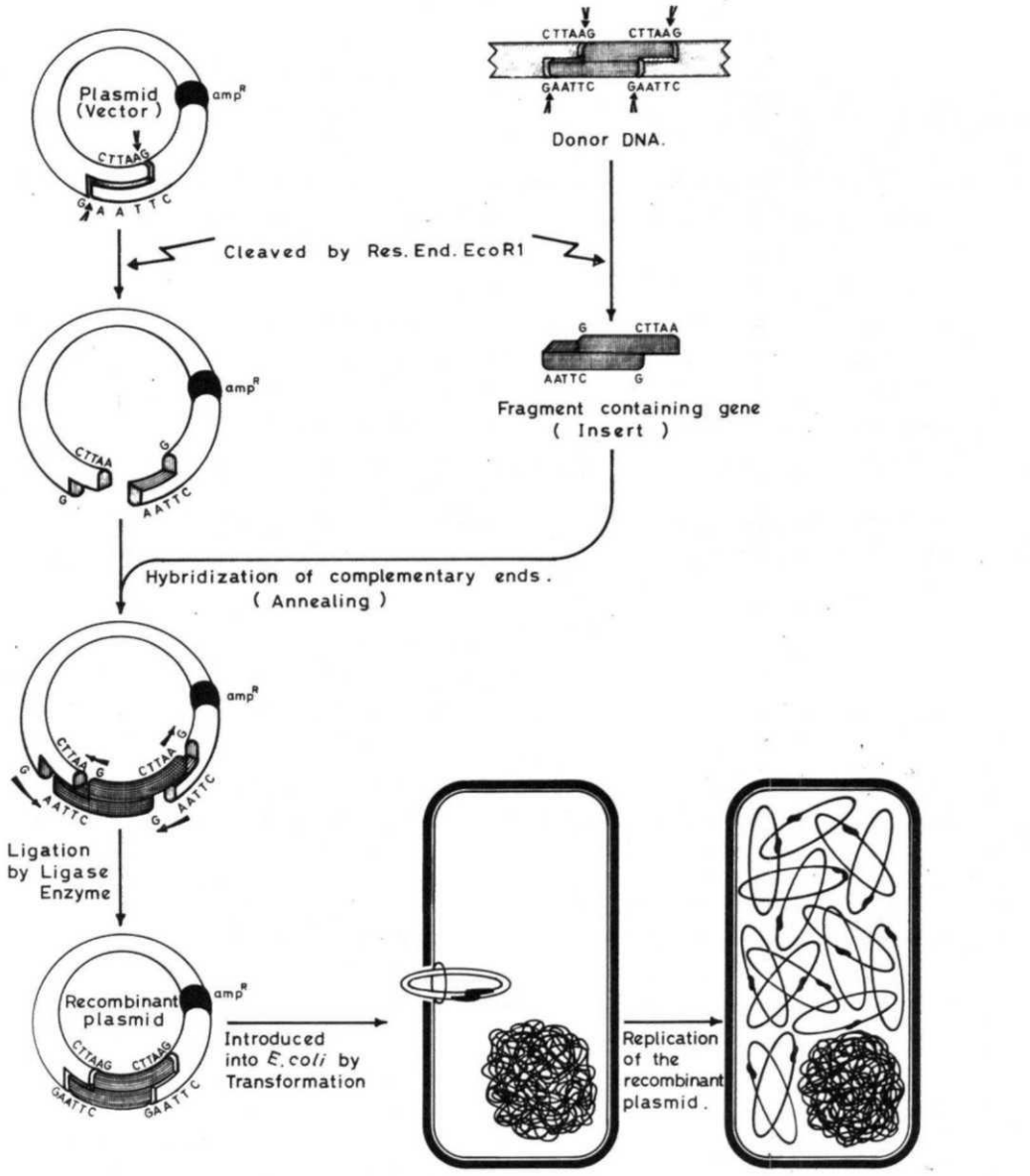
4- By a process of transformation, the recombinant plasmid is introduced into a suitable host (e.g. *E .coli* or yeast cells) in which it can autonomously replicate.

5- The host cell that acquires the recombinant plasmid can be identified by the new properties carried on the plasmid. For example, if the plasmid used as a vector carries the property of ampicillin resistance (amp^R) then the host cell that acquired the plasmid; can be grown and selected by culture on ampicillin containing media.

6- On replication of the host cell and the recombinant plasmid, large numbers of the insert, i.e. the desired gene, will be produced.

The recombinant plasmid is isolated and the inserted fragment is cut out and purified. This provides a large number of copies of the gene to be used in a bank of genes.

Alternatively, the host-vector system may be designed so as to permit expression of the gene product. For example, production of insulin by cloning the gene responsible for its production in a host bacterium.



(Fig. 16): Diagram showing steps of the recombinant DiNA technique.

Applications of Recombinant DNA Techniques:

Potential applications of recombinant DNA techniques are almost limitless. Achievements of medical interest include:

1- The techniques allow extensive chromosomal mapping and gene studies.

2- Preparation of "probes" that are used for several diagnostic and genetic studies.

3- The technique is also used for production of many proteins of medical importance in big amounts and with less cost, e.g. hormones (insulin, growth hormone etc.), interferons, interleukins, antibiotics, monoclonal antibodies, human antihaemophilic factor and erythropoietin.

4- Production of recombinant vaccines, e.g. hepatitis B surface antigen (HBsAg) vaccine. The gene coding for HBsAg is cloned in yeast cells where it is expressed. The gene product is extracted from the yeast cells, purified and used for immunization.

5- Gene therapy: Viruses are being used as genetic vectors to deliver new, functional genes to patients with genetic diseases. Retroviruses are excellent vectors because a DNA copy of their RNA genome is stably integrated into the host cell DNA and the integrated gene is efficiently expressed. Retroviral vectors are constructed by removing the genes encoding several viral proteins from the virus (so it does not replicate) and replacing them with the gene of interest, e.g. adenine deaminase (ADA) gene in patients with immunodeficiency due to defective ADA gene. Adenoviruses are also being used as gene delivery vehicles.

DIAGNOSTIC MOLECULAR BIOLOGY METHODS

These methods apply the principles of nucleic acid hybridization and amplification in the detection, identification and characterization of micro-organisms. Each bacterial species contains a specific DNA that is unique to it.

NUCLEIC ACID PROBES

Nucleic acid probes are short sequence of labelled single stranded DNA or RNA originally derived from the organism being sought. Probes are used in hybridization experiments to detect the presence of complementary sequence of microbial genes in clinical specimens or in cultures. They are usually synthesized commercially by DNA synthesizing machines and are labelled by radioactive isotopes or enzymes to facilitate their detection.

The steps of hybridization technique are:

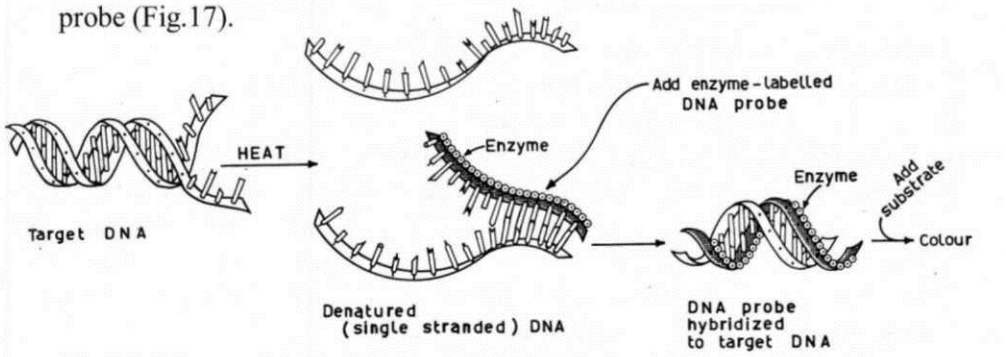
1- Extraction of target DNA present in clinical samples or culture.

2- The DNA is denatured to the single stranded form by alkali or heat and attached to a solid support e.g. nitrocellulose membrane.

3- The labelled probe is added to the target DNA and left to form hybrid duplex if the complementary base sequence is present in the specimen.

4- Excess unbound probe is removed by washing.

5- Formation of hybrid DNA is proved by detecting the label on the probe by autoradiography or enzymatic assay depending on the kind of label on the probe (Fig.17).



(Fig. 17): Diagram showing the steps of hybridization by enzyme labelled probe.

POLYMERASE CHAIN REACTION (PCR)

PCR technique involves amplification of a short sequence of target DNA or RNA leading to accumulation of several copies (billions) of that target. PCR is an automated temperature controlled recycling of three simple reactions each of which takes few minutes:-

1- DNA denaturation: By heating the sample containing the target DNA at 95°C, that will lead to separation of the 2 strands of the DNA (Fig. 18).

2- **Primer annealing:** The temperature is lowered (40°-60°C) and two short synthetic pieces of DNA termed primers* are added. The primers are complementary to the two ends of the opposite strands of the DNA to be amplified. Each primer anneals to its complementary sequence on the two single strands.

3- Primer extension: A heat stable Taq DNA polymerase included in the reaction mixture starts to add a string of nucleotides (included in the mixture) to both primers leading to formation of 2 complementary strands.

At the end of the first cycle there will be 4 strands of the target DNA which will serve as templates for the synthesis of new complementary strands in the next cycle, thus resulting in 8 strands and so on. One million copies can be produced in 20 cycles in a very short time.

The amplified nucleic acid product can be detected by:

1. Hybridization to specific probes.
2. Fractionation of the product by restriction endonucleases and its detection by agarose gel electrophoresis, staining by ethidium bromide and visualization under UV light.
3. Nucleic acid sequencing of the DNA produced.

Reverse Transcriptase PCR (RT-PCR) is used for amplification of **RNA.** That requires first formation of ssDNA complementary to the target RNA by the enzyme reverse transcriptase, followed by formation of dsDNA by the enzyme DNA polymerase.

Applications of Nucleic Acid Probes and PCR:

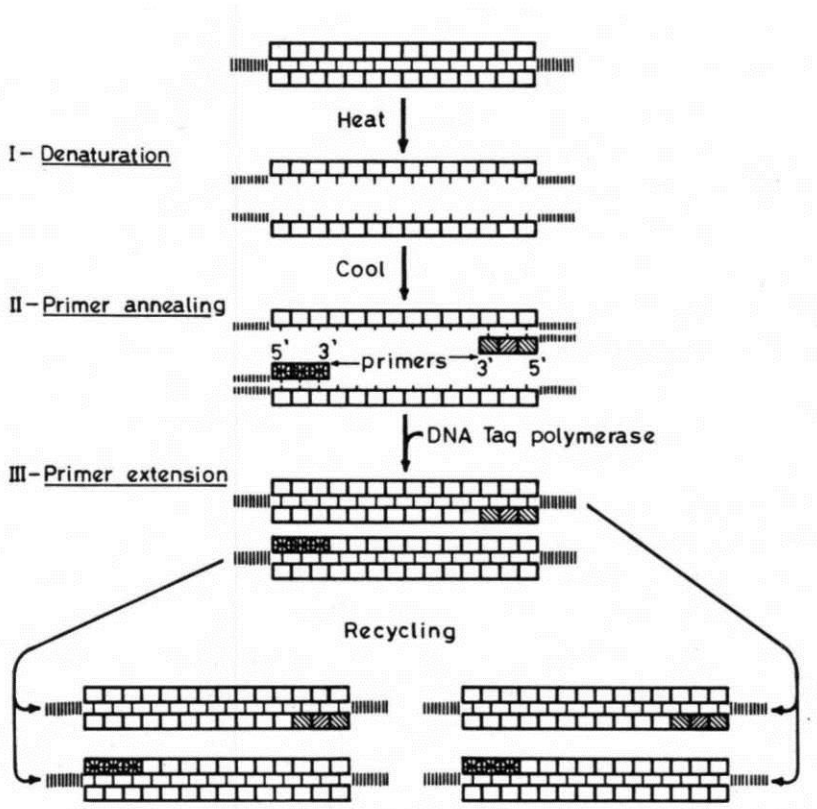
Probes may be used alone when the nucleic acid segments to be detected are abundant in the specimen. However, if these segments are scanty, preliminary amplification by PCR or other methods can facilitate their detection by probes or other detection methods in the following conditions;

1- Diagnosis of infections by detection and identification of pathogenic organisms that are present in low concentrations in specimens, non-cultivable or slow growing organisms or those that are difficult to grow e.g. *Tr. pallidum, M. leprae,* human papilloma virus, HIV, HBV, TB, CMV and *Chlamydia.*

2- Identification of antibiotic resistant strains of bacteria by detection of R genes.

3- Organ transplantation: HLA typing.

*** Designing of the correct primers requires knowing the nucleotide sequence of the target DNA.**



(Fig.18): Diagram showing the steps of the polymerase chain reaction (PCR)

4- Diagnosis of cancer by detection of latent viruses or the genetic changes in neoplastic cells e.g. human papilloma virus and EBV.
5- Diagnosis of inherited diseases due to genetic mutations e.g. thalassemia, haemophilia, sickle cell anaemia, cystic fibrosis and muscular dystrophy. Prenatal diagnosis of such diseases using foetal cells from amniotic fluid.
6- Forensic medicine e.g. proving paternity by DNA fingerprinting.
7- Basic biological and medical research e.g. genetic mapping of the human gene.

CHROMOSOMAL DNA RESTRICTION ENDONUCLEASE ANALYSIS

This method involves comparison of the number and size of fragments produced by digestion of DNA, from different sources, with restriction enzymes (RE).These fragments can be separated by agarose gel electrophoresis and stained by ethidium bromide then visualized under UV light. Alternatively, these fragments may be transferred to nitrocellulose membrane and detected by hybridization technique using specific probes.

Differences in the nucleotide sequence of the chromosome result in generation of fragments of variable number and length when digested with the same RE. This is known as *Restriction Fragment Length Polymorphism (RFLPs)* which can be used to distinguish different strains of a given bacterial species.

Refer to the* "Manual of Practical Microbiology" *by the same author. (Chapter 10)

CHAPTER 6

ANTIMICROBIAL CHEMOTHERAPY

Chemotherapy is the treatment of infectious diseases by administration of drugs which are lethal or inhibitory to the causative organisms.

An antibiotic is an antimicrobial substance produced by a living microorganism and is active in high dilutions. Many of these antibiotics have been chemically synthesized. The term is used for any antimicrobial chemotherapeutic agent whether naturally produced or chemically synthesized. Synthetic modifications of previously discovered drugs allowed the development of several new antimicrobial agents.

Bactericidal drugs have a rapid killing action of bacteria, which is irreversible. Those are particularly useful in certain infections, e.g. those that are immediately life-threatening as in severe leucopenic patients and endocarditis. Examples include penicillins, cephalosporins and aminoglycosides.

Bacteriostatic drugs merely inhibit bacterial multiplication, but do not kill them. The bacteria can grow again when the drug is withdrawn. In this case, host defence mechanisms, such as phagocytosis, are required to kill bacteria. Examples include sulphonamides, tetracyclines and chloramphenicol.

Spectrum of Action of Chemotherapeutics:

Broad-spectrum antibiotics; those are active against several types of microorganisms, both gram positive and gram negative e.g. tetracyclines, chloramphenicol and ampicillin.

Narrow-spectrum antibiotics are active against one or very few types, e.g. vancomycin is primarily used against certain gram positive cocci i.e. staphylococci and enterococci.

Mechanisms of Action of Chemotherapeutics

An ideal antimicrobial agent should have **selective toxicity,** i.e. it can kill or inhibit the growth of a microorganism in concentrations that are not harmful to the cells of the host. Disinfectants, e.g. phenol and antiseptics, e.g. alcohol and iodine, destroy bacteria but they are highly toxic to tissue cells and are unsuitable for use as chemotherapeutic agents.

Thus, the mechanism of action of a chemotherapeutic must depend on the inhibition of a metabolic channel or a structure that is present in the microbe (e.g. peptidoglycan) but not present in the host cell. Several mechanisms are known:

1- Inhibition of Bacterial Cell Wall Synthesis: Due to its unique structure and function, the bacterial cell wall is an ideal point of attack by selective toxic agents. Penicillin, cephalosporins and vancomycin, interfere with cell wall synthesis and cause bacteriolysis.

P-lactams e.g. penicillin and cephalosporins and the glycopeptides e.g. vancomycin inhibit peptidoglycan synthesis. However, **P**-lactams inhibit the final steps in synthesis of peptidoglycan by binding to receptors called penicillin-binding proteins (PBPs) in the cell wall. Vancomycin on the other hand, inhibits the early steps in peptidoglycan synthesis by a different mechanism. That is why vancomycin is effective in treatment of **P**-lactam resistant staphylococcal infections (MRSA).

2- Inhibition of Bacterial Cytoplasmic Membrane Functions: Some antibiotics cause disruption of the cytoplasmic membrane and leakage of cellular proteins and nucleotides leading to cell death. Polymyxins, amphotericin B, and nystatin are examples. These drugs are highly toxic as they have a narrow margin of selective toxicity.

3- **Inhibition of Bacterial Protein Synthesis:** Several drugs inhibit protein synthesis in bacteria without significantly interfering with protein synthesis in human cells. This selectivity is due to the differences between bacterial and human ribosomal proteins, RNA, and associated enzymes. Bacteria have 70S ribosomes (with 50S and 30S subunits), whereas human cells have 80S ribosomes (with 60S and 40S subunits). Chloramphenicol, erythromycin, linezolid and streptogramins (qumupristin/dalfopristin) act on 50S subunits, while tetracycline and aminoglycosides (gentamicin and arnikin) act on 3 OS subunits.

4- **ln hibition of Bacterial Nucleic Acid Synthesis:** These can act on any of the steps of DNA or RNA replication. Quinolones and novobiocin inhibit DNA synthesis by blocking DNA gyrase. Rifampicin inhibits RNA synthesis by binding to RNA polymerase. Trimethoprim and sulfonamides inhibit nucleotide synthesis.

5- Competitive Inhibition: In which the chemotherapeutic agent competes with an essential metabolite for the same enzyme. Para-aminobenzoic acid (PABA) is an essential metabolite for many organisms. They use it as a precursor in folic acid synthesis which is essential for nucleic acid synthesis. Sulphonamides are structural analogues to PABA so they enter into the reaction in place of PABA and compete for the active center of the enzyme thus inhibiting folic acid synthesis.

Mechanisms of Resistance to Antimicrobial Agents:

In the treatment of infectious diseases, one of the serious problems commonly faced with, is the development of bacterial resistance to the antibiotic used. The mechanisms by which the organism develops resistance may be one of the following:

1- Bacteria produce **enzymes that inactivate the drug,** e.g. production of **P**-lactamases that cleave **P**-lactam ring in penicillins and cephalosporins. Esterases hydrolyze the lactone ring of macrolides. Acetyl-transferase produced by gram negative bacilli inactivates chloramphenicol.

2- Bacteria **synthesize modified targets** against which the drug has no effect e.g. resistance to aminoglycosides is associated with alteration of a specific protein in the 30S subunit of the bacterial ribosome. Alteration of the penicillin-binding proteins is a mode of resistance to penicillin and methicillin by MRSA.

3- Bacteria develop **new metabolic pathways** that bypass the reaction inhibited by the drug, e.g. sulphonamide resistant bacteria acquire the ability to use preformed folic acid with no need for extracellular PABA.

4- Bacteria **decrease their permeability** to the drug such that an effective intracellular concentration is not achieved. Changes in porins (hollow membrane proteins) can reduce the amount of penicillin entering bacteria.

5- Bacteria **actively pump the drug out** across the cytoplasmic membrane using efflux pump or "multidrug resistance pump" (MDR). The MDR pump imports protons and, in an exchange-type reaction, exports a variety of foreign molecules including certain antibiotics, such that an effective intracellular concentration of the drug is not achieved, as in resistance to quinolones and macrolides.

Origin of Resistance to Antimicrobial Agents A-

Non-genetic Drug Resistance:

1- **Metabolic inactivity:** Most antimicrobial agents act effectively only on replicating cells. Non-multiplying organisms are phenotypically resistant to drugs. Tubercle bacilli survive for years in tissues and then-resistance to anti-tuberculous drugs is due in part to their metabolic inactivity (dormancy).

2- **Loss of target structure:** Protoplasts or L-forms of bacteria are penicillin resistant, having lost their cell wall which is the structural target site of the drug.

3- Bacteria may be **walled off** within an abscess cavity that the drug can not penetrate effectively.

4- Intrinsic natural resistance, e.g. mycoplasma are naturally resistant to penicillin because they lack a cell wall enterococci are naturally resistant to cephalosporins as they lack the receptor for the drug.

B- Genetic Acquired Drug Resistance:

1- Plasmid mediated resistance

Resistance (R) factors are a class of plasmids that mediate resistance to one or more antimicrobial agent. Plasmids frequently carry genes that code for the production of enzymes that inactivate antimicrobial agents, e.g. **P**-lactamase which destroys the pMactam ring in penicillin and cephalosporins. Plasmids may result in epidemic resistance among bacteria by moving from one to the other by conjugation, transduction or transformation.

2- Transposon and integron mediated resistance

Many transposons carry genes that code for drug resistance. As they move between plasmids and chromosomes they can transfer this property to bacteria. The process is called **transposition.** Five or more resistance genes may be contained in a single integron causing dissemination of drug resistance genes among gram negative bacteria.

3- Chromosomal drug resistance

This develops as a result of **spontaneous mutation** in a gene that controls susceptibility to an antimicrobial agent. The most common result of chromosomal mutation is alteration of the receptors for a drug. For example, streptomycin resistance can result from a mutation in the chromosomal gene that controls the receptor for streptomycin, located in the 30s bacterial ribosome.

Complications of Antibacterial Chemotherapy

1- Development of drug resistance:

This is one of the most serious complications of chemotherapy. The emergence of resistant mutants is encouraged by inadequate dosage, prolonged treatment, the presence of a closed focus of infection and the abuse of antibiotics without *in vitro* susceptibility testing.

The problem is more serious when resistant strains develop in hospitals. About 90% of hospital strains of *Staph, aureus* are resistant to penicillin.

2- Drug toxicity:

Many of the antibacterial drugs have toxic side effects. This can be due to overdosage, prolonged use or narrow margin of **selective toxicity,** Streptomycin affects the 8th cranial nerve leading to deafness. Chloramphenicol may cause bone marrow depression. Aminoglycosides,

(e.g. gentamicin, tobramycin) are nephrotoxic. Tetracyclines inhibit growth and development of bones and teeth in the developing foetusand infants.

3- Superinfection:

a- Superinfection may occur by pre-existing resistant strains present in the environment e.g. penicillin resistant *Staph, aureus* in hospital infections.

b- Another type of superinfection is due to suppression of normal flora by the antibiotic used and their replacement with drug resistant organisms which cause disease, e.g.:

i- Overgrowth of **Candida** in the vagina causing vaginitis or in the mouth causing oral thrush.

ii- Prolonged oral chemotherapy leading to suppression of intestinal flora and overgrowth of staphylococci causing staphylococcal enterocolitis orCV. *difficile* which causes pseudomembranous colitis

iii- Overgrowth of naturally drug resistant gram negative organisms, e.g. pseudomonas, proteus or enterobacter, may account for respiratory tract superinfection.

4- Hypersensitivity:

The drug may act as a hapten, binds to tissue proteins, and stimulates an exaggerated immune response leading to tissue damage, i.e. hypersensitivity. Any type of hypersensitivity reaction can occur with several antibiotics. The most serious is anaphylactic shock, this may occur with penicillin or cephalosporins. Milder manifestations may be urticaria, purpural eruptions, skin rash, diarrhoea, vomiting and jaundice.

Chemoprophylaxis:

Chemoprophylaxis is the use of antimicrobial agents to prevent rather than to treat infectious diseases. The following are principal conditions for which prophylactic antibiotics are positively indicated:

1- The use of benzathine penicillin G injections every 3-4 weeks to prevent reinfection with *Strept. pyogenes* in rheumatic patients.

2- A single large dose of amoxicillin given immediately prior to dental procedures is recommended for patients with congenital or rheumatic heart disease to prevent endocarditis.

3- The oral administration of rifampicin 600 mg twice a day for 2 days to exposed persons during epidemics of meningococcal meningitis.

4- Oral administration of tetracycline to prevent cholera.

5- Women identified as vaginal carriers of *Str. agalactiae* should receive ampicillin intravenously at least 4 hours before delivery to prevent occurrence of neonatal sepsis and meningitis.

6- Chemoprophylaxis in surgery is indicated in the following conditions:

a- Large bowel surgery, **b-** Major orthopedic and cardiac surgery.

c- Amputation of an ischaemic limb. **Clinical**

Use of Antibiotics:

The objective of antibiotic therapy is to cure the patient with minimal complications. At the same time, it is important to discourage the emergence of drug-resistant organisms. The following principles should be observed:

1- Antibiotics should not be given for trivial infections.

2- They should be used for prophylaxis only in special circumstances.

3- Treatment should be based on a clear clinical and bacteriological diagnosis. Suitable specimens should be sent to the laboratory before treatment is begun. However, **"empirical treatment"** can be started after taking the sample; but should be modified later according to results of antibiotic sensitivity testing *in vitro.*

4- Antibiotics for systemic treatment should be given in full therapeutic doses, by the proper route of administration and for adequate periods.

5- Combined therapy with two or more antibiotics is required in some conditions, e.g.:

a- Serious infections e.g. infective endocarditis or meningitis. **b-**In the treatment of T.B. to delay emergence of drug resistant mutants **c-** Sepsis by resistant organisms in immunocompromised patients, **d-** Febrile neutropaenic patients.

e-Severe mixed infections e.g. peritonitis following perforation of the colon or compound fractures.

Combination of two drugs may result in one of several interactions:-

1- **Indifference,** i.e., the combined action is no greater than that of the most effective agent when used alone. (1+1=1)

2- **Addition,** i.e., the combined action is equivalent to the sum of the actions of each drug when used alone. (1+1=2)

3- **Synergism,** i.e., the combined action is significantly greater than the sum of the two drugs acting separately, e.g. combination of penicillin and an aminoglycoside against enterococci, because penicillin damages the cell wall sufficiently to enhance the entry of the aminoglycoside. (1+1= >2)

4- One drug may **antagonize** the action of the other e.g. the use of penicillin combined with the bacteriostatic drug tetracycline in the treatment of meningitis caused by pneumococci. Tetracycline inhibits the growth of the organism, thereby preventing the bactericidal effect of penicillin, which kills multiplying organisms only. (1+1 = < 1)

Refer to the* "Manual of Practical Microbiology"** ***by the same author for detailed Methods for Antibiotic Sensitivity Testing (Chapter 11).

CHAPTER 7

STERILIZATION AND DISINFECTION

Sterilization is the killing of all living forms of microbes including their spores. It can be achieved by moist heat above 100°C (i.e. autoclaving) and by dry heat and ethylene oxide, plasma gas sterilizers, ionizing radiation, filtration and some chemicals used for liquid sterilization **(sterilants)** e.g. glutaraldehyde (contact time 10 hrs), liquid peracetic acid and hydrogen peroxide 6%.

Disinfection is the destruction of most but not necessarily all pathogenic microbes or their spores. It can be achieved by boiling, pasteurization, UV rays and chemical disinfectants. Disinfectants are categorized according to their spectrum of activity into high, intermediate and low level disinfectants (p. 144).

Antiseptics are chemical agents that are sufficiently non-toxic to living tissues. They can be safely applied to the skin and mucous membranes but are not suitable for systemic administration e.g. ethyl and isopropyl alcohol, and chlorhexidine.

Decontamination is a term applied to any procedure that reduces pathogenic microorganisms to a level where items are safe for handling, for use or disposal. It can be done by cleaning, disinfection or sterilization, (p. 144)

Cleaning is a process that removes foreign material (e.g. dirt, organic material, and some microorganisms). Cleaning must precede disinfection and sterilization. It is usually done with soap and water, detergents or enzymatic products.

I- STERILIZATION BY HEAT

Heat is the most efficient and inexpensive method of sterilization, and is the method of choice whenever possible. It can be used in two forms:

1- **Dry heat** kills by destructive oxidation of essential cell constituents. It is less efficient than moist **heat.** However, it is less expensive and is not corrosive.

Dry heat sterilizer is the main method of sterilization by dry heat. It is an isolated double walled metal chamber that is heated by electricity, and has a thermostat that maintains the chamber air constantly at the chosen temperature. It uses a temperature of 160°C for 2 hr or 170°C for 1 hr. This method is used for the sterilization of glass-ware, ointments, powders, oils and metallic instruments.

2- **Moist heat:** This method of sterilization kills microorganisms by protein denaturation. It is used as:

a) Moist heat at a temperature below 100°C: **Pasteurization**

Pasteurization of milk is the best example, by heating either at 63°C for 30 min. or at 72°C for 15-20 sec. and immediately cooling to below 10°C. This process will destroy all the non-spore forming pathogens that may be found in milk i.e. *M. tuberculosis, Br. abortus, Salmonella* and *Coxiella burnetti.*

b) Moist heat at a temperature of 100°C: **Boiling**

Boiling at 100°C for 20 min. is sufficient to kill all vegetative bacteria, and hepatitis B virus, but not all bacterial spores. This method may be used for the **disinfection** of surgical and medical equipment when sterility is not essential, and in emergency if no sterilizer is available.

Endotoxins are the most important cause of **endotoxic** or **septic shock.** All endotoxins produce generalized non-specific toxic effects in the form of fever, hypotension, disseminated intravascular coagulation (DIC), shock and sometimes death due to massive organ failure. Endotoxins cause these effects through **activation of;**

a- macrophages to produce IL-1, IL-6, TNF-a and nitric oxide which cause the **fever,** tissue damage, **hypotension** and **shock.**

b- complement to produce C3a and C5a which cause bradykinin-induced vasodilatation, increased vascular permeability, hypotension and shock.

c- Hageman factor; an early component of the coagulation cascade causing DIC, resulting in thrombosis, purpuric rash, and tissue ischaemia.

Differences Between Exotoxins and Endotoxins

Property	Exotoxins	Endotoxins
Location of genes	Plasmid, bacteriophage or PAIs	Bacterial chromosome
Composition	Proteins	Lipopolysaccharides (lipid A)
Action	Specific (binds to specific receptors	Non-specific (fever and shock).
	on specific host cells). No fever	No specific receptors
Heat stability	Labile, destroyed at 60°C	Stable at 100°C for 1 hr.
Mode of production	Secreted by living cells	Integral part of the cell wall
		Liberated when cells disintegrate
Immunogenicity	Strong, induce high titer of antitoxin	Weak immunogenicity
Toxicity	Strong	Weak
Convertibility to toxoid[1]	Yes	No
Produced by	Gram positive bacteria[2] mainly	Gram negative bacteria

1-Toxoid is toxin treated usually with formalin so that it loses toxicity but retains immunogenicity.

2- Peptidoglycan-teichoic acid, released when gram positive cells die, may also cause effects similar to

Koch's postulates: Robert Koch in 1877 proposed certain criteria to be used to prove that an organism is the cause of a disease. These are:-

1 - The organism must be isolated from every patient with the disease.

2- The organism must be grown in pure culture ***in vitro.***

3- The pure organism must cause the disease in a healthy susceptible animal.

4- The organism must be recovered from the inoculated animal.

Since the late 19th century, many microorganisms that do not meet the criteria of the postulates have been shown to cause disease, for example, those that can not be grown in culture e.g. *T. pallidum* or those that have no animal model e.g. *N. gonorrhoeae.* Modern-day microbial genetics and molecular guidelines are used to establish a causal relation of an organism to a disease, by detection of virulence genes and nucleic acid sequencing of the organisms.

IMMUNITY

The main function of the immune system is to prevent or limit infections by microorganisms such as bacteria, viruses, fungi and parasites. This defensive function is performed by various cellular and humoral components which interact with each other producing a coordinated immune response that eliminates the pathogen or mmimizes the damage it causes.

The immune system is divided into two functional divisions, namely the **innate immune system** and the **acquired (adaptive) immune system.** Innate immunity acts as a first line of defense against infectious agents which are checked before they cause overt infection. If these innate defenses fail, the acquired immune system is called upon.

However, it should be emphasized that innate and acquired immunity do not operate in total independence of each other. They cooperate in important ways to produce more effective immunity. The communication system through various cytokines and adhesion molecules, allow components of innate and acquired immunity to interact, send each other signals, activate each other and work in concert towards the final goal of destroying the invading microorganism.

INNATE (NON-SPECIFIC) IMMUNITY

Innate immunity is the natural inborn barrier against invasion by microorganisms. It is non-specific, acting against any foreign invader e.g. microorganisms. It is not acquired through previous exposure to infectious agents. It does not react against self molecules. It functions through:

I- First Line of Natural Defense:

1- Mechanical Barriers at the portal of entry. These include:

- The **intact skin** and **mucous membranes** are effective barriers against most microorganisms. Epithelia of the skin, GIT, and respiratory tract produce peptides that have natural antibiotic functions e.g. **defensins,** cryptocidins and cathelicidins. Epithelia also secrete cytokines e.g. IL-1.
- The **hair** at the nares, **coughing** and **sneezing** help to expel foreign particles.
- **Mucous secretions** trap many organisms which are pushed outside the body by the continuous movement of cilia in the respiratory tract.
- The **blinking reflex** and **tears** expel foreign particles or bacteria entering the conjunctiva.

2- Chemical Barriers at the portal of entry. These include:

- **Sweat** and **sebaceous secretions** in the skin have antimicrobial actions by virtue of their acidic pH and high fatty acids content.
- **Hydrolytic enzymes** in the saliva, **HCL** of the stomach, **proteolytic enzymes** in the small intestine are bactericidal.
- **Lysozyme,** an enzyme that dissolves bacterial cell walls (by breaking down peptidoglycan). It is present on the skin, in tears and cervical secretions.
- **Acidic pH** in the adult vagina is protective.

3- Normal microbiota present at the portal of entry suppress the growth of many pathogenic bacteria and fungi by competition for essential nutrients or by production of inhibitory substances such as colicins or acids. For example, in the adult vagina an acidic pH is maintained by normal microbiota namely lactobacilli, that interfere with the establishment of pathogenic organisms. Suppression of normal microbiota by antibiotics leads to superinfection with potential pathogens.

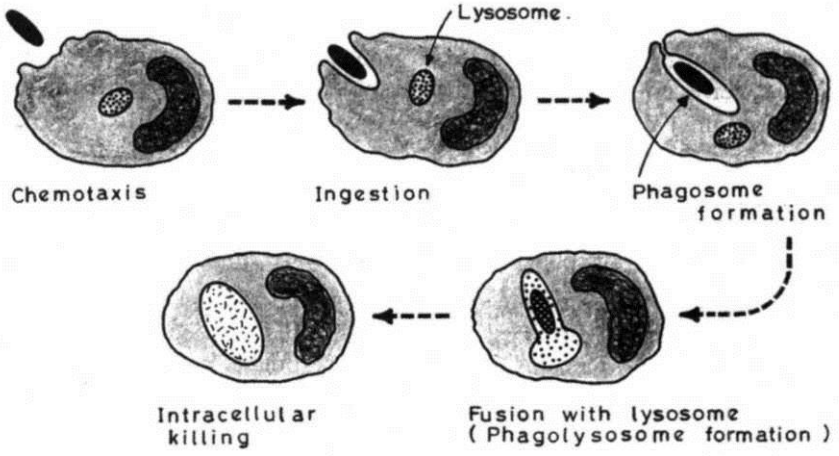
II- Second Line of Natural Defense:

If the invading organism gets through the first line of defense and enters the tissues, other non-specific host defenses operate. These include: **1- Circulating effector proteins** in serum and body fluids suppress the growth and promote killing of microbes. They include:

- **Lysozyme** present in all body fluids.
- **Complement:** It is a group of proteins in serum and body fluids. The alternate and lectin pathways of complement activation do not involve antigen-antibody complexes and so they act before antibodies appear as an initial defense mechanism against pathogens, promoting inflammation, phagocytosis (opsonization) and lysis of bacteria (chapter 15).
- **Acute phase proteins:** These are substances that increase in response to inflammation and include; C-reactive protein (CRP), fibrinogen serum amyloid A protein and plasma mannose binding lectins (MBL).

  They are synthesized in the liver in response to certain cytokines, namely, IL-1, IL-6 and TNF-a; these are produced by macrophages when stimulated by microbial products. It seems likely that the acute phase response achieves a beneficial effect through enhancing host resistance, mmimizing tissue injury and promoting the resolution and repair of the inflammatory lesion, e.g. CRP can bind to microbes resulting in activation of the complement and deposition of C3b on the surface of microbes and enhancing phagocytosis (opsonization). Measuring CRP is a useful laboratory test to assess activity of inflammatory diseases.

- **Interferons (IFNs):** These are a family of proteins which are important in the non-specific defense mechanisms against viral infections. They are released from virus-infected cells and, when taken up by other cells, protect them from infection not only by the same virus but also by other types of viruses. There is type I IFN which consists of IFN-a produced by mononuclear phagocytes and IFN-p produced by fibroblasts, and type II IFN or IFN-y produced by T cells (p.72).



(Fig. 19): The process of phagocytosis

2-**Cells of Innate Immunity** are phagocytes and natural killer (NK) cells

•• **Phagocytes** are body cells specialized for capture, ingestion and destruction of invading microorganisms i.e. **phagocytosis.** The **phagocytic cells** include the polymorphonuclear leucocytes (especially neutrophils) and mononuclear phagocytes (monocytes in the blood and macrophages in the tissues) and the dendritic cells (p.61-62).

The process of **phagocytosis** includes the following stages: (Fig. 19)

a- Chemotaxis, Migration and **Attachment:** If an infectious microbe breaches an epithelium and enters the sub-epithelial tissues, the resident macrophages are **attracted** to the site of inflammation by chemotactic substances liberated from microbes and damaged tissues. These include bacterial endotoxins (LPS), serum complement C5a, IL-8 (chemokine) and leukotrienes.

The macrophages respond by producing soluble proteins (cytokines) e.g. TNF-ct and IL-1. These activate the endothelial cells of the nearby venules to produce adhesion molecules and chemokines, which mediate the **migration** of leucocytes and monocytes from the blood through the endothelial wall of blood vessels to the tissues (i.e. diapedesis).

The phagocytes have many receptors on their surface e.g. Toll-like, scavenger and mannose receptors, Fc receptors and C3b receptors; these are called pathogen recognition receptors **(PRR).** These attachto molecular structures widely expressed on microbes but not on the host cells and are called pathogen associated molecular patterns **(PAMPs).** These may be sugars, proteins, lipids, nucleic acids or a combination of these molecules. PRRs on phagocytic cells recognize PAMPs directly or indirectly.

Attachment and ingestion is greatly enhanced if the organism is coated by its specific antibody, by activated complement C3b or by antibody and C3b (opsonins) and the process is called **opsonization** (Fig. 20).

b-Ingestion: The phagocytes proceed to engulf the organism by extending pseudopods around it. These fuse and the organism is included into a vacuole called phagosome. Lysosomal granules then fuse with the phagosome, forming the phagolysosome followed by digestion of organisms.

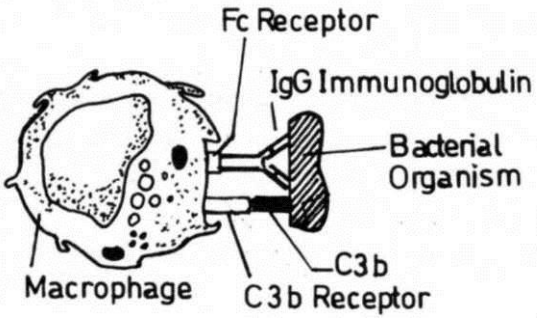
c- Intracellular killing or digestion: A number of antimicrobial and cytotoxic substances produced by activated macrophages can destroy phagocytosed microorganisms. This can occur through:

-The oxygen-dependent killing system in which oxygen is converted to superoxide anion, hydrogen peroxide, activated oxygen and hydroxyl radicals (oxygen burst). Myeloperoxidase produces hypochlorite from H_2O_2. Nitric oxide is also produced. All are powerful microbicidal agents.

-The oxygen-independent killing which is the result of lysozomal granules' content which include; lysozyme, lactoferrin, a group of cationic proteins (defensins), and a variety of hydro lytic and proteolytic enzymes.

It is to be noted that some species of bacteria resist destruction for long periods. Others may multiply within the phagocytes, e.g. *M. tuberculosis* and *M. leprae.* Wandering phagocytes can transport these organisms to new sites where they can set up foci of infection.

•• **Natural killer (NK) cells:** These are large granular lymphocytes. They have a non-specific cytotoxic activity on tumour cells, virus infected cells and graft cells and are important in innate immunity (p. 60).



(Fig. 20): Opsonization of bacteria with antibody and complement C3b.

3- Inflammatory Barriers:

Tissue damage by a wound or by an invading pathogen, induces a complex sequence of events collectively called "inflammatory response". This includes; vasodilatation of nearby capillaries leading to redness of tissues and increase of tissue temperature, increased capillary permeability and influx of fluids and cells (exudates) into the tissues causing oedema, and influx of phagocytes from the capillaries into the site of tissue damage, these engulf bacteria and release lytic enzymes that can damage nearby healthy cells forrning the pus.

A variety of chemical mediators are responsible for initiation of the inflammatory response. Some of these mediators are released from damaged tissues or invading microbes, some are generated by several plasma enzyme systems, and some are products of leucocytes. Among these chemical mediators are the acute phase proteins, histamine, kinins, fibrin and cytokines. These participate in destroying and removing the invaders and in healing of tissues.

4- Cytokines of Innate Immunity: Several **pro-inflammatory cytokines** play a key role in inflammation i.e. IL-1, IL-6, IL-8 and TNF-a. These are released from activated macrophages and increase extravasation of neutrophils to the site of inflammation, induce coagulation and increase vascular permeability. Other cytokine released from activated macrophages are IL-12 and IL-15 which activates NK cells. IFN-y produced by NK cells activates macrophages. IFN-a produced by viral infected cells inhibits viral replication and prevents spread of infection to uninfected cells.

Constitutional Factors that Modify Innate and Acquired Immunity

- **Species factors:** Some organisms are pathogenic only to certain species of animals, e.g. *M. leprae* affects man but not monkey.
- **Certain races** are more susceptible than others to certain microbes, e.g. negros and native Americans are more susceptible than the Caucasians to *M. tuberculosis.*
- **Individuals** in the same race may vary in their susceptibility to some organisms depending on their **genetic** make up, e.g. individuals who have genetic deficiency in G6PD enzyme are resistant to malaria. **Age** differences in susceptibility are met with, e.g. extremes of age are more susceptible to disease, due to immaturity of the immune system in children and aging of this system in the elderly. **Nutritional status;** under-nutrition increases susceptibility to disease. **Hormones** and corticosteroids increase susceptibility to infection. Diabetics are more susceptible to infection.

CHAPTER 10

IMMTJNOGENS OR ANTIGENS

Immunogens are any foreign substances which can stimulate a specific immune response (humoral or cell mediated immunity). **Antigens** have the ability to combine specifically with the antibodies produced or sensitized T lymphocytes induced. However, the two words, antigen and immunogen, are used almost interchangeably.

Haptens:

These are low molecular weight substances which, by themselves, are not capable of inducing an immune response. However, when coupled or conjugated to a larger carrier protein or if they autocouple spontaneously to tissue proteins following their administration into an animal, the haptens are transformed into antigenic deteirninants on the carrier molecule and are capable of inducing an immune response, i.e. they become immunogenic. Examples of haptens are simple chemicals and drugs, e.g. penicillin, tranquillizers, aspirin, neomycin skin ointments, cosmetics and soap.

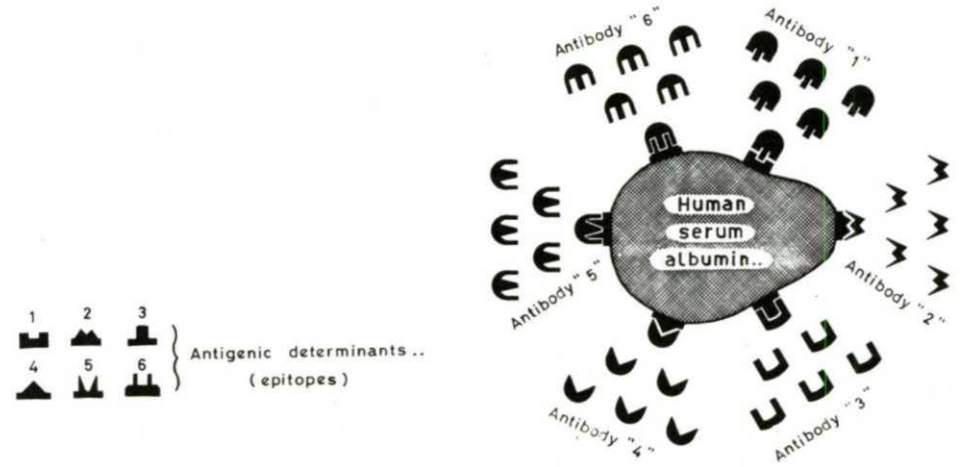
Epitopes or Antigenic Determinants:

Despite the fact that potent antigens are relatively large molecules, only small parts of the molecule can stimulate production of or bind to antibody. These parts are called "epitopes" or antigenic deteirninants. Each may be composed of 4-7 amino acids, or monosaccharide residues. The number of epitopes on a molecule varies with molecular size. Human albumin, for example, has at least six different epitopes, which means that at least six antibodies of different specificities can be produced after immunization of, for example, a rabbit (Fig. 21).

Factors that Influence Immunogenicity:

1- **Foreignness:** For a substance to be immunogenic it must be foreign to the animal in which it is introduced. The immune system of an individual can normally distinguish between body components, i.e. "self and foreign substances "non-self. Normally, the body is tolerant to its own components and does not initiate an immune response against them. Under certain circumstances, however, this auto-tolerance may be disturbed, permitting the individual to react against himself, leading to autoimmune diseases.

2- **Molecular size:** Small molecules such as amino acids or monosaccharides are usually not immunogenic. As a rule, molecules with a molecular weight of less than 5000-10,000, have no or only weak immunogenicity.



(Fig. 21): Diagram showing a molecule of human serum albumin with 6 antigenic determinants. When injected in rabbits, 6 different antibodies can be produced.

3- Chemical nature: The most potent immunogens are proteins. Some polysaccharides of high molecular weight are immunogenic. More complex proteins are more immunogenic i.e. globular proteins are more immunogenic than fibrillar proteins.

4- Degradability: For a substance to be immunogenic it has to be susceptible to partial enzymatic degradation that occurs during antigen processing by presenting cells such as macrophages (see p. 65). It has been found that peptides composed of D-amino acids, which are resistant to enzymatic degradation are not immunogenic, whereas their L-isomers are susceptible to enzymes and are immunogenic.

In general, a substance must have all the above four mentioned characteristics to be immunogenic. Other factors include:

5- Methods of administration of antigen:

a- Dosage: A state of antigen specific unresponsiveness (immunologic tolerance) can result if very high or very low doses of certain antigens are administered. The number of doses administered also affects the outcome of the immune response. Repeated administration of booster doses are required to stimulate a strong immune response, **b- Route of administration:** Parenteral routes are preferred to oral routes for experimental immunogens as they induce stronger immune response. I.V. injected antigens are carried to the spleen while subcutaneously injected antigens are carried to the local lymph nodes. Difference in the lymphoid cells that populate these organs may be reflected in the subsequent immune response.

c- Adjuvants: These are substances that, when mixed with an antigen before its administration will increase the immune response to that antigen.

Example of adjuvants are Freund's adjuvant (composed of killed tubercle bacilli, mineral oil and detergent), which is used for immunization of animals. Other microbial adjuvants include *B. pertussis*, BCG (attenuated *Mycobacterium)*, *C. parvum*, etc...

Aluminum hydroxide is used to enhance the immune response as in the alum precipitated toxoid used for vaccination against diphtheria.

The mechanism of action of adjuvants can be one or more of the following: **1-** A depot effect, by stimulating a granuloma around the antigen, thus slowing its absorption, i.e. prolonging antigen persistence and increasing stimulation of the immune cells. 2- They activate macrophages thus increasing phagocytosis, antigen processing and presentation and cytokines secretion such as IL-1. 3- They enhance co-stimulatory signal molecules leading to maximum activation of T cells. **4-** They stimulate non-specific proliferation of lymphocytes.

6- Host genetic factors: All individuals within a species will not show the same response to an antigen; some are strong responders and others are weak responders.

Antigen Antibody Binding (Fig. 22)

The binding of antigen to antibody binding site can be likened to a "lock and key" situation. The most efficient immunological responses occur when the antigen and antibody fit exactly (high affinity antibody). Sometimes an antigen can combine as a "poor fit" with an antibody (low affinity antibody) that was produced in response to an unrelated or partially related antigen which bears one or more epitopes in common. These are known as heterophile antibodies and heterophile antigens and the phenomenon is known as **"cross reactivity".**

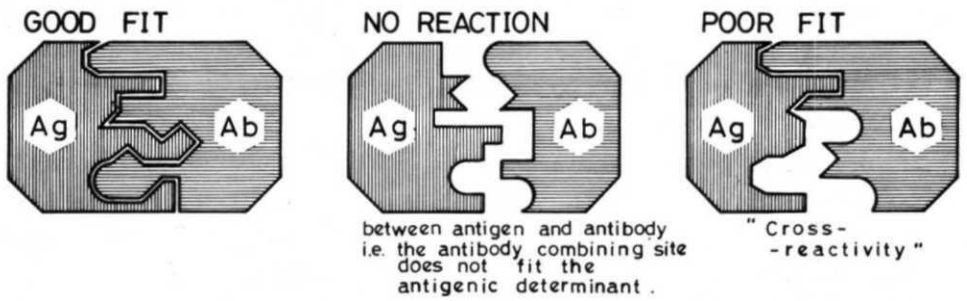
This can be met with ***in vivo*** in some infections of autoimmune nature, e.g. in acute rheumatic fever; it is thought that antibodies to *Strept. pyogenes* M antigen cross react with cardiac muscle proteins leading to rheumatic carditis and valve destruction.

In vitro several laboratory tests used for diagnosis are based on detection of heterophile antibodies in patients' sera e.g.:

1- **Paul Bunnel** test used in the diagnosis of infectious mononucleosis caused by Epstein Barr (EB) virus. In the sera of these patients, there is a heterophile antibody which agglutinates sheep RBCs.

2- **Cold agglutinins** in sera of *Mycoplasma pneumoniae* infected patients, which agglutinate human group O red cells at 4°C.

3- **The reagin** antibody in sera of syphilitic patients which reacts with cardiolipin antigen (alcoholic extract of beef heart muscle) used in the VDRL or RPR tests for diagnosis of syphilis.



(Fig. 22): Diagram showing antigen antibody binding.

Two terms are used to describe the strength of association between antibody and antigen; affinity and avidity. **Affinity** refers to the strength of association between one antigenic determinant and one antibody binding site and how good is the complementarity (fit) between the two. **Avidity** refers to the strength of association between multiple antibody binding sites and multiple antigenic determinants.

Types of Antigens

1- **Bacterial antigens:** These may be;
a-Soluble antigens which are products excreted into the environment, e.g. exotoxins, enzymes, haemolysins, etc... **b- Cellular antigens** which correspond to the different structures of the cell, e.g. **capsular** antigens in capsulated organisms, they are often polysaccharides as in *Strept. pneumoniae.* **Flagellar** or **"H"** antigens are present in flagellated organisms. **Somatic** or **"O"** antigens are part of the cell wall of gram negative bacteria (endotoxins). **"Vi"** or **virulence** antigens are surface antigens in recently isolated strains of, e.g. *S.* Typhi. **"K"** antigens are surface antigens in *E. coli.* **Fimbrial** antigens are surface antigens in fimbriated gram negative bacilli.

2- **Viral antigens:** These may be:
a- Protein coat viral antigens: Antibodies produced against these surface components can neutralize viral infectivity. **b- Soluble antigens** which diffuse in the surrounding fluids during virus growth, e.g. soluble nucleoproteins as in influenza and mumps viruses.

3- **Superantigens** (see p. 68).

4- **Human tissue antigens** (isoantigens):

a- Blood group antigens A and B as well as Rh antigens on red cells. These are of importance in blood transfusion reactions.

b- Histocompatibility antigens: These are glycoprotein molecules present on membranes of tissue cells. They are called the major histocompatibility complex (MHC) antigens or human leucocyte antigens (HLA). These antigens are coded for by MHC genes present on chromosome 6. There are two classes of MHC; class I MHC molecules are present on all nucleated cells, class II MHC molecules are present on immunocompetent antigen presenting cells (APCs) i.e. B cells, macrophages, dendritic cells and activated T cells. Individuals vary in their HLA types. Graft rejection is due to immunologic response of the recipient to tissue grafts from a donor with a different HLA type.

MHC has an important function in presentation of antigens to T cells. Helper T cells recognize foreign antigens on the surface of APCs, only when these antigens are presented in the groove of MHC-II molecule. On the other hand, cytotoxic T cells will only recognize antigens, on the surface of virus infected cells or tumour cells only when these antigens are presented in the groove of class I MHC molecules. This is known as **"MHC restriction"** (see chapter 11& 21).

Antigen Binding and Recognition Molecules in the Immune System:

Antigens are recognized by and bind to:

1- B cell receptors (BCR) which are membrane-bound immunoglobulins (IgM and IgD) on B cells. These have the same structure as other immunoglobulins (see p.76). They can bind unprocessed antigens of almost any chemical composition. BCRs can be secreted in the plasma as antibodies. BCR is associated with signal transduction molecules which are two single-chain immunoglobulin relatives known as Ig-a and Ig-P required for B cell activation (see chapter 11 & p. 138).

2- **T cell receptors (TCR)** which are composed of a and p chains anchored to T cells by their cytoplasmic tail (carboxy-tenriinal).There is a groove in the N- terminal of both chains which binds small peptides presented by MHC molecules on the surface of APCs. TCRs are cell-bound, their conformation is rigid as there is no hinge region like immunoglobulins. TCR is associated with a signal transduction molecule which is a multi-chain structure i.e. CD3 complex (see chapter 11 & p. 138).

3- MHC molecules are essential for presentation of peptides so that they can be recognized by and bind to TCRs.

CHAPTER 11

THE ACQUIRED (SPECIFIC OR ADAPTIVE)

IMMUNE RESPONSE

The acquired immune response is more specialized than the innate immune response. There are two mechanisms for the acquired (specific or adaptive) immune response:

I- **The Humoral Immune Response** in which immunoglobulins (antibodies) are produced by B lymphocytes. These have the ability to recognize and bind specifically to the antigen or immunogen that induced their formation and not other antigens.

II- The Cell Mediated Immune Response (CMI) which is mediated by certain types of lymphoid cells (T lymphocytes) that themselves have the ability, by means of surface receptors, to recognize foreign material, such as microorganisms or infected cells, and attack and destroy them directly or through the release of soluble mediators, i.e. cytokines (lymphokines).

It is important to realize that the normal acquired immune response involves a combination of both arms of immunity and that they can interact with one another to destroy microorganisms, infected cells or tumour cells.

The acquired immune response has several generalized features that characterize it:-

1- It is **highly specific** for the invading organism which is recognized by specific immune cells that are activated to produce a specific immune response.

2- Discrimination between "self and **"non-self,** i.e. the response only occurs to molecules that are non-self or foreign but not to those that are self, which is a cardinal feature of the immune response.

3- Diversity: It exhibits remarkable diversity and can respond to millions of different antigens. The lymphocyte population consists of many different clones (each of which is made up of one cell and its progeny), and each clone expresses an antigen receptor that is different from the receptors of all other clones and responds only to one antigenic epitope.

4- It is initiated by **recognition** of antigen by specific lymphocytes, these are **activated** then they **proliferate** and **differentiate** into **effector** cells that eliminate the antigen. This is followed by the return of homeostasis and development of **memory cells,** which evoke a more rapid and long lasting response on re-exposure to the same antigen. This explains the long lasting and sometimes life-long immunity to many infections.

The lymphoid system is composed of the **primary lymphoid organs,** i.e. bone marrow and thymus, in which haemopoiesis, lymphopoiesis and education and maturation of lymphocytes, to specifically recognize foreign antigen and distinguish self from non-self, occur. The **secondary lymphoid organs,** i.e. lymph nodes, spleen, tonsils, mucosa-associated lymphoid tissues (MALT) of the GIT and respiratory tract. These are the sites where naive mature recirculating lymphocytes will first be exposed to their specific antigens, presented to them by APC mainly by dendritic cells.

The cells that make up the immune system are distributed throughout the body but occur predominantly in the lymphoreticular organs mentioned above. The lymphocytes are the predominant immune cells but monocyte-macrophages, dendritic cells, eosinophils and mast cells play an important role in the immune response.

All the cells of the immune system arise from pluripotent stem cells in the bone marrow, that differentiate under the influence of soluble mediators (cytokines) mainly IL-7, IL-3 and colony stimulating factors, into cells of the lymphoid or myeloid series. The lymphoid stem cells differentiate into B lymphocytes, T lymphocytes and natural killer (NK) cells which are a third population of large granular lymphocytes. The myeloid stem cells differentiate tomonocyte-macrophages,eosinophilsandmast cells. Dendritic cells arise from the lymphoid or the myeloid stem cells.

Mature naive lymphocytes continuously recirculate from the blood and lymphatic vessels out into the tissues and lymphoid organs and return through the lymphatics. Microbial antigens are drained from sites of infection to local lymph nodes. Naive T lymphocytes traversing these organs, recognize their specific antigen displayed by APCs e.g. dendritic cells and are activated. They proliferate and differentiate to effector lymphocytes which migrate to sites of infection, where infectious microbes are eliminated.

B Lymphocytes

The lymphoid stem cells differentiate into B cells in the bone marrow, where B cell precursors mature, differentiate into immunocompetent B cells with a single antigen specificity. Immature B cells that express high affinity receptors for self antigens, that they encounter in the bone marrow, die or fail to mature i.e. **negative selection** or **clonal deletion.** This process induces central self tolerance and reduces the occurrence of autoimmune diseases.

Pre-B cells lack surface immunoglobulins, but they express IgM heavy chains mu (u) in their cytoplasm. **Immature B** cells express IgM receptors on the surface, while **mature B** cells express IgM and IgD molecules on

their surface that serve as receptors for antigens. These can directly recognize antigen, without the need for MHC determinants. **Memory B** cells express IgG or IgA or IgE on the surface.

B cells bear receptors for Fc portion of IgG (CD*32) and a receptor (CD21) for C3 component of the complement and Epstein Barr virus. They also express an array of molecules on their surface that are important in B-cell interactions with other cells such as MHC I and II, B7 and CD40. The latter is important for immunoglobulin class switching. CD 19 is used clinically to enumerate B cells in the blood.

B cells form about 30% of the re-circulating pool of small lymphocytes and their life span is short i.e. days or weeks. They are found in the germinal center of the lymph nodes, in the white pulp of the spleen and in the gut associated lymphoid tissue, awaiting the arrival of specific antigens.

B cells are important antigen presenting cells. Mature B cells differentiate on antigen stimulation to plasma cells that synthesize and secrete antibodies. Stimulation of B cells usually requires the cooperation of T cells (Fig. 29).

T Lymphocytes and their Maturation

The developing T cells migrate from the bone marrow to enter the thymus as thymocytes where they mature and acquire the following properties:-

1- In the outer cortex of the thymus they acquire **specific receptors** (TCRs) that commit each lymphocyte to a single antigen specificity, responding only to that antigen by proliferation and production of a clone of cells with the same specificity **(clonal selection).**They differentiate to express **CD3,** both **CD4** and **CD8** coreceptors and are called double positive (DP) cells.

2- As thymocytes move inwards to the thymus medulla, their TCRs recognize MHC molecules, loaded with normal self-peptides (p-MHC) which are presented to them on thymic dendritic cells. Those that have TCRs capable of binding with low affinity to p-MHC will receive **positive selection** signals to divide and establish clones that will eventually mature in the medulla. Thymocytes that bind too strongly to p-MHC will be induced to undergo apoptosis **(negative selection),** as these cells would have the potential to cause autoimmune diseases. T cells that fail to recognize self-MHC at all are useless and will die by apoptosis.

This selection process will ensure elimination of the potentially most harmful self reactive T cells, a property called **central self tolerance.** T cells with TCRs that weakly recognize self antigens in the thymus would strongly recognize and respond to foreign microbial antigens in the periphery.

*** CD = cluster of differentiation or cluster determinants.**

3- Immature T cells express both CD4 and CD8 (DP). As they mature, T cells with TCRs that have more affinity to bind to MHC class II will become helper T cells with CD4 molecules only. Those with TCRs that have more affinity to bind with MHC class I will become cytotoxic T cells (CTL) with CD8 molecules only. Hence there are two populations of T cells; **CD4 T cells** and **CD8 T cells.**

4- Mature positively selected T cells are **MHC restricted.** Thus, CD4 cells are MHC II restricted and CD8 cells are MHC I restricted. In addition, a single type of T cell **αβ** receptor responds to and recognizes only a specific combination of peptide (antigen) and MHC.

T cells constitute 65-80% of the recirculating pool of small lymphocytes. Mature naive T lymphocytes enter the circulation passing to the peripheral lymphoid organs, where they reside waiting for the antigen for which they express specific receptors. Their life span is relatively long, months or years.

T Cell Surface Markers are the molecules by which we can identify T cells and divide them to subsets. They are also required for interactions between T cells and APC and for antigen recognition. These are TCRs, CD3, CD4 or CD8, CD2, CD28, CD40L and FasL on activated T cells.

T Cell Receptor (TCR):

The TCR for antigen consists of two polypeptide chains **α** and **β**, linked by disulfide bonds. It is similar to a Fab fragment of an antibody molecule having variable and constant regions. The variable regions of both chains are located at the amino terminals of the polypeptide chains away from the cell membrane and both are required for recognition of antigen bound to MHC. The variable regions are highly polymorphic, so that within the entire T cell population there is a large number of different TCR **αβ** dimers which give T cells a remarkable ability to recognize millions of different antigens. An individual T cell contains only a single type of **αβ** dimer and recognizes and responds only to a specific combination of antigen and MHC.

The αβ dimer is non-covalently associated with **CD3** glycoprotein complex, which forms an intimate part of the receptor and is responsible for transducing the signal received by the TCR upon antigen/MHC recognition on APC leading to T cell activation (Fig.42 p. 138)

Besides the most common isotype, the ap TCR , there is a second isotype composed of y and 8 chains. The y8 TCRs are found mainly in the skin and at the mucosal surfaces. They constitute less than 5% of the total T cells.

CD28 are co-stimulatory molecules present on T cells and binds to B7 molecule present on APCs delivering the second signal for T cell activation.

CD40L is a molecule present on activated T helper cells and is involved in activation of B cells and binds to CD40 molecule on APCs.

CD4 and **CD8** are co-receptors that play a role with TCRs in antigen recognition. **CD3** are involved in transmitting signals from the TCR to the inside of the cells.

Fas Ligand (FasL) is present on activated TC cells and binds to Fas molecules present on various body cells during killing of target cells.

T Cell Subpopulations and their Effector Functions

I- CD4 T helper lymphocytes (TH) and their subsets and functions:

These constitute 65% of peripheral T cells and predominate in the thymic medulla, tonsils and blood. They are the cells attacked by the HIV virus. TH cells are the principal orchestrators of the immune response as they are needed for the activation of the major effector cells in the response, i.e. macrophages, cytotoxic T cells, NX cells, and antibody producing B cells.

TH lymphocytes recognize antigen on the surface of APCs in association with class II MHC molecules, they are activated and secrete several cytokines, which have several stimulatory and proliferative effector functions which differ according to the T cell subset.

TH cell subsets include TH1, TH2, regulatory T cells (Tregs) and TH17. All arise from the same precursor, the naive THO cells which are induced to differentiate to the different subsets by different stimuli. The two main subsets TH1 and TH2 produce different cytokines, and therefore promote different functions:

-**TH1:** IL-12 secreted by activated macrophages and IFN-y secreted by NK cells, during the innate immune response, induce the differentiation of naive THO to TH1 cells. TH1 secrete IL-2, IFN-y and TNF-**P** in response to viral and bacterial infections. These enhance cytotoxicity of TC cells, activate macrophages and NK cells and recruit inflammatory cells to the infected site i.e. CMI. They also enhance production of selected classes of antibody that participate in opsonization and antibody dependent cellular cytotoxicity (ADCC). IL-12 and IFN-y increase the production of TH1.

-**TH2:** IL-4 produced by several cells induces differentiation of naive THO to TH2 cells. TH2 produce IL-4, IL-5, IL-6, IL-10, IL-13 and TGF-p. They are mainly involved in B cell activation and differentiation and enhance production of all classes of antibodies including IgE. They stimulate eosinophils and mast cells. They respond mainly to allergic reactions and parasitic infections. IL-4 and IL-10 enhance the production of TH2. -Both subsets secrete IL-3 and GM-CSF. (Fig. 26 & table p. 136)

On the other hand, IFN-y inhibits production of **TH2,** whereas, IL^l and IL-10 inhibit production of **TH1,** i.e. cytokines produced by one subset can inhibit the production and hence function of the other subset, i.e. cross-regulatory activities.

Regulatory T cells (Trees) form 5-10% of TH cells they are CD4 +, CD25 + (IL-2Ra chain) and Foxp3 + (a transcription factor) a unique marker on Tregs and is necessary for its development and functions. They secrete the immunosuppressive TGF-P and IL10. They are essential for maintenance of peripheral self tolerance, by suppressing the activity of autoreactive T cells that escape thymic tolerance, *via* cell-cell contact. IL-2, TGF-P and Foxp3 control the activation, expansion and suppressive effector activity of Tregs. Their deficiency is associated with autoimmune /inflammatory diseases. *In vitro* expansion of antigen-specific Tregs and their transfer into patients suffering from autoimmunity is emerging as a promising new therapeutic approach for these diseases.

TH17 secrete IL-17 and IL-22 and other cytokines. Their differentiation from THO is, stimulated by antigen in presence of TGF-p, IL-6, IL-1 and, is inhibited by IFN-y or IL-4. They protect against extracellular bacterial and fungal infections. They may be important mediators of tissue damage in immune-mediated inflammatory or autoimmunity diseases.

II- CD8 Cytotoxic T lymphocytes (TC or CTLs)

These predominate in human bone marrow and gut associated lymphoid tissues and constitute 35% of peripheral T cells.

CTLs recognize antigen on the surface of target cells (may be an infected APC or any other infected nucleated cell) in association with MHC-I, they are activated and deliver a "lethal hit" that directly kills the target virus infected cell or tumour cell eliminating the reservoirs of infection. After delivering the lethal hit, CTL disengage from its target cell to kill other cells.

Mechanisms of target cell killing by CTLs;

1- CTLs release cytolytic proteins i.e. perforins, granzymes and serglycin. The perforins form pores in the cell membrane and facilitate granzymes entry into the cytoplasm of target cells. The granzymes initiate several pathways of apoptosis.

2- On activation, CTLs express a membrane protein Fas ligand (FasL), that binds to its target protein Fas, which is expressed on many cell types, and triggers apoptosis of these cells by degrading DNA of target cells as well as the DNA of the intracellular microbe.

Natural Killer (NK) Cells are large granular lymphocytes which lack most surface markers of B and T cells. In contrast to T cells, they are CD3-ve, CD16+ve, CD56+ve. They form 5-10 % of the peripheral lymphocytes. They function mainly in innate immunity. They have spontaneous non-specific cytotoxic activity on virus infected cells, tumour cells and graft cells. They are not MHC restricted, and MHC-I inhibits their killing functions.

The mechanism of NK mediated cytolysis is the same as that of CTLs. NK cells have granules that contain perforins which create pores in target

cell membranes, and granzymes, which enter through the pores and induce apoptosis of target cells.

By killing of cells infected by viruses and intracellular bacteria, NK cells eliminate reservoirs of infection. They kill virus infected cells very early in infection before antigen-specific CTLs are activated.

NK cells are activated and expanded by cytokines of innate immunity IL-12, IL-15 and IFN-a and B. They secrete IFN-y that activates macrophages to destroy phagocytosed microbes.

IL-2 activated NK cells are called lymphokine activated killer (LAK) cells. These cells have been used in cancer immunotherapy; however, they have shown variable results in clinical trials to treat metastatic cancer.

NK cells differ from CTLs in the following;

1- They are non-specific i.e. one NK cell can kill many different abnormal cells.

2- They act spontaneously without prior recognition or activation.

3- They do not require antigen presentation by MHC i.e. not MHC restricted.

4- They destroy cells coated with antibodies, a mechanism called antibody dependent cellular cytotoxicity (ADCC). NK cells have Fc receptors (CD 16) for IgG, which bind to antibody coated cells and kill these cells. The mechanism of killing involves lytic enzymes, TNF and perforin. ADCC provides a bridge between innate and acquired immunity. Other cells that possess Fc receptors and kill by ADCC are neutrophils, macrophages and eosinophils (Fig.31 p.79).

NK cells activation and recognition of infected cells is regulated by a combination of killer activating receptors (KARs) and killer inhibitory receptors (KJJls) on NK cells. KIRs bind to self class I MHC on normal cells and inhibit NK cells killing action; that is why NK cells do not kill normal host cells. They kill cells with reduced or lost expression of MHC-I as virus infected cells and tumour cells. At the same time, activating signals are induced by binding of KARs to stress molecules (MICA and MICB) that are expressed on the surface of infected cells, thus the infected cells are killed (Fig.41 p. 137).

NKT cells are a distinct subset of T cells that share markers and functional characters with NK. Like T cells they develop in the thymus and express rx**(3** TCRs that recognize lipid related molecular fragments presented on the non-classical MHCI (CDId). They recognize stress molecules on infected or injured cells and proceed to kill them. They secrete several cytokine mainly IL^t. They may play a role in defense against microbes rich in lipids e.g. M. tuberculosis

Macrophages:

They are derived from myeloid stem cells in the bone marrow. They exist as free cells in the blood e.g. monocytes and fixed cells in tissues e.g. Kupffer cells of the liver. CD 14, the endotoxin receptor, is the best marker for macrophages. Other surface markers include; receptors for Fc of IgG, complement receptors CR1 and CR 3. MHC-II and I, and B7 are expressed on activated macrophages.

Macrophages are an important link between the innate and the acquired immune responses. They are activated and attracted to the site of foreign material by the action of different cytokines including IFN-y. Fractions released on complement activation e.g. C5a attract macrophages to the site of inflammation. Their main functions are: **1-** Phagocytosis (p. 47).

2- Opsonization (p.48): Macrophages have surface C3b and Fc receptors that interact with Fc portion of IgG thereby enhancing the uptake of organisms coated with antibody alone or antibody and complement.

3- Macrophages are important APCs. They ingest foreign material, process it, and fragments of antigen are presented on their surface (in association with MHC molecules) for interaction with T cells.

4- Macrophages may also kill antibody coated infected cells or tumour cells (ADCC) through the release of lytic enzymes at the site of Fc-mediated contact and secretion of TNF-a resulting in cytotoxicity.

5- They secrete IL-1, IL-6, IL-12, IL-15, TNF-a, IFN, and chemokine IL-8.

6- They secrete prostaglandins and synthesize complement components.

Professional APCs are the dendritic cells, macrophages, and B lymphocytes

Dendritic cells are the most efficient APCs. They are particularly important because they are the main inducers of the primary immune response, presenting antigen to and activating naive T cells in the recognition phase. They express class I and II MHC molecules. The name dendritic describes their many long narrow processes which make them very efficient at making contact with foreign material. Dendritic cells are primarily located under the skin (e.g. Langerhans' cells in the skin) and mucosa of most organs where they capture foreign antigens and transport these antigens to local lymph nodes, where they present antigen to naive helper T cells.

Adhesion Molecules:

These are groups of molecules that are involved in the adhesion of cells to each other or with the extra-cellular environment. They have several important functions in immunity since they mediate: **a-** Migration of leucocytes out of the circulation and into the tissues in response to inflammatory stimuli by increasing their adherence to endothelial cells, as well as their movement in tissues through adhesion to extra-cellular matrix components (fibronectin, laminin and collagen).

b- Homing of lymphocytes to secondary lymphoid tissues, e.g. lymph nodes.

c- Cell-cell interactions in the immune response, e.g. helper T cells and APC, helper T cells and B cells, and cytotoxic T cells or natural killer (NK) cells and target cells. They include 3 major "families" of proteins:-

1- Cell adhesion molecules (CAMs): These have a domain structure similar to immunoglobulins. They are widely distributed in tissues and include CD2 and leucocyte function associated antigen-3 (LFA-3 recently named CD58) molecules that aid in TH interaction with APC, and B cells as well as intercellular adhesion molecules-1 (ICAM-1 recently named CD54) and ICAM-2 which aid in homing of lymphocytes and their migration to inflammatory sites.

2- **Integrins** are two-chain **(aP)** molecules. The pi integrins mediate leucocyte interactions with the extra-cellular matrix. The **P2** integrins CR3 (Mac-1), CR4 and LFA-1 mediate tight leucocyte adhesion to endothelial cells and their migration out of the blood into the tissues. CR3 and CR4 are also receptors for the activated complement fragments C3b that enhance phagocytosis (opsonin receptors).

3- **Selectins** are lectins (carbohydrate binding proteins) that allow cells to bind to the carbohydrate regions of glycoproteins or oligosaccharides on cells. They mediate binding between lymphocytes, neutrophils, platelets and endothelial cells, and their migration to inflammatory sites.

CELL MEDIATED IMMUNITY

Host defenses against extracellular infectious agents are mediated mainly by antibody, complement, and macrophages. However, once the infectious agent invades the host cell, cell mediated immunity (CMI) is required for recovery from these intracellular infections. CMI is responsible for a wide variety of protective mechanisms, e.g.:

- Resistance to many infectious agents especially intracellular pathogens,
  e.g. viruses, *M. tuberculosis, M. leprae*, *Brucellae* and *Listeria.*
- Resistance to fungal and protozoal infections.
- Resistance to tumours.

However, CMI may play a role in some harrnful conditions e.g.:

- Hypersensitivity reactions type IV e.g. contact dermatitis.
- Graft rejection, and autoimmune diseases.

CMI destroys the intracellular infectious agent, by killing the host cell that harbours it. In many cases, this will also kill the pathogen, which may require the host cell for its own reproduction. Combating viral and intracellular bacterial infections require the induction of cell mediated cytotoxicity, mediated by CTLs, NK cells, or activated macrophages. The same mechanisms may be effective against most tumour cells.

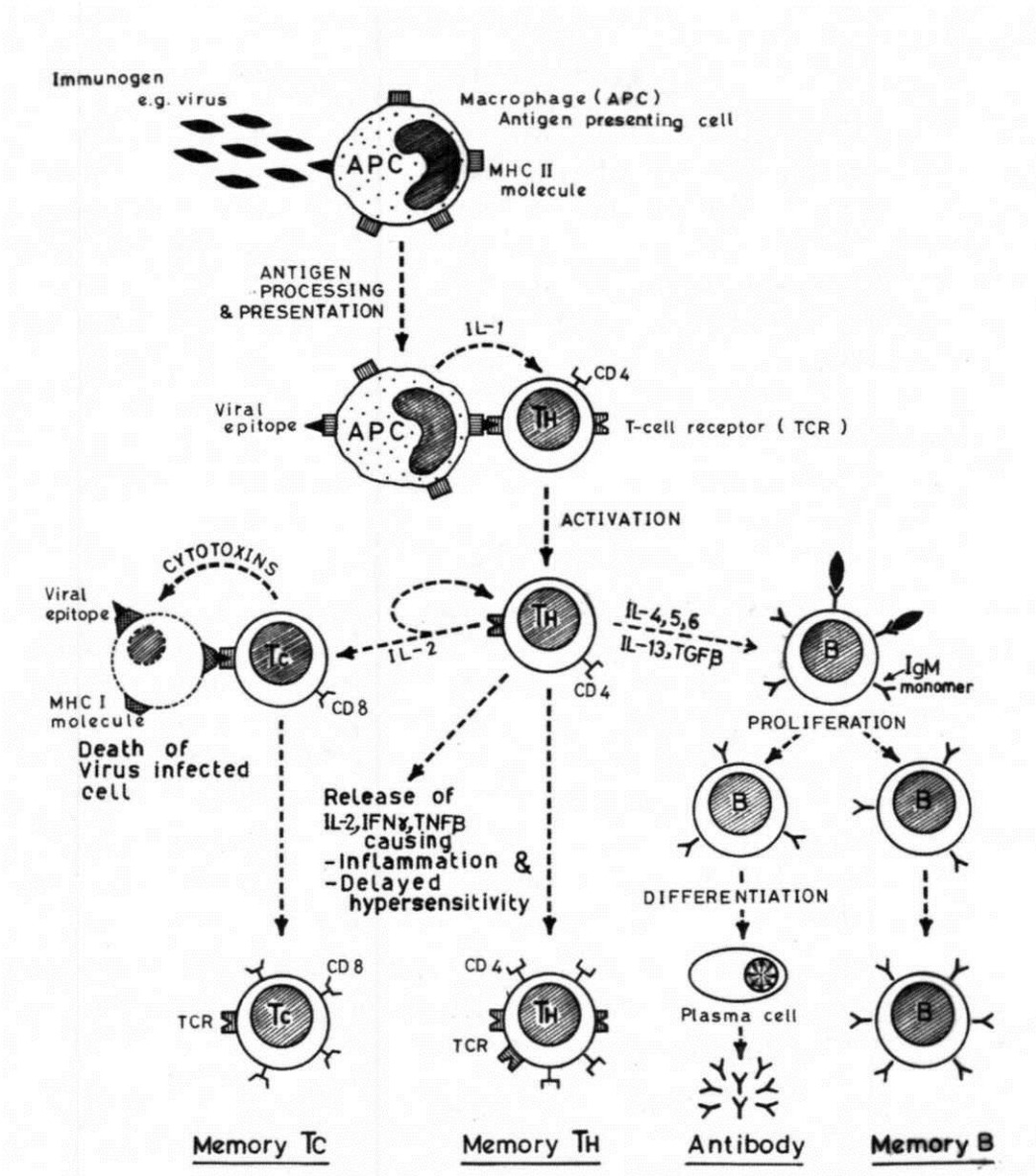
Characters of CMI

Generally speaking, the cellular immune response is principally mediated by subpopulations of T lymphocytes, macrophages and their products.

Macrophages present antigen *via* their surface MHC to T cells which **recognize** antigen through their specific receptors (TCR) and a specific T cell clone becomes **activated** and begins to proliferate.

Activated TH lymphocytes become **effector** cells that secrete soluble mediators i.e. cytokines which stimulate other effector cells of the CMI and humoral immune response and mediate the following responses:

1- They attract monocytes, macrophages and lymphocytes to the site.
2- They activate macrophages to kill intracellular microbes.
3- They promote the activity of CD8 CTLs, which directly kill virus infected cells, tumour cells and graft cells through several mechanisms.
4- They activate NK cells increasing their cytotoxic functions.
5- They stimulate B cells that are specific for the same antigen, to differentiate into plasma cells that secrete antibodies.



(Fig. 23): Scheme of the acquired immune response.

- **Immunogen, (e.g. virus) is processed and presented (in association with MHC-II) to TH cells.**
- **Activated TH1 cells release cytokines, INF-y, IL-2, TNF-P which stimulate effector cells of CMI and activate inflammatory cells, e.g. macrophages and NK cells.**
- **Released IL-2 activates TC cells which kill cells infected with virus after recognition of viral epitopes on infected cells in association with MHC-I.**
- **Activated TH2 cells release IL-4, IL-5, IL-6, IL-13, TGF-p\ These stimulate proliferation and differentiation of specific B cells to antibody forming plasma cells.**
- **IL-2 auto-activates TH cells.**

The Phases of CMI:

1- Antigen Processing and Presentation

Protein antigens have to be processed and converted to peptides then bind to MHC molecules on APCs to be presented to T cells:

Extracellular (exogenous) proteins (e.g. extracellular microbes or their products) are internalized into the **vesicular compartment** of APCs where they are degraded enzymatically in endosomes and lysosomes to generate peptides, which bind into the groove of class II MHC molecules in the endosomes. Peptide-MHC II complex is transported to the surface of APCs to be presented to CD4 TH cells (Fig. 43 & table p. 139).

Endogenously synthesized proteins, in the **cytosol** of nucleated cells; i.e. intracellularly synthesized microbes mainly viruses and tumour antigens are proteolytically degraded to peptides in the proteasome, found in the cytoplasm. They bind to class I MHC in the endoplasmic reticulum (ER). Peptide-MHC I complex is expressed on the surface of nucleated cells to be presented to CD8 cytotoxic T cells.

CD4 TH cells recognize vesicular peptides associated with MHC-II and CD8 TC cells recognize cytosolic peptides associated with MHC-I. These pathways of MHC-restricted antigen presentation ensure that most of the body's cells are screened for the possible presence of foreign antigens.

2- **Activation of T cells:**

Naive* CD4 and CD8 T cells are activated by two signals.

The **first signal** is the recognition of antigenic peptide-MHC complex on the surface of APC by TCR-CD3 complex. CD4 and CD8 molecules are co-receptors that stabilize the interaction of TH cells and TC cells, respectively, with APCs. CD3, CD4 and CD8 act as signal transduction molecules.

The second co-stimulatory signal is the interaction of CD28 on T cells with B7 (includes B7-1/B7-2 some times called CD80/CD86) On APCs. Without this signal, exposure of T cells to an antigen may lead to anergy.

- CTLA-4 is a molecule closely related to CD28 and is induced on T cells after activation and interacts with B7 giving a signal that is believed to turn off the synthesis of IL-2, limiting the extent of the immune response and leading to the differentiation of T cells into memory cells.

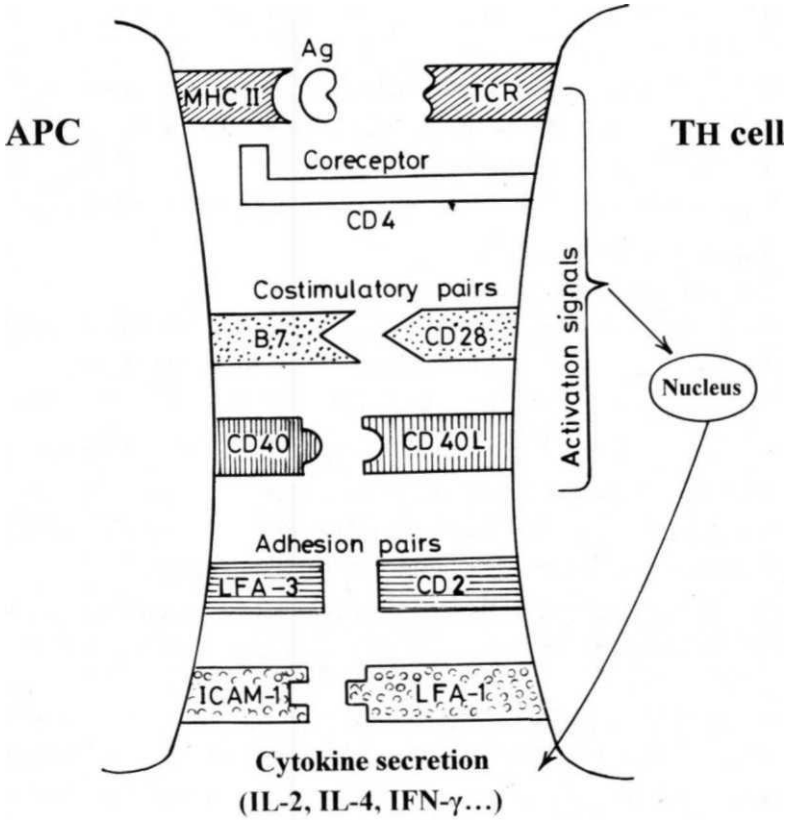
Other molecules may also serve as either co-stimulators or amplifiers of the immunologic synapse; among these is the interaction of CD40 on APCs with CD40L (CD 154) on activated T cells. Other surface proteins and adhesion molecules** play an accessory role in such interactions (Fig.24)

*** Naive T cells are mature T cells that did not encounter their specific antigen yet. **Adhesion molecules namely leucocyte function associated antigen (LFA-1) and CD2 on T cells interact with intracellular adhesion molecules (ICAM-1, recently named CDS4) and LFA-3 (CD58) on APC, respectively, thus enhancing the binding between these cells.**

TH cells express IL-2 receptors and secrete cytokines including IL-2 which auto-activate TH cells. The APCs also release IL-1 which acts on both APC and TH cell to promote their activation.

All the above mentioned interactions lead to **activation** of naive TH cells which proliferate and differentiate into **effector** antigen specific TH cells releasing cytokines. Some of them become **memory** cells which provide the secondary immune response in which they do not need the early recognition phase. The **cytokines** released from activated TH cells activate macrophages, NK cells and B cells.

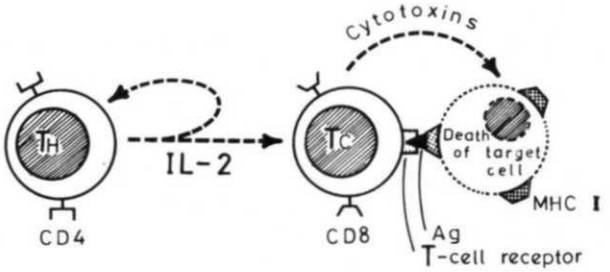
The activated CD8 TC cells proliferate and differentiate into a clone of **effector** CTLs which **kill** target cells i.e. nucleated cells (expressing MHC-I) infected with viruses, tumour cells or graft cells. Note that activation of CTLs is promoted by IL-2 secreted by activated TH cells (Fig.25).



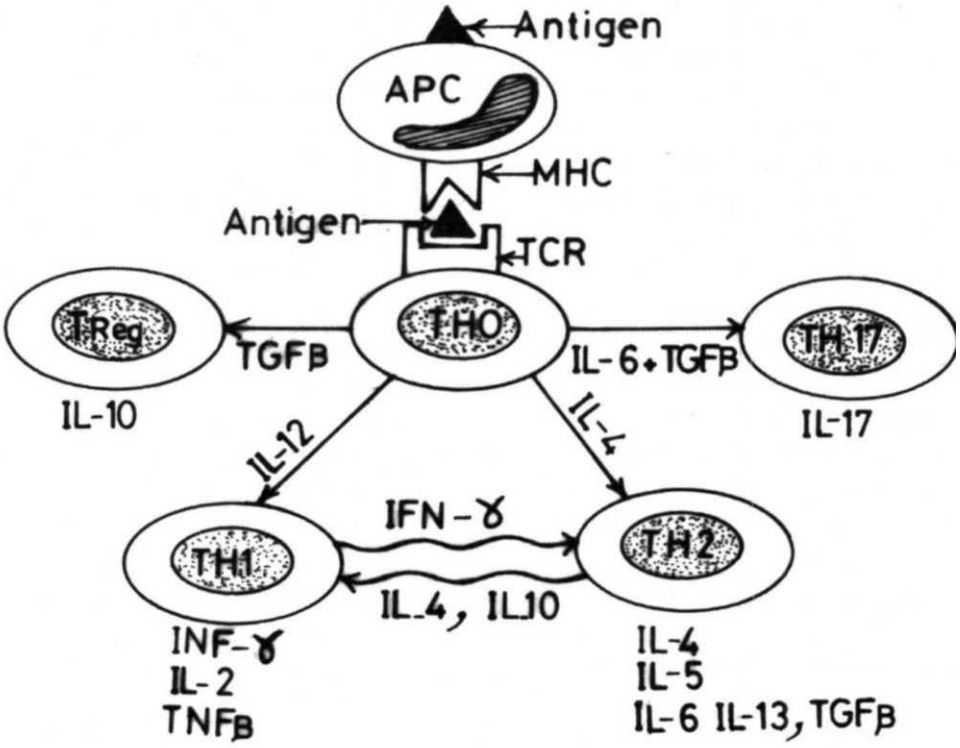
(Fig. 24): Key cell surface interactions that lead to TH cell activation and cytokine secretion.

3-**Activation of Macrophages and Delayed Type Hypersensitivity (DTH):**

Activated TH cells (TH1) secrete IFN-y which activates macrophages and increases their ability to kill ingested intracellular pathogens.



(Fig. 25): Activation of TC cell, and the consequence of activation.



(Fig. 26): TH cell subsets: T_{HI},$T_{H}2$, Tregs, TH17 their induction and cytokines released. IFN-y suppresses $T_{H}2$ production. IL-4 and IL-10 suppress production of T_{HI}.

The process of activation of macrophages, NK cell and cytotoxic T cells, infiltration and proliferation of inflammatory cells, stimulated by the cytokines released from TH cells (THI), is a very important protective mechanism when directed against an intracellular pathogen (CME).

Activated macrophages can also kill some abnormal host cells (infected or tumour cells). Its cytotoxicity is non-specific and is mediated by TNF, nitric oxide, enzymes and oxygen metabolites (H_2O_2, +02-).

If the infection is not fully resolved, activated macrophages cause tissue injury and fibrosis i.e. DTH reaction.

Polyclonal activation of lymphocytes:

A specific antigen stimulates only a small number of B and T cells to divide and differentiate. Some molecules have been discovered that stimulate a large number of cells without antigen specificity. These include:

a- Plant lectin mitogens that stimulate non-specific T cell division, e.g. phyto-haemagglutinin (PHA) and concanavalin A (CONA). Pokeweed mitogen (PWM) is also a polyclonal B cell stimulator. These are used for assessment of lymphocyte functions (p. 133). b- Epstein-Barr virus (EBV) is a polyclonal B cell activator, **c-** Endotoxin (LPS) is a polyclonal B cell and T cell stimulator, **d-** Superantigens (SAgs):

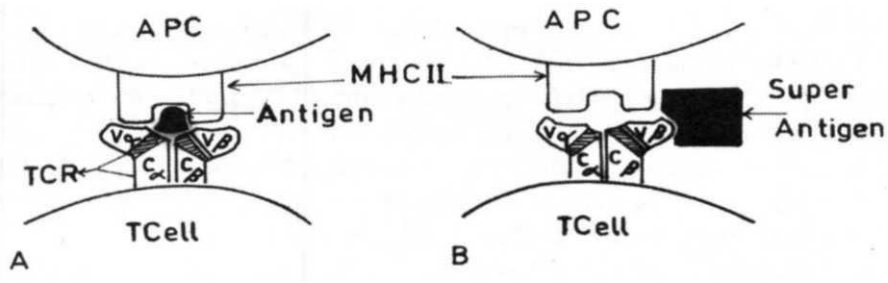
SAgs are antigens that activate multiple clones of T lymphocytes. These include a class of bacterial toxins, e.g. *Staph, aureus* toxic shock syndrome toxin (TSST) and enterotoxins, *Strept. pyogenes* pyrogenic toxin A, as well as some viral SAgs, e.g. those produced by a murine retrovirus -the mouse mammary tumour virus- and the nucleoprotein of rabies virus. The gpl20 of HIV is postulated to act as a SAg.

SAgs have the remarkable ability to bind both class II MHC molecules and the TCR **P** chain. They act as a clamp between the two, providing a signal for T cell activation (Fig. 27). The differences between the classic antigens and SAgs are:-

SAgs are not processed. They interact with the MHC molecule outside the peptide-binding groove. On the T cell side, SAgs bind to the variable segment of the **P** chain **(VP)** only, but not the a chain of the TCR.

They are active at very low concentrations and cause a large percent of T cells expressing a particular **VP** segment, irrespective of their antigenic specificity, to be stimulated causing the release of large amounts of cytokines including IL-1, IL-2 and TNF. This method of stimulation is not specific for the pathogen and does not lead to acquired immunity, i.e. no memory.

The massive T cell activation and release of large amounts of cytokines that cause systemic toxicity, explains to a large extent the pathogenesis and manifestations of diseases caused by organisms having SAgs, e.g. the toxic shock syndrome by *Staph, aureus* toxin (TSST-1) which stimulates T cells that bear Vp2. After the initial proliferation and release of cytokines, the affected lymphocytes become "exhausted" and refractory to further stimulation, i.e. anergic, and the host becomes clinically immunodeficient to many different infectious agents (see table p. 140).



(Fig. 27): The interaction of superantigen as compared to classic antigen with TCR and APC.

CHAPTER 13

CYTOKINES

Cytokines, include the previously known lymphokines (secreted by lymphocytes), monokines (secreted by macrophages), interleukins (secreted by some leucocytes and act upon other leucocytes), interferons and chemokines. They are low molecular weight soluble proteins (polypeptides) produced in response to microbes and other antigens. They act *via* cell surface receptors to mediate and regulate the amplitude and duration of the irnmune-mflammatory responses, through activation of macrophages, controlling growth and differentiation of T and B cells, etc...

General Properties of Cytokines:

- Cytokine secretion is a self limited event **(transient).**
- They are potent in minute amounts.
- Their action is **pleiotropic** i.e. one cytokine can act on different cells, and **redundant** i.e. multiple cytokines may have the same functional effects.
- Cytokines often influence the synthesis and actions of other cytokines. One cytokine may **stimulate** the production of another. Two cytokines may **antagonize** each other's action, produce **additive** or **synergistic** effects.
- Actions of cytokines may be local or systemic. Most cytokines act close to where they are produced, either on the same cell that secretes them **(autocrine action)** or on a nearby cell **(paracrine action).**When secreted in large amounts they may enter the circulation and act at a distance from the site of production **(endocrine action).**
- Their action is not antigen specific. It is initiated by binding to specific cytokine receptors on the membrane of target cells.
- They act as intracellular messengers, and the cellular response to most cytokines is the expression of new functions and sometimes proliferation of the target cells.

Functional Categories of Cytokines:

Cytokines can be classified according to their principal biologic actions to the following three groups; however, many of them mediate more than one of these functions.

1- Mediators and regulators of **innate immunity:** These are produced by activated macrophages and NK cells in response to microbial infections. They act mainly on endothelial cells and leucocytes to stimulate the early inflammatory reactions to microbes. They include; IL-1, IL-6, IL-10, IL-12, IL-15, IL-18, IL-23, IL-27, TNF-a, chemokines, type I interferon (IFN-a and IFN-p).

TNF-a, IL-1, IL-6 and the chemokines are known as the **proinflammatory** cytokines.

2- Mediators and regulators of **acquired (adaptive) immunity:** These are produced mainly by T lymphocytes in response to specific recognition of foreign antigens. They include IL-2, IL-4, IL-5, IL-13, IL-17, IFN-y, transforming growth factor-B (TGF-**P**) and lymphotoxin (TNF-**P**).

3- Stimulators of **haematopoiesis:** These are produced by bone marrow stromal cells, leucocytes, and other cells. They stimulate the growth and differentiation of immature leucocytes. These include; stem cell factor, IL-7, IL-3 and GM-CSF.

Interleukin-1 (IL-1) is produced mainly by activated macrophages. Its production is stimulated by bacterial products such as LPS and by TNF.

1- Its principal function is as a mediator of the host inflammatory response to infection by recruiting and activating macrophages and neutrophils at the site of infection, in innate immunity.
2- It stimulates the hypothalamus, inducing fever (endogenous pyrogen).
3- It acts on the liver and induces production of acute phase proteins.
4- It activates TH cells which then release lymphokines including IL-2.
5- It stimulates proliferation of B cells.
6- It stimulates macrophages to secrete prostaglandins, cytokines and chemokines.

Interleukin-2 **(IL**-2) is produced by activated T cells mainly TH1. It plays several important roles in the acquired immune responses:

1- It stimulates proliferation of, and antibody secretion by B cells.
2- It stimulates proliferation of T cells and increases cytokine secretion by activated TH cells.
3- It enhances cytolytic action of NK cells. IL-2 activated NK cells are called LAK cells. IL-2 and LAK cells are used in cancer therapy.
4- It activates macrophages.
5- It terminates the immune response by stimulating the function of regulatory T (Tregs) cells and by rxrtentiating apoptosis of activated T cells.

Interleukin-3 (IL-3) is secreted by TH cells and stimulates bone marrow stem cells.

Interleukin-4 (IL-4) is produced mainly by TH2 and mast cells.

1- It stimulates the development of TH2 from naive TH cells and inhibits development of TH1.
2- It stimulates B cell immunoglobulin class switching to IgE and promotes mast cell/eosinophil-mediated reactions and thus predisposes to the occurrence of type I hypersensitivity reaction.

3-It inhibits IFNy-mediated activation of macrophages and thus inhibits cell mediated immunity.

Interleukin-5 (IL-5) is produced by TH2 cells and activated mast cells.

1- It stimulates proliferation and activation of eosinophils which kill helminthes.

2- It stimulates proliferation of B cells and the production of IgA.

InterIeukin-6 (IL-6) is produced by macrophages, TH2 cells and others. It functions in both the innate and the acquired immune responses.

1- It stimulates acute phase proteins production by the liver.

2- It stimulates production of neutrophils from bone marrow progenitors.

3- It stimulates B cell growth and differentiation.

Interleukin-7 (IL-7) is produced by bone marrow stromal cells and fibroblasts. It stimulates survival and expansion of immature precursors committed to the B and T lymphocyte lineage.

IL-8 and other chemokines are a family of cytokines whose members have a chemo-attractant activity for leucocytes and fibroblasts. They are important mediators of acute inflammatory reactions, leucocyte adhesion, migration, activation and differentiation. They include IL-8, macrophage inflammatory proteins MlP-la and p and others. They are secreted by T cells, and monocytes.

Two of the chemokine receptors, CCR-5 on macrophages and CXCR4 on lymphocytes are co-receptors needed with CD4 for attachment of HIV to target cells. People with genetic defect in these receptors are resistant to HIV infection.

IL-10 is produced mainly by Treg cells and macrophages. It inhibits production of IL-12, IFN-y and TH1 cells. It inhibits expression of MHC-II on macrophages and dendritic cells and decreases their antigen presenting abilities as well as other functions, i.e. it **down- regulates** innate and CMI.

IL-12 is produced mainly by activated macrophages and dendritic cells. It is an important link between the innate and acquired immune responses.

1- It promotes the production of TH1 cells from naive THO cells.

2- It induces IFN-y production by T cells and NK cells.

3- It increases the cytolyic functions of activated NK cells and CD8 T cells.

IL-13 is produced by TH2 cells. It inhibits macrophage activation and antagonizes IFN-y. It stimulates mucous production by pulmonary epithelial cells and may play a role in asthma.

IL-17 see page 60.

Transforming Growth Factor B (TGF-B) is produced mainly by regulatory T cells (Tregs) and monocytes. It is viewed as an **"anti-cytokine".** It inhibits growth and activation of T cells as well as many functions of macrophages, B cells, neutrophils and NK cells i.e. it dampens or **suppresses** the immune response when it is no longer needed. However, it promotes wound healing and enhances IgA production.

Granulocyte Macrophage-Colony Stimulating Factors (GM-CSF) are produced by TH1 and TH2 cells and macrophages; they support growth of bone marrow stem cells.

They stimulate growth of granulocyte and macrophage colonies. They activate macrophages, eosinophils and neutrophils.

Tumour Necrosis Factor (TNF or TNF-a) is produced by macrophages and T cells. It is the principal mediator of the acute inflammatory response to gram negative bacteria and other microbes and is responsible for many of the systemic complications of severe infections. The main biologic actions are:

1- In low or moderate quantities, it stimulates the recruitment of neutrophils and macrophages to the sites of infection and activates these cells to eradicate microbes (local inflammation). It also induces apoptotic death of some cells.

2- In severe infections, TNF is produced in large amounts causing systemic clinical pathologic abnormalities. The principal systemic actions are:

a- It stimulates the hypothalamus, inducing fever (endogenous pyrogen).

b- It acts on the liver and induces production of acute phase proteins.

c- It causes septic or endotoxic shock i.e. vascular collapse, intravascular coagulation and decreased glucose levels. This syndrome is due to induced production, of TNF, IL-12, IFN-y and IL-1, by the LPS of gram negative bacteria.

d- Prolonged production causes muscle and fat wasting i.e. cachexia, **e-** It causes necrosis of tumours (which is the basis of its name).

Tumour necrosis factor-B (TNF-**P**) or **lymphotoxin** (LT) is produced by T lymphocytes and other cells. It has biologic effects similar to TNF, however, it is produced in much smaller quantities and is a locally acting cytokine.

Interferons (IFNs) (see p. 97):

IFNs are a large family of proteins secreted by most cells of vertebrates in response to viral infections or other selected stimuli. **Type I** interferon i.e. **IFN-a** is produced by mononuclear phagocytes and **IFN**-P is produced by fibroblasts. Both are induced by viral infections or by double stranded RNA.

Type II interferon or **IFN-y** is also called immune interferon and is produced byTHl cells and NK cells.

Actions of Type I interferon (IFN-a and IFN-0):

1- Anti-viral activity: Prevents viral replication early in infection (p. 97).

2- Increases MHC-I expression on virus infected cells, helping their recognition by CD8 TC cells which kill virus infected cells.

3- Increases cytotoxic action of NK cells.

4- Antiproliferative actions: inhibit cell proliferation and tumour growth.

Actions of Type II interferon or IFN-y:

1- It is the principal macrophage- activating cytokine.

2- It increases expression of MHC-I and II and co-stimulatory molecules on APCs.

3- It enhances cytotoxic actions of NK cells.

4- It promotes production of TH1 and inhibits production of TH2.

5- It stimulates B cells to secrete IgG2a in mice.

AT?: *Note that this chapter includes the most important cytokines. Many other cytokines are not discussed.*

Therapeutic Uses of Cytokines:

1- GM-CSF induces increase in white cell count hence it is used:
   a-To restore the leucocytic count after cytotoxic chemotherapy.
   b- After bone marrow transplantation to improve regeneration of cells.
   c- To correct AIDS - associated leucopenia.

2- IL-11 is used to shorten periods of neutropenia and thrombocytopenia after chemotherapy to treat cancer.

3- Interferon in treatment of viral diseases, cancer and other diseases (p.97).

4- IL-2 and LAK cells in treatment of cancer (p. 103).

5- TNF-a targeted to tumour cells in "magic bullet therapy" (p. 102).

6- IFN and TNF-a are used to activate NK and T cell for treatment of various tumours and immuno-deficiency diseases.

7- IL-12 enhances anti-tumour T cell and NK cell activity and is under trial in treatment of advanced cancer patients.

Anti-cytokine and anti-cytokine receptors are used for therapy

1- Anti-IL-2R a (anti-CD25 also known as anti-Tac) is used in treatment of adult T cell leukemia induced therapeutic responses in 1/3 of patients.

2- Anti-IL-2R a (anti-Tac) is used to reduce graft rejection.

3- Anti-TNF is used in treating patients with septic shock.

4- Anti-TNF is approved for use in rheumatoid arthritis.

5- Anti-IL-4 and anti-IL-5 in treatment of allergies e.g, asthma and rhinitis.

6- Anti-IL-17 is being developed to treat rheumatoid arthritis, psoriasis and other autoimmune diseases.

CHAPTER 14

HUMORAL IMMUNE RESPONSE

Humoral immunity is mediated by antibodies synthesized by B lymphocytes and secreted by their fully differentiated end cells, the plasma cells. This response is directed towards defense against extracellular microbes and their toxins leading to their extra-cellular elimination or enhancing their destruction *via* phagocytosis.

The antibodies are a group of glycoproteins present in the serum and tissue fluids of all mammals. They are mainly found in the gamma globulin fraction of the serum. They bind specifically to the antigen that induced their production.

Clonal Selection: Each individual has a large pool of B lymphocytes, each of which is programmed to make only one antibody and it expresses this immunoglobulin (IgM monomer) on its outer surface to act as an antigen receptor. When the antigen enters the body, it selects the B lymphocyte that has the specific receptor, binds to it, and stimulates its proliferation to give a clone of B cells which differentiate to plasma cells that secrete antibody specific to that antigen (Fig. 28).

Activation of B Cells and Production of Antibodies

Antibody production to protein antigens introduced into the body requires the direct contact of B cells with TH cells and their cytokines. These are called thymus dependent or **T-dependent antigens.**

Antibody responses to non-protein antigens, such as polysaccharides and lipids, do not require antigen specific helper T cells for antibody production. These are called thymus independent or **T-independent antigens.** These will be discussed later.

The process of activation of B cells and the generation of antibody producing cells in response to protein antigens consists of sequential phases:

1- Mature naive antigen specific B cells, after leaving the bone marrow, populate the peripheral lymphoid tissues, where they meet their specific antigens. Antigens are recognized by, bind to and cross-link the specific receptors (IgM-IgD) on the surface of B cells. This provides the **first signal** of B cell activation. Then the antigen is internalized by B cells and processed and presented on its surface in association with MHC-II to specific TH cells (Fig. 29).

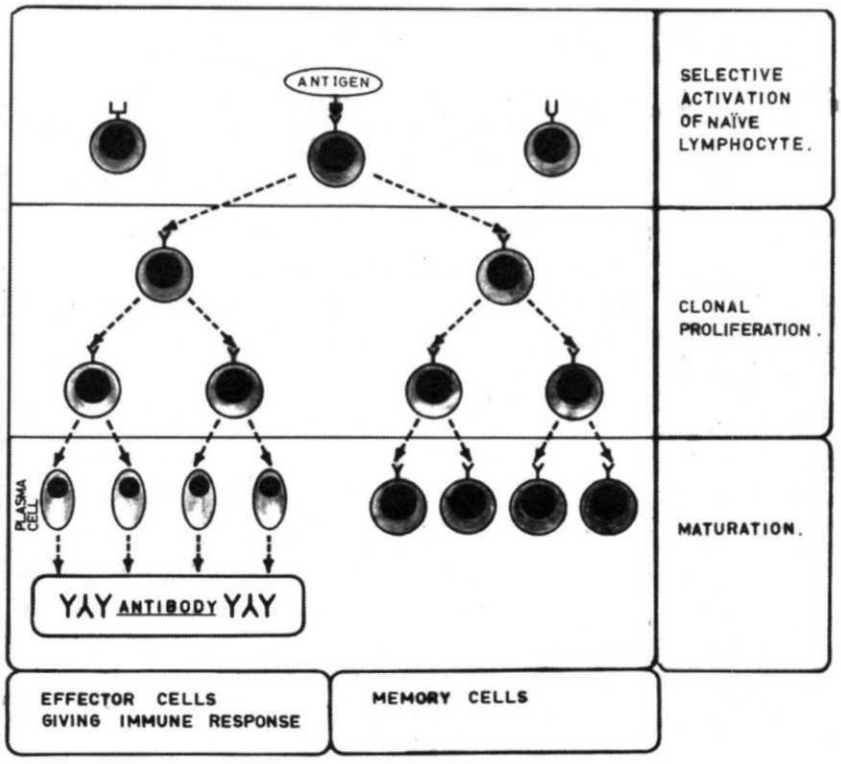
2- B7 molecules **are** induced on B cells and interact with CD28 on T cells. This second co-stimulatory interaction is needed for proper antigen presentation to TH cells and their activation.

3- TH cells activated by antigen and B7 co-stimulation express a surface molecule called CD40L which interacts with CD40 molecule on the B cells presenting the antigen. This signal is essential for secretion of cytokines and for immunoglobulin class switching.

4- The direct contact of B and TH cells and secretion of cytokines by TH cells (IL-4, 5, 6, 13 and TNF-B) provide the final signal for B cell activation, proliferation and differentiation to effector plasma cells secreting antibodies.

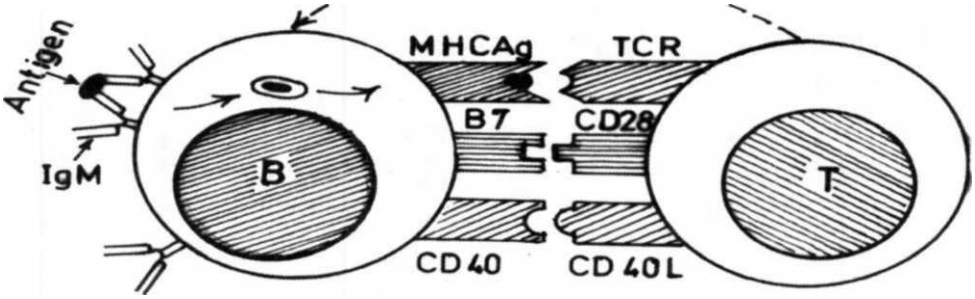
The cytokines secreted induce immunoglobulin heavy chain class switching from IgM, which is the first antibody produced, to IgG, IgA or IgE.

5- Some activated B cells differentiate into long-lived plasma cells and memory cells that remain quiescent for long periods but are capable of being activated rapidly upon re-exposure to antigen. The presence of these cells explains the rapid appearance of antibody in the secondary immune response and are responsible for long-lasting immunity after some infections e.g. measles and poliomyelitis.



(Fig. 28): Clonal selection and generation of effector plasma cells and memory cells after primary contact with antigen.

IL-4,5, 6/13, TGF3^



(Fig. 29): Activation of B cells. T cell - B cell cooperation.

Non-peptide antigens activate B cells directly without the help of T cells and are called **T cell independent (TI) antigens.** Such antigens are generally large polymers with repeating antigenic epitopes, such as bacterial capsular polysaccharides. The multiple, identical epitopes can cross-link surface receptors on B cells and stimulate their proliferation and IgM production (see table p. 140).

In the T cell dependent response, all classes of antibodies are made (IgG, IgM, IgA, IgE), whereas in the TI response only IgM is made (this indicates that cytokines produced by T cells are needed for class switching). T cell dependent response generates memory B cells, whereas the TI response does not, so a secondary antibody response does not occur in the latter.

Structure of Immunoglobulins or Antibodies: (Fig. 30)

The basic immunoglobulin unit (monomer) consists of 4 polypeptide chains: two identical heavy chains and two identical light chains held together by disulphide bonds.

Light chains (M.W. 25,000): There are two antigenic types called kappa (**κ)** and lambda *(k).* Only one type of light chain is found in any individual molecule.

Heavy chains (M.W. 50,000-75,000): There are five main types; gamma (y), alpha (a), mu (u), delta (8) and epsilon (s) corresponding to the five major classes or isotypes of immunoglobulins IgG, IgA, IgM, IgD and IgE, respectively.

Papain, a proteolytic enzyme, splits the antibody molecule, at the hinge region, into three fragments. Two identical pieces called **Fab** fragments each containing 1 antigen binding site. The amino end of the Fab fragment contains the variable (V) region of the molecule in which the amino acid sequences vary greatly from one molecule to another, thus allowing the binding sites to adapt to the shape of different epitopes. The rest of the chains have a relatively constant sequence.

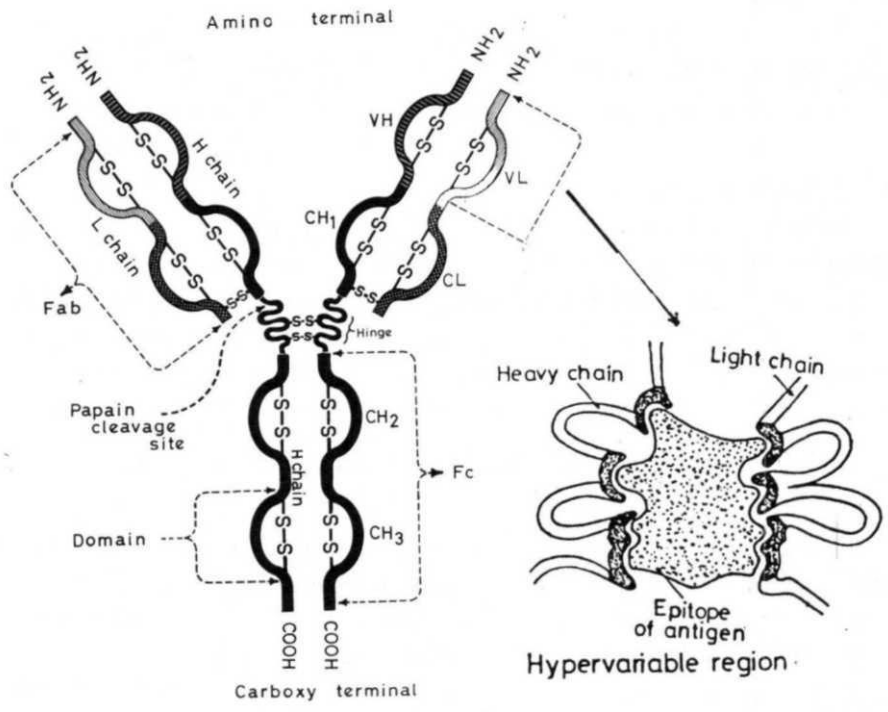
The third piece is called **Fc** fragment (so called as it is easily crystallized). The function of that fragment is to direct the biological activity of the different immunoglobulin classes. This is determined by the presence of Fc receptors on various body cells with which the Fc fragment reacts. For example, placental cells (trophoblasts) have Fc receptors for Fc portion of IgG only. That is why it is the only immunoglobulin that crosses the placenta.

Domains: (Fig.30)

Both light and heavy polypeptide chains consist of subunits or domains. Each domain consists of 110 amino acids held together in a single loop by an intra-chain disulphide bond. The NH_2 terminal domains of each light and heavy chains show marked degree of variation and are termed VL and VH respectively. The other domains are constant and are termed CL, CHI, CH2 and CH3. Separate domains serve separate functions.

Hypervariable Regions: (Fig. 30)

In the variable regions of both L and H chains there are 3 regions with extremely variable (hypervariable) amino acid sequences that form the antigen-binding site.



(Fig. 30): Structure of the basic immunoglobulin unit (monomer), i.e. IgG molecule.

The hypervariable regions form the region complementary in structure to the antigenic determinant or epitope. These regions are involved in the formation of the paratope.

The paratope is the area of the immunoglobulin molecule that interacts specifically with the epitope of the antigen. It is created by folding of the polypeptide chains which bring the hypervariable regions of the VH and VL domains into close proximity, resulting in a three dimensional structure that is complementary to the epitope (Fig. 30).

Isotype is the class of an antibody. IgG, IgM, IgE, IgA and IgD are different isotypes. The isotype is determined only by the structure of the constant domains of H chains of the immunoglobulin molecule. Immunoglobulin subclasses are also examples of different isotypes.

Idiotypes: These represent the antigen-binding specificities of immunoglobulins and are associated with the hypervariable regions which determine antibody specificity. Idiotypic determinants may be shared by different immunoglobulin classes.

Effector Biologic Functions of Immunoglobulins by which they contribute to immunity include:

1- Agglutination or clumping of pathogens (bacterial adhesion) to prevent their dissernination and facilitate their removal by phagocytic cells.

2- Neutralization of toxins or viruses and preventing them from binding to target cells.

3- Opsonization (see p. 48).

4- Complement activation leading to cell lysis or opsonization.

5- Antibody dependent cellular cytotoxicity (ADCC) which means the destruction of antibody coated target cells (tumour or virus infected cells) by NK cells, macrophages or polymorphs. These cells have receptors for Fc portion of antibody so they can bind to the antibody found on the surface of a target cell and exert their cytotoxic effect leading to cell lysis (Fig. 31).

Different isotypes mediate different functions (see table p. 141).

IgG

It is the major immunoglobulin in normal serum accounting for 75% of the total immunoglobulin pool. It is a monomeric unit -2 heavy and 2 light chains-(M.W. 160,000). It can bind two antigen molecules. Four subclasses are known IgGi, IgG_2, IgG3 and IgG4. Properties and biologic activities:-

1- Its half life time is 23 days and is the longest of all immunoglobulins.

2- It is the major antibody in the secondary immune response.

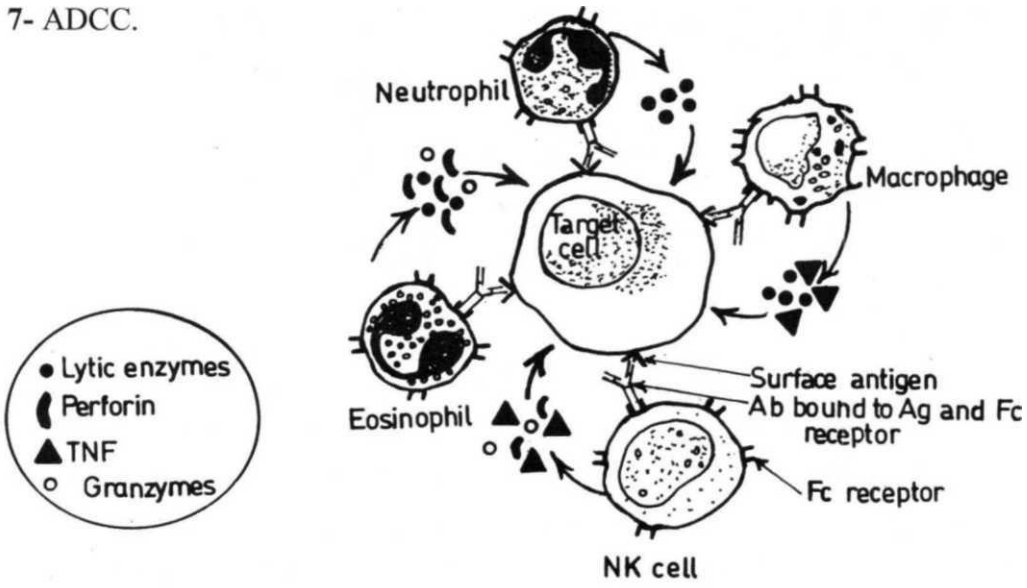
3- It is the only antibody that can cross the placenta and is excreted in milk and provides neonatal immunity during the first months after birth.

4-It diffuses into the extravascular spaces neutralizing bacterial toxins (antitoxin). It clumps bacteria and neutralizes viruses.

5- It enhances phagocytosis (opsonization) by coating bacteria and attaching by its Fc portion to Fc receptors on phagocytic cells. It holds the microbe close to the phagocytic membrane, facilitating its engulfment.

6- It can activate the complement.

7- ADCC.



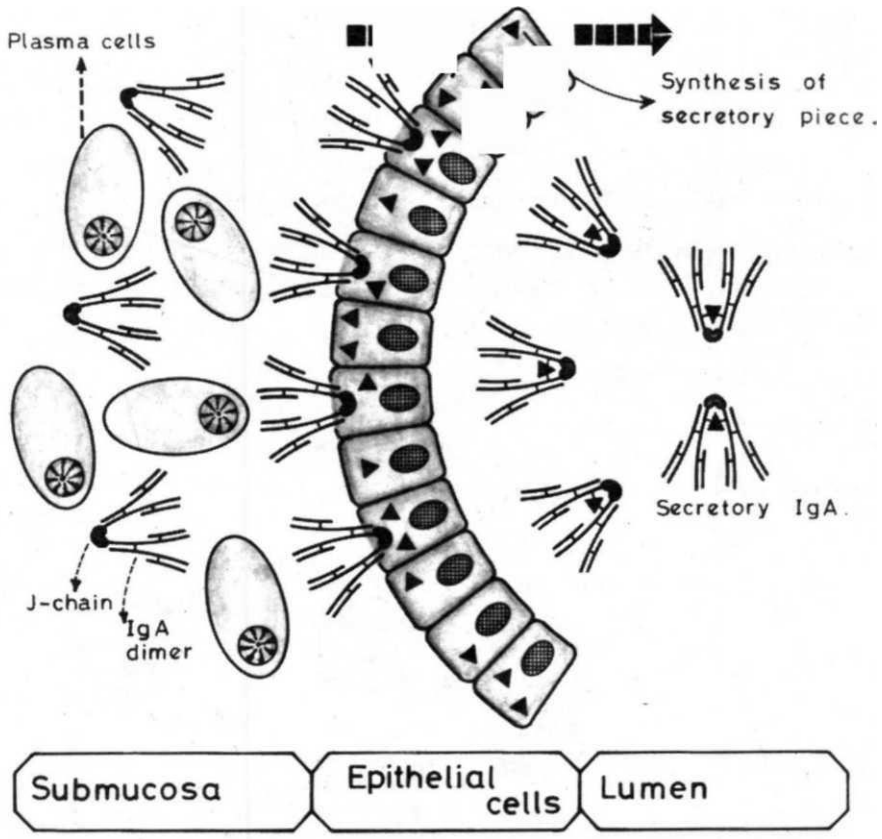
(Fig. 31): Antibody dependent cellular cytotoxicity (ADCC).

IgA

This class of immunoglobulin is found both in serum and in body secretions. Most IgA is found in secretions. **Serum IgA** is mainly monomeric and its biologic significance is uncertain.

Secretory IgA is found in mucous secretions of the respiratory, gastrointestinal and genitourinary tracts. It is also found in tears, sweat, saliva, colostrum and milk. It is dimeric (Fig. 32), consisting of 2 monomer units joined by a short polypeptide chain called **J** chain. It is synthesized locally by submucosal plasma cells. As it passes to the lumen of the organ in which it was produced, it acquires a **"secretory piece"** which is a polypeptide synthesized by epithelial cells. The latter facilitates transport of secretory **IgA** into secretions and protects it from being digested by proteolytic enzymes

There are two subclasses IgAi and IgA_2. IgA does not fix the complement. It does not cross the placenta.



(Fig. 32): Secretory IgA, and synthesis of secretory piece in the epithelial cells, i.e. mucosa.

The main function of secretory IgA is to provide local immunity at mucous surfaces; by coating microorganisms -viruses or bacteria- preventing their adherence to mucosal cells (neutralization), thus preventing their entry into body tissues. IgA in colostrum and milk protects the newly born.

IgAi can be inactivated by an IgA protease produced by gonococci, pneumococci and *H. influenzae;* therefore, IgA_2 is more important in mucosal immunity against such pathogens.

IgM

This is the largest immunoglobulin molecule (M.W. 900,000) consisting of five monomers (pentamer) joined together by a single **J** chain at the Fc region and stabilized by disulphide bonds. Properties and biologic activities:-

1- It is the antibody that appears during the primary immune response. Since it is short-lived, its presence indicates recent infection.

2- IgM molecule possesses 5-10 antigen binding sites hence these antibodies are extremely efficient agglutinating and complement activating agents.

3- The monomeric form serves as the antigen receptor on the B cell surface.

4- It does not cross the placenta hence a raised IgM level in a newborn indicates intrauterine infection e.g. syphilis, rubella, toxoplasma and cytomegalovirus. It is the first antibody that an infant makes.

5- IgM is the only antibody made to certain carbohydrate (TI) antigens such as the ABO blood group antigens on human erythrocytes. AB iso-agglutinins are of the IgM type, while Rh antibodies are of the IgG type.

IgD is monomeric and is present in very small amounts in serum. It is co-expressed with IgM on the surface of mature B cells. Like IgM it functions as an antigen receptor.

IgE is monomeric and is found in very low concentrations in normal serum. Elevated levels in serum may be detected in patients with type I hypersensitivity reactions (e.g. asthma or hay fever), where it plays a pathogenic role. It has the ability to bind to the Fc receptors on the surface of mast cells and basophils, and when IgE is cross-linked with the specific antigen, it triggers the release of inflammatory mediators (see chapter ***20).*** High levels are also detected during parasitic infestations. IgE provides immunity against parasites by binding to Fc receptors on eosinophils and triggering them to release toxic substances on the surface of the parasite. IgE does not activate the complement or cross the placenta.

Immunoglobulin (IG) Genes (Antibody diversity)

To produce the very large number of antibody molecules (10^6-10^9) that develop in the host in response to antigen stimulation without requiring excessive number of genes, special genetic mechanisms, e.g. DNA rearrangement and RNA splicing are used.

For each type of immunoglobulin chain, i.e. kappa light chain (κL) and lambda light chain ***(X L)*** and the 5 heavy chains (yH, aH, pH, eH and 8H), there is a separate pool of gene segments located on chromosomes 2, 22, and 14, respectively. Each pool contains a set of different V (variable) gene segments separated from C (constant) gene segments. During B cell differentiation, the DNA is rearranged to bring the selected gene segments adjacent to each other in the genome.

The V region of each L chain is encoded by two gene segments (V + J). The V region of each H chain is encoded by three gene segments (V+D+J). The segments are united into one functional V gene by DNA rearrangement. Each assembled V gene is then transcribed with the appropriate C-constant gene and spliced to produce an mRNA that codes for the complete peptide chain. L and H chains are synthesized separately on polysomes and finally assembled in the cytoplasm to form H_2L_2 units by means of disulfide bonds. The carbohydrate moiety is then added, and the IG molecule is released from the cell.

The gene rearrangement mechanism permits the assembly of an enormous variety of IG molecules with different specificity. **Antibody diversity** depends on:-

(1) Multiple gene segments. (2) Their rearrangement into different sequences (3) The combining of different L and H chains in the assembly of IG molecules. (4) Somatic hyper-mutation. (5) Junctional diversity, applies only to the heavy chain, which occurs by the addition or deletion of new nucleotides in the splice junction between the V-D and D-J gene segments.

Immunoglobulin Class or Isotype Switching:

During the immune response plasma cells switch from producing IgM to IgG or to other immunoglobulin classes (IgA or IgE). There is no alteration in the L chain or in the variable portion of the H chain thus there is no change in antigen binding specificity. The switch involves a change in the H chain constant domains (CH). This occurs through a process of DNA rearrangement and RNA splicing of the responsible gene resulting in the production of antibodies of the same antigenic specificity but different immunoglobulin class with different effector functions.

Class switching happens after antigenic stimulation and requires the interaction of CD40 on B cells with its ligand CD40L on T cell. The cytokines produced by T cells determine the isotype of the antibody produced e.g. IL-4 enhances IgE production, IL-5 in concert with TGF-6 stimulate and induce IgA production. Switching to IgG subclasses (IgGi, IgG_2, IgG_3 and IgG_4) is induced by different cytokines i.e. IFN-y, IL-4 and others.

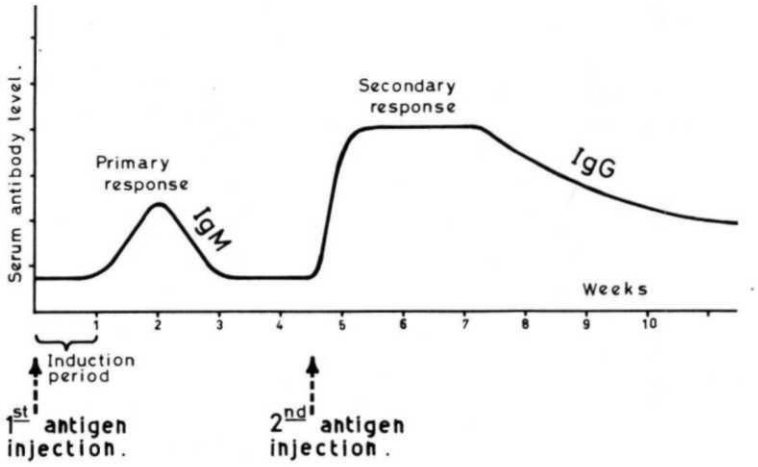
Primary and Secondary Antibody Response: (Fig. 33)

When an animal or a human being is exposed to foreign antigen for the first time, antibodies start to appear in the serum after 7-10 days. This is called the "induction period". The antibody concentration then rises to a peak within 1-2 weeks, then declines rapidly to undetectable levels. The antibody thus formed is of the IgM type.

On re-exposure to the same antigen (weeks, months or even years after primary exposure), there is a more rapid antibody response (induction period several hours) which reaches very high concentrations and high affinity*. The antibody formed is of the IgG type and it persists for long periods may be up to several years. If the antigen is administered *via* a mucosal route (oral or inhaled), IgA is usually the predominant antibody produced.

The long induction period in the primary immune response corresponds to the time during which antigen is being processed by APC and recognized by B and T lymphocytes. The very short induction period in the secondary response is due to presence of "memory cells" that are stimulated immediately to form antibodies. This "immunologic memory" is the cause for the long lasting immunity after several diseases **e.g.** diphtheria, mumps or measles. Most vaccines are given in two or more doses to stimulate higher levels of antibody with strong affinity (see table p. 141)

* During the initial antibody response, antibody affinity maturation occurs, so that memory B cells, which can make antibodies with stronger affinity for the antigen, are formed.



(Fig. 33): The primary and secondary antibody response.

Monoclonal Antibodies

These are highly specific antibodies produced against a single epitope by cells derived from a single clone of immortal cells.

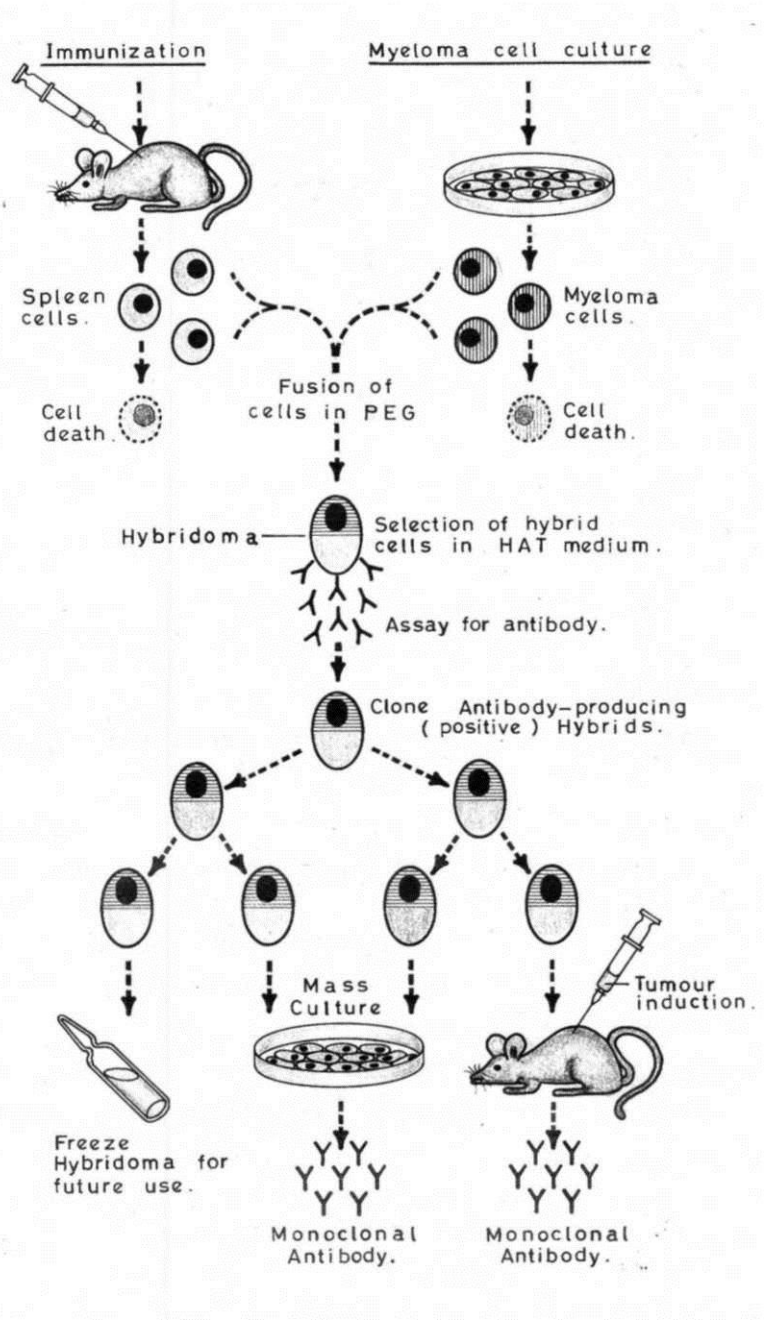
They are obtained by fusion of myeloma cell (malignant plasma cell) with a B cell producing antibody against a single epitope (derived from the spleen of mice immunized with this epitope) (Fig.34). The resulting cell called "hybridoma" acquires the property of being immortal from the myeloma cell and the single antibody specificity from the B cell. It can produce unlimited quantities of a highly specific monoclonal antibody.

Uses of Monoclonal Antibodies

The potential for their use is unlimited. Some of these applications are:

A- Diagnostic uses:

1- For identification of differentiation antigens on cells and microbes.
   a- Lymphocyte subsets determination (CD markers), b- Tissue typing (HLA typing), c- Identification and typing of viruses and bacteria, d- Detection of tumour related antigens.
2- Detection of CEA, alpha foeto-proteins and other tumour markers.
3- Hormonal assay.
4- Radiolabeled monoclonal antibodies are used *in vivo* to detect or locate tumour antigens, e.g. for detection of breast cancer metastases.



(Fig. 34): Production of monoclonal antibodies: Spleen cells from mice immunized with antigen, carrying the epitope of interest, are grown with myeloma cells in presence of PEG which promotes fusion of cells. HAT culture medium is used as it supports the growth of fused hybrid cells but not single parental cells which die out. Resulting hybridoma are screened for antibody production. Positive clones producing the antibody of interest are propagated in culture or in mice peritoneal cavity or freeze dried and stored at -70°C.

B- Therapeutic uses:

1- Anti-tumour therapy, by using monoclonal antibodies to tumour specific antigens alone or after coupling to cytotoxic agents, e.g. radioactive isotopes or TNF ("magic bullet therapy").

2- Anti-CD20 (a chimeric antibody) used in treatment of B cell lymphoma.

3- Omalizumab used for treatment of atopy, is a humanized chimeric monoclonal antibody that binds to IgE and prevents it from binding to mast cells.

4- Immunosuppressive therapy, e.g. the use of monoclonal antibodies to T cell, e.g. anti-CD3 to prevent graft rejection.

5- Anti-TNF (a chimeric antibody) used in treatment of rheumatoid arthritis.

6- In reversal of toxicity of drug, e.g. to neutralize digitalis toxicity and snake venom toxicity.

7- Anti-Rh D to prevent Rh-incompatibility.

8- Passive immunotherapy for protection against viral infections e.g. varicella zoster and cytomegalovirus (CMV) and in treatment of immunodeficiency diseases.

Mouse monoclonal antibodies used in humans can cause complications because of the host response to the foreign mouse antibodies. In an attempt to overcome this problem, "humanized" chimeric monoclonal antibodies are now-generated by molecular genetic techniques. Briefly, the gene segments for human constant regions are combined with the gene segments encoding the mouse monoclonal variable regions to create a chimeric antibody with mostly human sequences; this mmimizes the part of the antibody that could be recognized as foreign by humans.

Immunoglobulin Superfamily:

The immunoglobulin molecule (Ig) is a member of a very large family of proteins that share a similarity of structural features of immunoglobulin heavy and light chains which include the immunoglobulin-fold domains. Most of these have been found to be membrane-bound glycoproteins. Each molecule contains the characteristic Ig-fold structure (loops) formed as a result of intrachain disulfide bonds and consisting of approximately 110 amino acids. These Ig-fold domains are believed to facilitate interactions between membrane proteins i.e. cellular interactions (e.g. TCR on TH cells and MHC-II on APC) and most of them serve as **recognition molecules.**

Examples are: Ig, TCR, MHC-I and II, CD4, CD8, CD2, CD28 and CD3. Various cell adhesion molecules, including ICAM-1, ICAM-2, and LFA-3.and Ig-a /Ig-B heterodimer of the B cell receptor.

THE COMPLEMENT SYSTEM

The complement system is a complex enzymatic system composed approximately of 20 protein components synthesized in the liver. They are present in an inactive form in serum and tissue fluids of man and all warmblooded animals (not in CSF or urine).

The complement system plays a major role in host defense and in the inflammatory process which is an integral part of all immune responses. One of the major mechanisms for initiating inflammation is the activation of the complement cascade, which results in the production of powerful opsonins, chemo-attractants, and anaphylatoxins and can directly mediate cell killing through lysis.

Activation of Complement

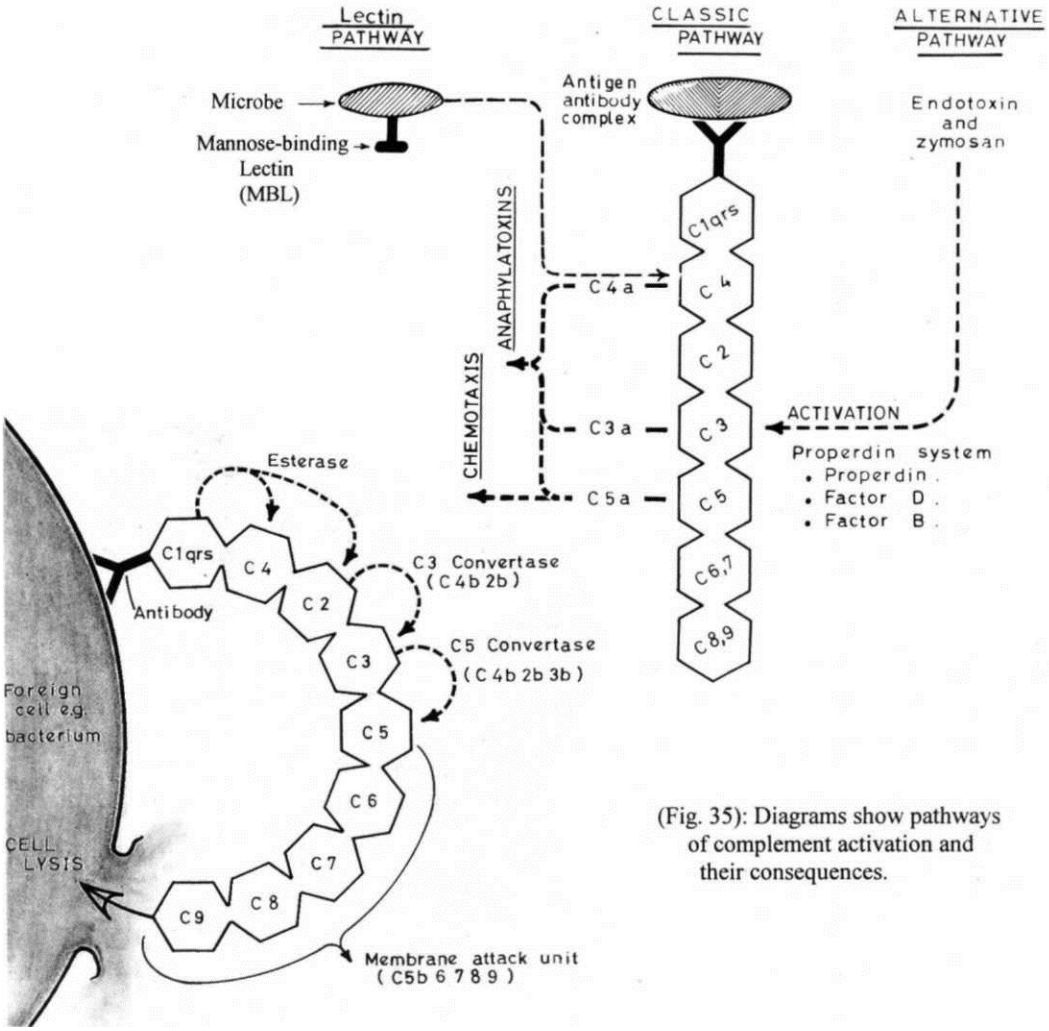
The complement is activated sequentially in a cascading manner. Each protein activates the protein that directly follows it in the sequence. The complement is activated through the classic pathway, the alternative pathway or the lectin pathway. The three pathways differ in how they are initiated, but they share the late steps and perform the same effector functions.

I- The Classic Pathway

Activation is initiated by antigen antibody complexes, only IgG and IgM activate the complement. The reaction starts by binding of the first complement component CI to Fc portion of the antibody molecule attached to the antigen (e.g. bacterial cell). CI* is activated and forms an esterase which activates C4 followed by C2 forming C3 convertase**, which activates and cleaves C3 to C3a and C3b. The latter reacts with the preceding complex to form C5 convertase** that activates and cleaves C5 to C5a and C5b. The latter binds to C6, C7, C8 and C9 to form the **"membrane attack complex"** (C5b6789) or MAC, which causes cell lysis.

Note that during activation, the complement components are cleaved into 2 fragments. The larger one designated **"b"** joins the preceding activated components to form a new enzyme. The smaller fragments designated **"a"** are released in the serum or body fluids and they frequently have important inflammatory properties. For example, C3a, C4a, C5a are anaphylatoxins. C5a is also chemotactic.

* Cl is composed of 3 proteins Clq,Clr,Cls. Clq binds to the Fc portion of IgG and IgM. ** C3 convertase = C4bC2b complex. C5 convertase = C4bC2bC3b complex.



(Fig. 35): Diagrams show pathways of complement activation and their consequences.

II- The Alternative Pathway

The alternative pathway is activated in the absence of antibody as a result of binding of C3b (a spontaneous short-lived break down product of C3 hydrolysis in serum) to the surfaces of microbes, i.e. bacterial endotoxins bacterial polysaccharides, and zymosan (yeast cell wall), with which it forms stable covalent bonds. Microbe-bound C3b becomes a substrate for the binding of factor B leading to formation of C3 convertase. This is followed by activation of the complement components in the same order as in the classic pathway to produce MAC. Factor D[1] and properdin participate in the process. The alternative pathway does not involve CI, C4, C2 or MBL2.

1 MBL= mannose binding lectins.
2 MBL= mannose binding lectins.

Differences between the classic and alternative pathways:

1- The classic pathway is specific acquired immunity. The alternative pathway provides non-specific innate immunity in absence of antibody.
2- The classic pathway is primarily initiated by antibody, usually bound to antigen, whereas the alternative pathway is initiated primarily by microbial cell surface components binding to C3b.
3- The classic pathway requires the interaction of all components, whereas the alternative pathway does not require CI, C4 and C2.
4- The alternative pathway involves the properdin system.

Ill- The Lectin Pathway :

Initiation occurs when a plasma protein, mannose-binding lectin (MBL), binds to terminal mannose residues on the surface glycoproteins or glycolipids of microbes. Then a MBL associated protease activates C4 and C2 and proceeds as in the classic pathway, i.e. the subsequent steps are essentially the same. It is initiated in the absence of antibody i.e. it is a component of innate immunity.

Therefore, some of the complement mediated defense mechanisms are nonspecific, and can take place before the appearance of specific antibody through the alternative or lectin pathways (see table p. 142).

Regulation of the Complement System:

Complement activation is associated with potent biological functions that, if left unchecked, would exhaust the complement system and cause significant damage to the host. Regulation is achieved by:

1- The natural instability and short active life of some of the activated components.
2- Serum inhibitors, e.g. CI inhibitor, C3b inactivator, C6 inactivator and anaphylatoxin inactivator.

Deficiency of these inhibitors can result in certain diseases e.g. congenital absence of CI inhibitor results in familial hereditary angioneurotic oedema.

Biological Activities of the Complement: (Fig. 36)

1- **Cytolysis:** This occurs when the antigen is on the cell wall of bacteria or tissue cell e.g. RBCs or tumour cells. Insertion of the "membrane attack complex (C5b6789) into the cell membrane; results in its disruption and the entry of water and electrolytes into the cell leading to its lysis.

Several cells have receptors for various complement fragments e.g. RBCs, phagocytes, mast cells, basophils and B cells which participate in the following activities:

2- **Opsonization:** Cells, antigen-antibody complexes and other particles are phagocytosed much better when coated with C3b. This is due to the presence of C3b receptors on the surface of phagocytes (see p. 48).

3- Inflammatory reaction: Small fragments released during the course of complement activation have several inflammatory actions:

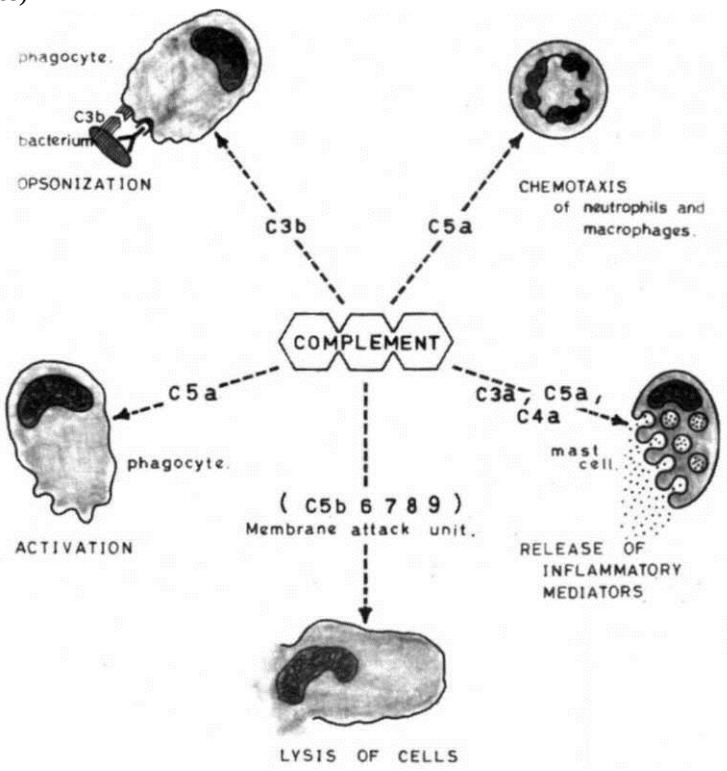
a- C5a is **chemotactic,** it attracts phagocytes to the site of inflammation and **activates** them increasing their bactericidal or cytocidal actions.

b- **C3a, C4a** and **C5a** are **anaphylatoxins** i.e. cause degranulation of mast cells and release of histamine and other inflammatory mediators, leading to increased vascular permeabilityand promoting the inflammatory reaction.

4- Immune complex clearance: Immune complexes with C3b on their surfaces become bound to C3b receptors (CR1) on erythrocytes and are carried through the circulation to the liver and spleen to be taken into phagocytes and destroyed. C3 deficiency is associated with immune-complex disease and susceptibility to recurrent bacterial infections.

5- Enhancement of antibody production: Binding of C3d (a break down product of C3b) to CR2 receptors on activated B cells greatly enhances antibody production. Patients deficient in C3 produce much less antibody than normal individuals and are more susceptible to pyogenic infections.

The above mentioned activities, though they are protective, yet they can cause unwanted damage as they may contribute to hypersensitivity and autoimmune disorders. (See chapter 23 for complement deficiencies)



(Fig. 36): Biological activities of the complement.

CHAPTER 16

ANTIGEN ANTIBODY REACTIONS

Reactions of antigens and antibodies are highly specific in the sense that an antibody can react only with the antigen which induced its formation. These interactions are widely used *in vitro* for diagnostic purposes i.e. for the detection and identification of either antigen or antibody and are termed **serologic reactions.** In these tests one of the reactants should be known while the other is unknown.

The interactions of antigen with antibody may result in a variety of consequences, including agglutination if the antigen is particulate, precipitation if the antigen is soluble, or activation and fixation of complement. The extent of the reaction depends on the proportions of the interacting antigen and antibody. At high antibody levels, i.e. antibody excess, the reaction may not occur. This is referred to as a "prozone" effect.

AGGLUTINATION

The antigen in the agglutination reaction is in the form of particles, e.g. suspensions of microorganisms, cells (e.g. red blood cells), or latex particles coated with antigens. When mixed with the specific antiserum, these particles become clumped, i.e. agglutinated.

If one of the reactants (antigen or antibody) is known, the reaction can be used for the identification of the other. So known antisera are used to identify microorganisms isolated from clinical specimens. On the other hand, known organisms can be used to detect antibodies in sera of patients. There are several types of the agglutination reaction:-

1. Direct Agglutination; can be done in small tubes or on a slide.

The slide method is rapid and useful in identification and typing of isolated organisms by mixing a drop of the bacterial suspension with known antisera. Clumping occurs if the serum is specific to the organism under test. The slide method is also used in blood grouping.

The tube method is a quantitative test, which is used to determine the amount of antibodies in the serum of patients. It is done by mixing serial dilutions of the patient's serum (1/10, 1/20, 1/40, 1/80, etc.) with a constant amount of a known bacterial suspension, suspected to be the cause of the disease. The last tube showing visible agglutination will reflect the serum antibody titer of the patient.

The classical application of the tube method is the Widal test used to detect antibodies to salmonella in the serum of patients suspected to have enteric fever. The agglutination tests are also used in diagnosis of brucellosis, and other diseases.

In diagnosis of such infectious diseases, two serum samples separated by 7-10 days interval should be tested. A rising antibody titer of two folds or more is diagnostic.

2. **Antiglobulin Agglutination test = Coomb's test:**

This test is used to deterrriine the presence of Rh incompatibility which causes ***"erythroblastosis foetalis".***

Most anti-Rh antibodies are incomplete IgG (unagglutinable) antibodies which can only coat the Rh positive RBCs, but can not bridge between two RBCs to cause agglutination. These antibodies can be detected by the **antiglobulin Coomb's test** which is performed in two ways:

Indirect Coomb's test: The mother's serum -containing incomplete anti-Rh antibodies- is mixed with Rh-positive RBCs (group O). After incubation, the mixture is centrifuged. The deposit containing red cells coated with incomplete antibodies is washed, and antihuman globulin is added, and the tubes incubated. The antihuman globulin causes agglutination by linking together the incomplete antibody molecules.

Direct Coomb's test: This test can detect incomplete Rh antibodies coating the RBCs of the newborn in *erythroblastosis foetalis.* The antihuman globulin is added directly to a washed suspension of the newborn RBCs, agglutination occurs.

Both the direct and indirect Coomb's tests are also used to detect incomplete antibodies in autoimmune haemolytic anaemias.

3. **Latex or Passive Agglutination:**

It is an agglutination reaction in which inert particles, e.g. latex or RBCs are coated with various antigens or antibodies. These particles are aggregated in the presence of specific antibody or antigen, respectively. Examples of passive agglutination include the following:-

Immunologic pregnancy test: It depends on the appearance of human chorionic gonadotrophic hormones (HCG) in the urine of pregnant females. The test is done by adding a drop of latex particles coated with anti-HCG to a drop of urine; agglutination occurs if HCG is present in urine.

Similarly **Rheumatoid factor, C-reactive protein (CRP)** and **anti-streptolysin 'O' (ASO)** tests, used for diagnosis of acute rheumatic fever, can be done by the passive agglutination reaction.

4. **Haemagglutination Inhibition** assays are used to determine whether an individual has been exposed to certain types of viruses that cause agglutination of red blood cells. The patient serum containing specific antiviral antibodies is mixed with a known virus, then RBCs are added, the antibodies will bind to the virus and interfere with haemagglutination of RBCs by the virus i.e. **virus haemagglutination inhibition.**

PRECIPITATION

This is an antigen antibody reaction in which the antigen is soluble. Precipitation reactions can be done in solution or in agar gel.

A- **Precipitation in solution:** This reaction can be made quantitative; i.e. antigen or antibody can be measured in terms of micrograms of nitrogen present. It is used primarily in research.

B- Precipitation in agar: In this technique, diffusion of antigen and antibody is allowed to occur in agar gel. It can also be done in presence of an electric field

1- **Double diffusion (Ouchterlony):** The antigen and antibody are placed in different wells punched in the agar. Both will diffuse in the agar and where they meet at optimal proportions, precipitation bands will appear. This method can show whether antigens are identical, related but not identical, or not related.

A clinical application for this method is the **Elek's test** which is used to prove the toxigenicity of diphtheria bacilli:

A strip of filter paper, saturated with diphtheria antitoxin, is embedded in serum agar. A heavy inoculum of the diphtheria bacillus to be tested is inoculated at right angle to the strip of filter paper. Plates are examined after 2 days incubation at 37°C. If the organism is toxigenic, the toxin will diffuse sideways from the inoculum and the antitoxin diffuses from the filter paper, and where they meet at optimal concentrations, a precipitate is formed which will appear as fine white lines.

2- Single diffusion: The antibody is incorporated in the agar before pouring it in the plates. The antigen is placed in a well punched in this agar. The antigen diffuses in all directions, and where its concentration is optimal in relation to the antibody, a precipitation ring will form around the well. The diameter of the ring depends on the antigen concentration. This technique is used to estimate the quantity of the various immunoglobulin classes, complement components and other substances in human serum.

3- **Precipitation in agar with an electric field:**

Immunoelectrophoresis: A serum sample is placed in a well in agar fixed on a slide. A current is passed through the agar, and the proteins move in the electric field according to their charge and size. Then a trough is cut into the agar and filled with antibody. As the antigen and antibody diffuse toward each other, they form a series of precipitation arcs. This permits the serum proteins to be characterized in terms of presence, absence, or unusual pattern (e.g. human multiple myeloma proteins).

Western Blots (immunoblots): In this test, an antigen (or a mixture of antigens e.g. HIV proteins) is first separated electrophoretically on an SDS-polyacrylamide gel. The separated material is then "blotted" (transferred) onto a nitrocellulose sheet to which the antigen bands bind strongly. The test serum sample, containing the unknown antibody, is added onto the separated bands and any existing antibody will bind to its specific antigen.

A second antibody (anti-human Ig), which is radiolabeled or enzyme labelled, is added to localize the first antibody that attached to the antigen. The result is read by autoradiography or ELISA, respectively. This test is used to confirm the diagnosis of HIV infection.

This procedure may also be used to identify a specific antigen in a mixture.

COMPLEMENT FIXATION (CF)

This is an antigen antibody reaction that occurs in the presence of a third component known as the complement. The antigen unites with its specific antibody and the resulting complex fixes the complement.

The CF test is used for diagnosis of many diseases by detecting complement fixing antibodies in the serum of patients, as in syphilis, whooping cough, chronic gonorrhoea, typhus, small pox and other diseases.

N.B. This test is infrequently used nowadays and is replaced by more sensitive and faster tests.

IMMUNOFLUORESCENCE

These are antigen antibody reactions in which we use fluorescein labelled antibodies. Fluorescein is a dye which emits greenish fluorescence under ultraviolet light (UV), and it can be tagged to immunoglobulin molecules. There are two ways for doing the test:

1- Direct Immunofluorescence:

In this test, fluorescein labelled antibodies are layered over the antigen fixed on a slide. They are left to react for some time then the excess unattached antibody is washed thoroughly. The slide is then examined by the fluorescent microscope under ultraviolet light. The site where the antibody adheres to its specific antigen will be seen as apple green fluorescence. This method can be used to detect bacteria, viruses and antigens in tissues or in pathological samples.

2- **Indirect Immunofluorescence:**

The test is used to detect antibodies in serum of patients. The serum is layered on the antigen preparation which is fixed on a slide; they are allowed to react for some time, then the excess is washed. Whether there is antibody in the serum, that adhered to the antigen, or not is determined by adding a fluorescein labelled anti-globulin which will attach to the antibody if present in patient's serum and give positive fluorescence under UV light.

This method is used in the serologic diagnosis of syphilis (caused by *Treponema pallidum)* to detect anti-treponemal antibodies. This is known as **Fluorescent treponemal antibody (FTA)** test.

ENZYME-LINKED IMMUNO SORBENT ASSAY (ELISA)

This technique is very sensitive and does not require specialized equipment and avoids the hazards of radioactivity.

The method depends on conjugation of an enzyme to either antigen or antibody, then the enzyme activity on a substrate is used as a quantitative measure.

To measure antibody, the **indirect method** is used. **A** known antigen is fixed to a solid phase (e.g. plastic cup or microplate), incubated with the test serum, then washed to remove excess unattached antibody and re-incubated with antiglobulin

labelled with a suitable enzyme (e.g. horseradish peroxidase). The labelled antiglobulin will attach to the antibody bound to the fixed antigen. After washing, the enzyme activity is measured by adding a specific substrate and measuring the degree of colour change.

To measure an antigen, the **double antibody technique** is used. A known antibody is fixed to the solid phase. The test material containing antigen is added and the excess washed. A specific known antibody labelled with enzyme is added. After washing, a substrate is added and the enzyme activity measured colourimetrically and related to antigen concentration.

RADIOIMMUNO ASSAY (RIA)

This is a sensitive method used to measure antigens or antibodies that can be radioactively labelled. There are many variations of the test. However, the solid phase RIA is a popular method. The principle of the assay method is that radio-iodine (^{125}I) labelled antigen (e.g. thyroid hormones T3 or T4, hepatitis B antigen) competes with a non-labelled antigen in a test sample, for a fixed amount of a specific antibody in a limited time.

The test is done by adding the serum sample (test antigen) to antibody adsorbed to solid phase (tubes or beads), then the labelled antigen is added and incubated for a limited time. After decanting the supernatant, the remaining bound radioactivity is measured in a gamma counter and the percentage bound labelled antigen is calculated. The concentration of test antigen is deduced from a standard curve drawn between concentrations of known standards (run at the same time with the test) and the percentage bound radiolabeled antigen.

N.B. The shelf life time of the reagents used in RIA is 2 weeks to 2 months as compared to that of the reagents used in the ELISA which is 6-12 months. In addition, the RIA reagents are hazardous while ELISA reagents are not.

FLOW CYTOMETRY

Flow cytometers are instruments capable of analyzing properties of single cells, in a fluid sheath, as they pass through an orifice at high velocity (50,000 cells/minute). They can sort out and count cells with certain characteristics from the general population. Properties measured include physical characters such as size, volume, refractive index, viscosity and chemical features such as content of DNA or RNA, proteins and enzymes.

These properties are detected by measuring light scatter, cell volume and fluorescence after staining the cells with fluorescein labelled monoclonal antibodies to any specific cell surface markers.

*For more illustrative details on this subject see chapter 13, in the "**Manual of Practical Microbiology** " by the same author.*

CHAPTER 17

PROTECTIVE IMMUNITY TO MICROBES

Defense against microbes is mediated by the effector mechanisms of innate and adaptive immunity through both humoral (antibodies) and cell mediated immune (CMI) mechanisms.

ANTI-BACTERIAL IMMUNITY Immunity

to Extracellular Bacteria:

The **innate** immune mechanisms to these organisms are mainly through complement activation, phagocytosis and the inflammatory response.

The **acquired** immune responses to extracellular bacteria are mediated mainly by the humoral mechanisms.

Antibodies induce immunity through the following mechanisms:

1- Neutralization of bacterial toxins produced by some bacteria, e.g. diphtheria and tetanus.

2- Antibodies can attach to the surface of bacteria and:

a- Act as opsonins and enhance phagocytosis (opsonization).

b-Prevent the adherence of microorganisms to their target cells e.g. by IgA on mucosal surfaces, **c-** Activate the complement leading to bacterial lysis, **d-** Agglutinate bacteria, preventing their spread and enhance phagocytosis.

CMI plays a less important role. Microbes are internalized by APCs and presented to TH cells. These are activated and release cytokines which: a- Activate phagocytes increasing their microbicidal functions, b- Stimulate antibody production, c- Induce local mflammation.

Immunity to Intracellular Bacteria:

Innate immunity to intracellular bacterial infections, e.g. tuberculosis, leprosy, and listeriosis, is mainly by NK cells. They kill infected cells and secrete IFN-y which activates phagocytes to kill intracellular organisms.

Acquired immunity to these organisms is mainly CMI, through activation of macrophages to kill the organisms they harbour and lysis of infected cells by cytotoxic T cells (CTLs). However, most of these organisms are resistant to phagocytosis, cause chronic infections and granuloma formation.

ANTI-VIRAL IMMUNITY Innate irnmunity to viruses is mediated by type I IFN and NK cells. **Acquired** immunity against viral infections is mediated by humoral and CMI and by IFN type I and II.

A- Humoral immunity:

1- Virus neutralization is the most important mechanism:

Antibodies can neutralize virus infectivity by preventing its attachment to receptor sites on susceptible cells. **IgG** and **IgM** neutralize viruses which pass through the blood stream -causing viraemia- before they reach the target organ as in poliovirus infections, mumps, measles and rubella. In these diseases, one attack is liable to give long lasting immunity. Artificial active immunization by respective vaccines is very effective in their prevention.

The situation is completely different with viruses that cause superficial non-viraemic infections e.g. influenza and common cold where the target tissue is the respiratory mucosa at the portal of entry. Secretory **IgA** neutralizes virus infectivity at the mucous surfaces. Parenteral vaccines are not effective in prevention of superficial viral infections. Such infections are not followed by long lasting immunity and one may get several attacks during the same season due to:

a- The weak nature of the immune mechanisms involved.

b- The presence of several antigenic types.

c- The emergence of antigenic variants as in influenza virus.

2- Antibodies may destroy free virus particles directly through:

a- Aggregation of virus and opsonization.

b-Complement mediated lysis which is mainly effective on enveloped viruses.

Both mechanisms can also act on virus infected cells (Fig. 37).

B- Cell mediated immunity:

Protection against virus infection virtually always requires the induction of cell mediated cytotoxicity, mediated by cytotoxic T cells (CTLs), NK cells, or activated macrophages. CMI acts on virus infected cells through the following mechanisms:

1- CTLs kill virus infected cells directly after recognition of viral antigens on the cell surface in association with MHC-I.

2- Helper T cells stimulated by viral antigens release cytokines, which attract and activate macrophages to kill virus infected cells.

3- NK cells destroy virus infected cells early in infection before appearance of antibodies. This activity is enhanced by IFN-y and IL-2.

4- ADCC: Antibody binds to virus infected cells, such cells are then lysed by NK cells, macrophages or polymorphs.

C- Interferons (IFNs) actions in virus infections: (see cytokines)

1- Anti-viral activity by **type 1 IFN:** Virus infected cells produce type I IFNs which inhibit intracellular replication of viruses in neighbouring cells by inducing proteins that prevent the translation of viral mRNA and block viral protein synthesis. IFNs have no direct effect on extracellular virus.

They are produced soon (less than 48 hrs) after viral infection in contrast to antibodies which appear several days after infection. Hence IFNs may act in the early phase of viral diseases before appearance of antibody and limit the spread of virus.

Interferon activity is not specific, i.e. an interferon induced by one virus can inhibit replication of any other virus. However, they are species specific, i.e. an interferon produced in mice will only be active in mice.

2- Both type I and II IFN increase expression of MHC-I leading to better presentation, recognition and killing of virus infected cells by CTLs.

3- Both types activate NK cells and macrophages to kill virus infected cells.

Therapeutic Uses of Interferons: Type I IFN inhibit cell proliferation and tumour growth i.e. **anti-proliferative action.** For this action and for the activating action of both types on macrophages and NK cells they are used in cancer therapy. Large quantities of purified recombinant preparations of IFNs are now available and are used in therapy.

Trials to use IFN-a in treatment of viral diseases gave promising results with some viruses e.g. papilloma virus, chronic hepatitis due to hepatitis B or C viruses and for topical application in herpetic kerato-conjunctivitis.

Trials to treat cancer patients with IFN-a showed marked recession and sometimes disappearance of some tumours, e.g. non-Hodgkin lymphoma, cutaneous T cell lymphoma, hairy cell leukemia, Kaposi sarcoma, chronic myeloid leukemia and melanoma. IFN-J3 in multiple sclerosis caused clinical improvement. IFN-y in chronic granulomatous diseases reduced the incidence of infections. However, IFNs have toxic side effects on the gastrointestinal tract, the C.N.S., and may cause bone marrow suppression.

ANTI- FUNGAL IMMUNITY

Protective responses to fungi consist mainly of innate immunity, mediated by neutrophils and macrophages. Neutropenic patients are very susceptible to fungal infections. CMI is the main acquired protective mechanism to fungi. It acts in a manner similar to its action against intracellular bacteria. Fungi are usually readily eliminated by phagocytes and a competent immune system, that is why disseminated fungal infections are seen mostly in immunodeficient persons.

CHAPTER 18

TUMOUR IMMUNOLOGY

Transformation of normal cells to malignant cells can occur by spontaneous mutation that occurs daily during division of body cells. It may be induced by chemical or physical carcinogens or by viruses. Such cells become antigenically different from normal cells, hence they are recognized and destroyed by the immune system (particularly NK cells, macrophages and CTLs) before they can develop into clinically detectable tumours. This is called **"immune surveillance"** mechanisms. However, most tumours are weakly immunogenic and factors that lower the immune capabilities predispose the individual to malignancy.

Tumour antigens:

On changing to malignant cells, there is alteration of the antigenic profile of the cells. Normally occurring antigens may be lost while new epitopes emerge. The modern classification of tumour antigens relies on the molecular structure and source of the antigen. The main classes of tumour antigens are:

1- Products of mutated oncogenes and tumour suppressor genes:

These are antigens encoded for by genes that are expressed in normal cells but are over-expressed in tumour cells due to gene mutation, deletion, translocation or amplification.

A number of tumours have been shown to express antigens encoded by cellular oncogenes, e.g. human breast-cancer cells exhibit elevated expression of the oncogene-encoded Her-2/Neu protein, a growth factor receptor, whereas normal adult cells express only trace amounts of that protein.

P53 is a tumour suppressor gene. Mutations of p53 gene are the most commonly seen in tumours of humans and experimental animals. The protein products of mutation were found to stimulate an immune response by CTLs.

2- Products of mutated self genes, also called **tumour specific transplantation antigens (TSTAs):**

These are mutants of various host genes and are mainly due to chemical carcinogens and radiation. The resulting antigens are extremely diverse because the carcinogens can randomly mutagenize any host cell.

Such cancers are often accompanied by the emergence of completely unique tumour associated antigens. Even when the same chemical carcinogen induces two separate tumors at different sites in the same animal, the antigens are distinct and the immune response to one tumour does not protect against the other.

3- **Over-expressed and abnormally expressed cellular proteins:**

Tumour antigens may be normal cellular proteins that are abnormally expressed in tumour cells and elicit immune response e.g. melanoma antigen genes (MAGE). These antigens are encoded for by families of genes that are normally silent in all normal tissues except human testis. They include the MAGE, BAGE or GAGE families of genes, which code for proteins found on the surface of a large number of tumours, including melanomas, bladder, breast, skin and prostatic cancer. They are now classified in the cancer-testis (CT) antigen family.

4- **Tumor antigens encoded by genomes of oncogenic viruses:**

Cells infected by oncogenic viruses usually display new antigens on their surface. These are either viral proteins or new enzymes induced in the cell to aid in replication of the virus. Burkitt's lymphoma cells have been shown to express a nuclear antigen of the Epstein-Barr virus (EBNA). Human papilloma virus E6 and E7 proteins are found in more than 80% of invasive cervical cancers.

5- **Oncofoetal antigens:**

These antigens are present during normal foetal development but are lost during adult life. However, these antigens may reappear in cancer cells. Examples are alpha foeto-proteins (AFP) in hepatic carcinoma and carcino-embryonic antigen (CEA) in cancer of the intestine. The serum levels are increased in these patients. The level of CEA is used to monitor the persistence or recurrence of the tumour after treatment.

6- **Tissue-specific differentiation antigens:** A tumour arising from a particular tissue may express normal differentiation antigens specific for that tissue, e.g. prostatic tumours may carry prostatic specific antigens (PSA) which are also released into the serum and can be measured as a screening test for prostatic cancer.

Immune Response to Tumour Antigens: (Fig. 37)

Both specific and non-specific immune responses -humoral and cell mediated- are involved in destruction of tumour cells:

1- T cells play the major role in defense against tumour growth.

a- CTLs killing of tumour cells is the principal mechanism of tumour immunity. CTLs recognize tumour antigens in association with MHC-I on the surface of tumour cells and kill them.

b- TH activated by tumour antigens release cytokines e.g. IL-2, IFN-y and TNF. These help the differentiation of CTLs and increase the expression of

MHC-I on tumour cells, to be more sensitive to lysis by CTLs. They also activate NK cells and macrophages.

2- Macrophages when activated can kill tumour cells by two mechanisms:
a- Cell contact cytotoxicity through the release of hydrogen peroxide, proteolytic enzymes and TNF which is cytotoxic to certain tumours, b- Antibody dependent cellular cytotoxicity (ADCC).

3- NK cells can cause spontaneous lysis of a variety of tumours. IFN-y activated NK cells show more efficient lysis of tumour cells. Interleukin activated NK cells have a wider range of action and are called lymphokine activated killer (LAK) cells.
- NK cells can also kill tumour cells through ADCC.
- Class I MHC molecules deliver inhibitory signals to NK cells. The loss of class I MHC molecules from the surface of tumour cells makes them particularly good targets for NK cells (Fig. 41 p. 137).

4- Humoral mechanisms may participate, though to a minor degree, in tumour cell destruction. Anti-tumour antibodies bind to tumour cells and lead to:
a- Tumour cell killing by NK cells, macrophages or polymorphs i.e. antibody dependent cellular cytotoxicity (ADCC). b- Complement activation and cell lysis, c- Opsonization of tumour cells by macrophages.

Evasion of immune response by tumours:

There are many mechanisms by which tumours escape immune destruction leading to enhancement of growth and spread of cancer. Some of these mechanisms are:

1- Reduced levels or absence of MHC-I molecules on tumour cells, so that they cannot be recognized by CTLs.

2- Some tumours stop expressing the antigens that are the targets of immune attack. These tumours are called "antigen loss variants". This is common in rapidly growing tumours.

3- Tumour cells have an inherent defect in antigen processing and presentation due to lack of co-stimulatory signals e.g. B7. They also lack molecules for T cell activation especially MHC-II and adhesion molecules

4- Certain tumours produce immunosuppressive factors e.g. transforming growth factor (TGF-**P**). Fas ligand expressed on some tumours may induce apoptotic death of lymphocytes.

5- Tumour antigens may induce specific immunologic tolerance.

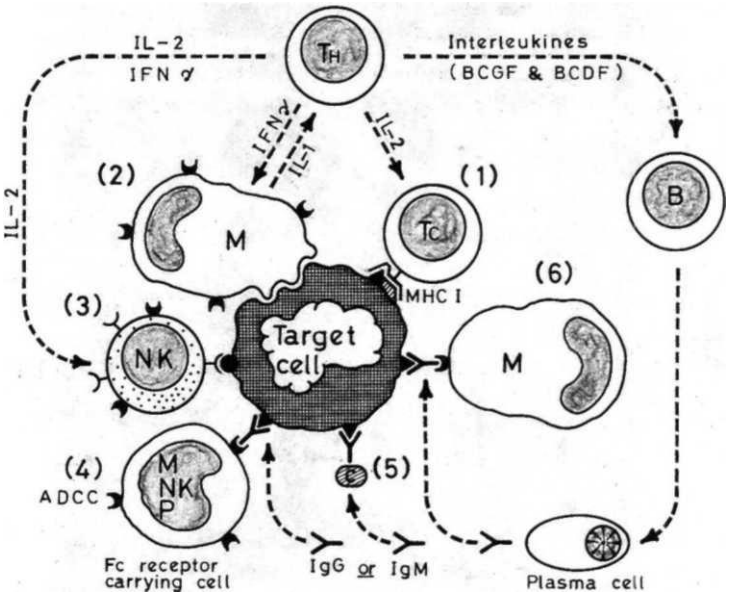
6- The antigens on the surface of tumours may be masked by sialic acid-containing mucopolysaccharides.

7- Blocking antibodies: These are non-complement fixing anti-tumour antibodies that bind to antigen sites on the surface of tumour cells, and render them inaccessible to cell mediated destruction.

8- Soluble antigens shed by the tumour acting as free antigens or forming immune complexes may mask the tumour antigen recognition sites of T cells, thus preventing T cells from recognizing and attacking the tumour cells. The complexes also may inhibit ADCC by binding to Fc receptors on NK cells or macrophages and blocking their activity.

9- Regulatory T cells (Treg) may suppress T cell responses to tumours. The number of Treg cells is increased in cancer patients.

10- Immune suppression of the host as in transplant patients who show a higher incidence of malignancy.



(Fig. 37): Mechanisms of target cell destruction, e.g. tumour cells, graft cells or virus infected cells.

P = polymorphs. M = macrophages. NK = natural killer. C = complement.

1- Direct killing by cytotoxic T cells.
2- Activated macrophages by IFN- y are cytotoxic.
3- Killing by NK cells; enhanced by IL-2 & IFN.
4- ADCC by M, P & NK cells.
5- Complement mediated cell lysis of antibody coated cells. 6-
Opsonization of antibody coated cells by macrophages.

Approaches to Cancer Immunotherapy

Reduction of the tumour mass by surgery, irradiation or chemotherapy is very essential before immunotherapy.

1- Specific Active Immunotherapy

Many **vaccines** are under clinical trials. The following are examples;

a- Vaccination with killed tumour cells or cell lysates plus adjuvants. In some leukemias, the patient is immunized with his own inactivated tumour cells together with an adjuvant such as BCG vaccine.

b- Viral vaccines against oncogenic viruses will reduce the incidence of these tumours. HBV vaccination will preventoccurrenceofhepatocellular carcinoma. HPVvaccine will prevent the occurrence of cervical carcinoma

c- **Dendritic cell vaccines** are made by growing dendritic cells (APC) derived from cancer patient's blood, and incubated with cells or antigens from the tumour. These antigen-pulsed APCs are used for vaccination. Dendritic cells may be transfected with tumour antigen genes and re-infused in the patient.

d- Immunization by injection of naked DNA plasmid constructs (DNA vaccines) with the goal of having the unique tumour antigen encoded and expressed by muscle cells.

e-**Cytokine and costimulatory genes enhanced vaccines:-**

-Tumour cells are removed and transfected with cytokine genes. When the cells are re-infused, they secrete cytokines e.g. IL-2 or IFN-y: these will enhance T cell activation. Once T cells respond to the transfected cells and become memory cells, they will acquire the ability to kill non-transfected tumour cells.

-Tumour cells can also be transfected with costimulatory genes e.g. B7 needed for T cell activation.

- APCs can be trasfected with cytokine genes and pulsed with tumour antigens.

f- Viral vector vaccines e.g. adenovirus or vaccinia virus encoding rumour antigens and /or cytokines.

2- **Specific Passive Humoral Immunotherapy (immunotoxins)**

The use of monoclonal antibodies directed against tumour antigens, and linked to cytotoxic drugs, toxins, radioactive isotopes or TNF i.e. **"magic bullet therapy".** These carry tumour destroying agents specifically and directly to tumour cells with minimal effects on normal cells.

Humanized anti-CD20 monoclonals labelled with radioiodine can cure or reduce 50% of B cell lymphoma.

Humanized monoclonal antibody specific for the oncogene product Her2/ Neu, which is expressed at high levels in some tumours, is now approved for use in breast cancer patients.

3- Non-Specific Immunostimulation

a-The use of certain bacterial products, e.g. BCG vaccine which stimulates CMI non-specifically, activating macrophages and NK cells. BCG is used

for treatment of superficial bladder cancer, by local injection after removing the tumour mass.

b-Cytokine therapy: Systemic administration of IL-2, IFN and other cytokines, singly or in combination is under trial. These have several immunostimulatory effects. However, they have toxic side effects.

4- **Adoptive Cellular Immunotherapy**

Re-infusion of patients' own peripheral lymphocytes after their activation *in vitro* by IL-2, "LAK cell therapy". Encouraging results were observed specially when IL-2 was simultaneously given to patients. However, this approach is effective in a minority of tumours and has considerable side effects.

A variation of this approach is the use of tumour infiltrating lymphocytes (TILs). These lymphocytes-removed from biopsied tumour, expanded in *vitro* with IL-2 and re-infiised into the tumour bearing individual, have an anti-tumour activity higher than LAK cells.

Another approach is to extract TILs from the tumour and then to stimulate them in the presence of tumour antigens, (e.g. tyrosinase in the case of melanoma) and stimulatory cytokines, e.g. IL-2. When the TILs have increased in number and become effective killers, they can be re-infused.

Tumour markers

These are enzymes or tumour products secreted in the blood and are used to confirm the diagnosis of certain tumours and to monitor the response to therapy. The following are examples:

1- Carcinoembryonic antigen (CEA) in gastrointestinal tumours.

2- Alpha-foeto proteins (AFP) in hepatoma.

3- **P**-subunit of human chorionic gonadotrophin (**P**-HCG) in cases of choriocarcinoma.

4- Prostatic specific antigen (PSA) and prostatic acid phosphatase (PAP) in prostatic tumours.

5- CA 125 in ovarian carcinoma.

6- CA 19-9 in colon and pancreatic tumours.

7- CA 15-3 in breast cancer.

8- Pancarcinoma antigen (TAG-72) is used to localize occult tumour secondaries.

9- Bence-Jones proteins in urine for diagnosis of multiple myeloma or plasma cell myeloma.

CHAPTER 19

IMMUNOPROPHYLAXIS

Protection against infectious diseases by immunoprophylaxis (immunization) represents one of the greatest accomplishments of biomedical science. One disease, smallpox, has been completely eradicated by the use of immunization and the incidence of other diseases has been significantly reduced. Protection against disease may be acquired by the individual either actively or passively (see table p. 142).

I- Active Acquired Immunity: On exposure to foreign antigen in vaccines or on infective agents as in disease, these stimulate specific B and T cells leading to active production of protective antibodies or CMI. Immunity develops slowly; however, it lasts for a long time due to development of immunologic memory. It can be naturally acquired or artificially induced.

a- Natural active immunity occurs following clinical or subclinical infections e.g. after measles or mumps, in which immunity is long lasting.

b- Artificial active immunity occurs after vaccination with live attenuated or killed infectious agents or their products (see next page).

II- Passive Acquired Immunity: In which ready-made antibodies are transferred to an individual. This gives rapid protection; however, immunity is short lasting. There are 2 types:

a- Natural passive immunity as occurs when antibodies are transferred from mother to foetus through the placenta (IgG) or in the colostrum (IgA). This type of immunity is responsible for protection of the newborn against several diseases during the first 6 months after birth.

b- Artificial passive immunity as in prophylaxis or treatment of several diseases, for example:

- Injecting antitoxic serum for treatment of diphtheria or tetanus.
- Prophylactic injection of immunoglobulins to premature infants exposed to measles prior to vaccination or to hypo-gamma- globulinaemic children.

DH- Passive Active Immunity; involves giving gamma globulins or specific immune globulins which provide immediate protection and a vaccine to provide long term protection as in the case of, infants born to HBV-positive mothers, or after needle prick injury with HBV positive blood and to individuals susceptible to tetanus or rabies.

These should be given at different sites in the body to prevent the antibodies from neutralizing the immunogens in the vaccine.

Types of Vaccines: The different types of vaccines are:

1- ***Killed or inactivated vaccines*** e.g. TAB vaccine for typhoid and paratyphoid, Salk vaccine for poliomyelitis and human rabies vaccines.

2- ***Living attenuated vaccines*** e.g. Sabin vaccine for poliomyelitis, BCG vaccine for tuberculosis, 17-D vaccine for yellow fever, MMR vaccine for measles, mumps and rubella and rabies vaccine for animals.

3- ***Toxoids:*** These are vaccines prepared by detoxifying the toxins of some bacteria, e.g. toxoid vaccines for diphtheria and tetanus.

4- ***Vaccines prepared from bacterial fractions or viral components*** e.g.:-

a) The capsular polysaccharide vaccine of meningococci, pneumococci, and *H. influenzae* type b. **b)** Acellular vaccine of *B. pertussis* containing the purified proteins from the organism, **c)** Purified surface antigens of hepatitis B virus, **d)** The split virus vaccine of influenza virus containing haemagglutinin and neuraminidase, e) Capsid proteins of types 6, 11, 16, and 18 of human papilloma virus (HPV) vaccine.

5- ***Recombinant vaccines*** prepared by recombinant DNA technology, e.g. hepatitis B virus vaccine and HPV vaccine.

Some Concerns About Vaccines

A- Killed Vaccines

1- The killed vaccines elicit primarily antibody responses. They do not stimulate CME, since the virus does not replicate inside cells and viral epitopes are not presented to CTLs in association with MHC-I.
2- They are less effective (if any) in stimulating local immunity.
3- Immunity is short lasting and needs boosting.
4- However, they are safe and can be given to pregnant women or immunocompromised patients.

B- **Living Attenuated Vaccines**

1- Though live attenuated vaccines are more effective in stimulating both humoral and cell mediated immunity, both local and systemic, yet they may revert to virulence.
2- They may cause diseases in immunocompromised hosts and should not be given to such persons or to pregnant women because of potential damage to the foetus.
3- The live vaccine can be excreted by the immunized person. This is a double-edged sword; it is advantageous if the spread of the virus successfully immunizes others (by the feco-oral route) as occurs in live oral poliovirus

vaccine (herd immunity). However, it could be a problem if the strain reverts to virulent wild type organism.

4- While killed vaccines are heat stable; live vaccines are heat labile and should be properly refrigerated to remain effective.

5- Vaccines prepared in chick embryo may cause allergic reactions in persons allergic to eggs.

C- Capsular Polysaccharide Vaccines alone are poor immunogens in children below 2 years of age, e.g. *H. influenzae* b vaccine. They do not respond to T cell independent antigens inspite of their early *(in utero)* capacity to generate IgM. Conjugation of such polysaccharides to carrier proteins such as diphtheria toxoid, has provided vaccines that are effective in this age group. Capsular polysaccharide vaccines produce anti-capsular opsonizing antibodies.

D- Combined Immunization, e.g. DTP or MMR is widely used and a proper immune response occurs to all injected antigens; no interference was observed. Now combinations of 4 in 1, DTP Hib or DTP IPV or a combination of 5 in 1 DTP, Hib, IPV are being used for child vaccination

Many of the disadvantages of current vaccines will be bypassed when purified viral antigens are produced from genes cloned in bacteria or yeast. These will not contain viral nucleic acids and can not replicate or revert to virulence and can be produced in large amounts.

Approaches to Develop New Vaccines

Advances in recombinant DNA technology and in the technology of rapid, automated synthesis of peptides and other areas of bioengineering hold promise for improvements in available vaccines, and new approaches to the production of vaccines.

1- **Subunit Vaccines:** In which microbial polypeptides are isolated from the infective material (e.g. hepatitis B and influenza).

2- **Recombinant DNA-Derived Antigen Vaccines:** In which antigens are synthesized by inserting the coding genes into *E. coli* or yeast cells. Examples are HBV vaccine and several others are available.

3- **Recombinant Avirulent Vectors Vaccines:** In which the gene coding for the antigen (protein) of interest is inserted into the genome of an avirulent vector such as vaccinia virus or BCG that can be then administered as a vaccine for HSV, HIV, HBV and rabies virus.

4- **Synthetic Peptides Vaccines:** It is now technically possible to synthesize short peptides that correspond to antigenic determinants on a viral or bacterial protein to be used as vaccine (e.g. cholera toxin and poliovirus).

However, these need to be coupled to larger proteins to induce antibody response.

5- **Induction of Attenuated Non-Reverting Mutation:** Genetic engineering techniques are used to attenuate a virus irreversibly by selectively removing genes necessary for virulence.

6- **DNA Vaccines:** Plasmid vectors carrying microbial DNA encoding antigenic proteins is injected directly into the muscle of the recipient. The DNA is taken up by muscle cells and the encoded protein antigen is expressed, leading to both humoral and cell mediated immunity. The DNA appears either to integrate into the chromosomal DNA or to be maintained for long periods in an extra-chromosomal form. The viral antigen is expressed not only by muscle cells but also by dendritic cells.

7- **Mucosal Vaccines (locally administered):** Intranasally administered aerosol vaccines are being developed, particularly for viral respiratory disease and measles in the hope of stimulating local antibodies at the portal of entry.

8- Membrane Proteins from Various Pathogens, including influenza virus, measles virus, hepatitis B virus, and HIV have been incorporated into micelles, liposomes, and immunostimulatory complexes (ISCOMs) and are currently being assessed as potential vaccines. In addition to their increased immunogenicity, liposomes and ISCOMs appear to fuse with the plasma membrane to deliver the antigen intracellularly, where it can be processed by the cytosolic pathway and thus induce a cell mediated immune response.

Schedule for Active Immunization of Children in Egypt

Age	Vaccine
At birth-1 month	BCG.
2 months	DTP, OPV, HBV.
4 months	DTP, OPV, HBV.
6 months	DTP, OPV, HBV.
9 months	measles vaccine.
15 months	MMR.
18 months	DTP, OPV.
4 -6 years	DTP, OPV.
10 years and every 10 years thereafter	Td.

A country-wide campaign is run in an attempt to eradicate poliomyelitis from Egypt by immunizing all children from 0-5 years every 2 years. Local campaigns in areas where poliomyelitis cases appear are also in progress.

The WHO recommends the use of the immunization schedules mentioned bellow, used in USA and approved by several committees.

Schedule for Active Immunization of Children in USA

Age	Vaccine	
At birth	HBV.	
2 months	HBV	DTaP, IPV, Hib, PCV, Rota
4 months		DTaP, IPV, Hib, PCV, Rota
6 months	HBV	DTaP, IPV, Hib, PCV, Rota
12-15 months		DTaP, Hib, PCV. 1 MMR, Varicella, HAV.
4-6 years		DTaP, IPV, MMR, Varicella.
11-12 years		Tdap. HPV

-Human papilloma virus (HPV): Two vaccines are licensed, a quadrivalent vaccine (HPV4) for the prevention of cervical, vaginal and vulvar cancers (in females) and genital warts (in females and males), and a bivalent vaccine (HPV2) for prevention of cervical cancer in females. Both vaccines are most effective if given before exposure through sexual contact. HPV4 or HPV2 is administered to females in 3 doses, starting at age 11-12 years and 2 and 6 months after the first dose. HPV4 may be administered in a 3-dose series to males aged 9-18 years to reduce their likelihood of acquiring genital warts.

-Hepatitis A vaccine is recommended in 2 doses one at 12- 23 months and the second 6 months later. It may be given to older children at risk of infection.

-Influenza vaccine is recommended annually for children between age 6 months and 5 years and for their contacts.

-Meningococcal conjugate vaccine (MCV4) is recommended for children 2-10 years with terminal complement deficiencies, asplenic or other risk factors. It is recommended at age 11-12 years and to college freshmen living in a dormitory if not previously vaccinated. MPSV4 is an acceptable alternative.

DTP = Diphtheria, tetanus, pertussis.
DTaP = Diphtheria, tetanus, acellular pertussis OPV = Oral poliovirus vaccine.
MMR = Mumps, measles, rubella. IPV= Inactivated polio vaccine
BCG = T.B. vaccine. HBV = Hepatitis B vaccine.
Hib = *H. influenzae* b polysaccharide conjugated vaccine. HAV= Hepatitis A vaccine. PCV = Pneumococcal conjugate vaccine (PCV13). Rota virus attenuated oral vaccine

These immunization schedules are approved by the Advisory Committee on Immunization Practice, the American Academy of Pediatrics and the American Academy of Family Physicians 2013.

Recommended Vaccinations for Health Care Workers (HCWs):

HCWs are at high risk of contracting diseases from patients in hospitals. They can also be a source of infection for patients. Therefore they should be immunized by the following vaccines; **hepatitis B, measles, mumps** and **rubella** mainly for females, **varicella,** and **influenza** current yearly vaccine, **tetanus** and **diphtheria** toxoid. **Zoster** and **HPV** vaccines are given if the HCW is in the recommended age group. **Pneumococcal** and **meningococcal** vaccines are given if the HCW has a risk factor. Microbiologists routinely exposed to *N. meningitides* should receive the vaccine. It is recommended to start vaccinating HCWs against **smallpox** as they will be the first to be exposed to any emerging cases due to bioterrorism or biologic warfare.

CHAPTER 20

IMMUNOLOGIC MECHANISMS OF TISSUE DAMAGE (Immunopathology)

Although the immune response generally aims at protection, yet inappropriate or exaggerated immune response may lead to different forms of tissue damage, i.e. immunopathology. This may occur due to:

1- An overactive immune response, which produces more damage than it prevents, e.g. **hypersensitivity reactions** and **graft rejection.**

2- Failure of appropriate recognition as in **autoimmune diseases.**

HYPERSENSITIVITY REACTIONS

When an immune response results in exaggerated or inappropriate reactions harmful to the host, the term hypersensitivity or allergy is used. Gell and Coombs defined four types of hypersensitivity reactions. Types I, II and III are antibody mediated while type IV is T cell mediated and is called delayed hypersensitivity reaction.

TYPE I: IMMEDIATE (ANAPHYLACTIC) HYPERSENSITIVITY

These reactions occur when an antigen **(allergen)** reacts with cell fixed antibody **(IgE)** leading to release of soluble molecules **(mediators)** that cause the manifestations of the disease. According to the target organ or tissue affected the manifestations may range from the systemic life threatening anaphylactic reactions to the local atopic allergies, e.g. bronchial asthma, hay fever and food allergies.

Pathogenic Mechanisms (Fig. 38)

There must be a **first exposure** to the allergen* which stimulates formation of antibody of the IgE type which is cytotropic, i.e. tissue fixing. It fixes, by its Fc portion, to mast cells and basophils (which possess receptors for Fc of IgE) especially those in the bronchial tree. On **second exposure** to the same allergen, it bridges between IgE molecules fixed to mast cells leading to activation and degranulation of mast cells and the release of the mediators.

*On first exposure to the allergen, APCs mainly dendritic cells and macrophages capture the allergen, process it and present its epitopes to TH2 cells which are activated and produce IL-4. The latter stimulates class switching of B cells to produce IgE.

There are Three Classes of Mediators Derived from Mast Cells:

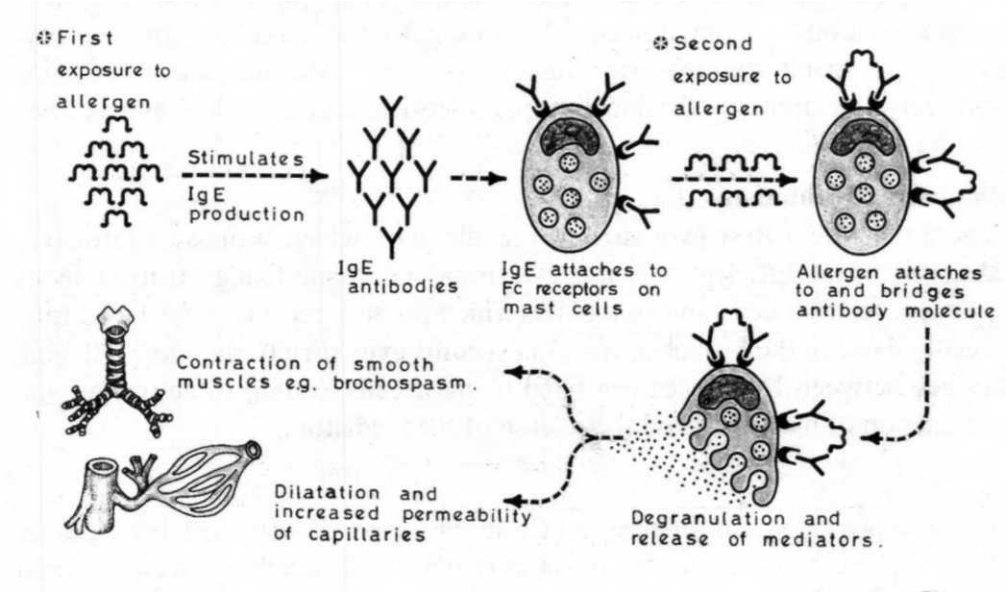
1- **Preformed Mediators** stored in the granules and released immediately i.e. histamine, which is responsible for the wheal and flare reaction, which occurs within 15-20 min, in response to the intradermal injection of an allergen.

2- **Newly Synthesized Mediators** i.e. leukotrienes and prostaglandins derived from arachidonic acid, and the platelet activating factor (PAP).

3- **Cytokines** produced by activated mast cells (and basophils) e.g. TNF, IL-3, IL-4, IL-5, IL-13 and chemokines. TH2 cells that are recruited to the site of the reaction also secrete cytokines.

These mediators cause the immediate reactions which include; increased smooth muscle contraction and mucus secretions, vasodilatation and increased vascular permeability of capillaries and oedema.

A **late phase inflammatory reactions** occur 2-4 hrs later. The inflammation is maximal by 24 hrs and then gradually subsides, and it consists of accumulation of inflammatory leucocytes, including neutrophils, basophils, eosinophils and TH2 cells. The cytokines contribute to the late reaction, through the recruitment of inflammatory cells, increasing IgE production and activation of eosinophils, and may lead to tissue damage.



(Fig. 38): Pathogenic mechanisms of type I hypersensitivity reactions.

Clinical Types:

1- **Anaphylaxis:** It is the systemic form of type I hypersensitivity reactions. It occurs on exposure to an allergen to which a person is previously sensitized. It manifests by shock due to sudden decrease of blood pressure, respiratory distress due to bronchospasm, cyanosis, oedema, urticaria, etc... Shock may lead to death.

The common allergens are drugs, e.g. penicillin or serum injections e.g. anti-diphtheritic or anti-tetanic serum, anaesthesia or insect venom.

The condition should be rapidly treated by corticosteroid injection which is life saving as well as epinephrine and antmistaminics.

2- **Atopy:** It is the local form of type I hypersensitivity reactions affecting one organ. It occurs on exposure to certain allergens that induce production of specific IgE. These are usually common environmental proteins and chemicals and include:

-Inhalants, e.g. dust mite faeces found in bedding and carpet, tree or grass pollens, moulds spores, etc... -Ingestants, e.g. milk, wheat, egg, fish, strawberries, chocolate, etc... -Contactants, e.g. wool, animal fur, nylon, etc...

-Injectants, e.g. drugs (penicillin, salicylates, anaesthetics), foreign serum, insect venom etc...

There is a strong **familial predisposition** to the development of atopic allergy. The predisposition is genetically determined (several genes associated with atopy have been identified). Atopic individuals produce **high levels of IgE** in response to environmental allergens, as compared to normal individuals who will produce mainly IgG and little IgE.

Different members of a family may manifest in different ways. The manifestations depend on the site of antigen-antibody reaction. These are bronchial asthma in the bronchial tree, rhinitis or hay fever in the nose, diarrhoea and vomiting in the GIT, urticaria and skin rash in the skin, and conjunctivitis in the eye.

Methods of Diagnosis:

1- History taking is important for determining the allergen involved.

2- Skin tests: These are done by the skin prick or patch tests of a battery of different groups of allergens. A wheal and flare (erythema) develops within 15-20 minutes at the site of the allergen to which the individual is allergic. The reaction is due to the release of Mstamine and other mediators at the site.

3- Determination of total serum IgE level which is usually high in atopic individuals by using radioimmunosorbent test (RIST).

4- Determination of specific IgE levels to the different allergens by using the radioallergosorbent test (RAST) or enzyme RAST.
5- Provocation tests may be used to test food allergies.

Management:

1- Avoidance of the specific allergen responsible for the condition e.g. food and drug allergies.

2- Hyposensitization is a form of immunotherapy. It is done by injecting the patient subcutaneously with gradually increasing doses of an extract of the allergen. The beneficial effects of hyposensitization may be due to:

a) It may induce regulatory T cells (Tregs) to switch the immune response from TH2 to TH1 resulting in decrease of IgE production.
b) The method stimulates the production of IgG blocking antibody, which binds the allergen and prevents its combination with IgE fixed to cells.
c) It may induce T cell tolerance.

3- **Drug therapy** aiming at inhibition of mast cell degranulation, antagonizing the effects of mast cell mediators, and reducing inflammation. It includes:

- Corticosteroids, antmistamines, epinephrine and theophylline.
- Sodium cromoglycate stabilizes mast cells and reduces degranulation.
- **P**-adrenergic agonists are bronchial dilators that relax smooth muscles in airways, e.g. albuterol or terbutaline.
- Montelukast (Singular 10) is a specific receptor antagonist that blocks the effects of leukotrienes, thus reduces the amount of airways inflammation in asthma.
- Omalizumab (Xolair) is a humanized monoclonal anti-IgE antibodies that bind to IgE and prevent its binding to the Fc receptors on mast cells. It prevents both the immediate and the late phases of asthma.
- Antagonists against IL-4 and IL-5 are in clinical trials.

TYPE II: CYTOTOXIC OR CYTOLYTIC REACTIONS

These occur when an antibody (IgG or IgM) reacts with antigen on the cell surface, whether this antigen is part of the cell membrane or it is a circulating antigen (or hapten) that attaches to the cell membrane. Cell lysis results due to any of the following mechanisms.

Mechanisms of Cytolysis:

1- The complement is fixed to the antigen antibody complex on the cell surface; the activated complement will lead to cell lysis by the membrane attack unit (C5b6789).
2- Phagocytosis is enhanced by the antibody (opsonin) bound to cell antigen leading to opsonization of the target cell.

3- Antibody dependent cellular cytotoxicity (ADCC): Antibody coated cells e.g. tumour cells, graft cells or infected cells can be killed by several cells which possess Fc receptors; a process different from phagocytosis and independent of the complement. The cells most active in ADCC are: **a-** NK cells.

b- Macrophages and neutrophils.

c- Eosinophils can kill antibody coated schistosoma parasites. **Clinical Conditions due to**

Cytotoxic Reactions:

1- Transfusion reactions due to ABO incompatibility which can be prevented by proper cross matching.

2- Haemolytic disease of the newborn due to Rh incompatibility.

3- In many of the autoimmune diseases, the mechanism of tissue damage is cytotoxic reactions, e.g. SLE, autoimmune haemolytic anaemia, idiopathic thrombocytopenic purpura, myasthenia gravis, Goodpasture syndrome, nephrotoxic nephritis, and Hashimoto's thyroiditis.

4- A non-cytotoxic type II hypersensitivity is Graves' disease which is a form of thyroiditis in which antibodies are produced against TSH surface receptors. These do not lead to the destruction of the cell but mimic the effect of TSH and stimulate the cells to over-produce thyroid hormones.

5- In graft rejection, cytotoxic reactions are one of the mechanisms of tissue damage. In hyperacute rejection, the recipient already has preformed antibody against the graft. Rejection occurs within hours.

6- Drug reaction, e.g. penicillin, phenacetin and quinidine may attach as haptens to the surface of red cells and induce antibodies which are cytotoxic for the cell-drug complex leading to haemolysis. Quinine may also attach to platelets; and the antibodies induced cause platelet destruction leading to thrombocytopenic purpura.

TYPE III: IMMUNE COMPLEX MEDIATED REACTIONS

When antibodies (IgG or IgM) and antigen coexist, immune complexes are formed. Normally, they are promptly removed by the reticuloendothelial system. However, some immune complexes of a certain size* escape phagocytosis and are deposited in tissues on the basement membrane of blood vessels and cause tissue injury.

*Large immune complexes are rapidly eliminated (several minutes) mainly by the Kupffer cells in the liver. However, small or soluble complexes (due to low-affinity antibody as those formed to autoantigens) require hours to several days for their removal. Circulation of high levels of these immune complexes will overwhelm the ability of the phagocytic system to remove them and the excess complexes then deposit in various tissues.

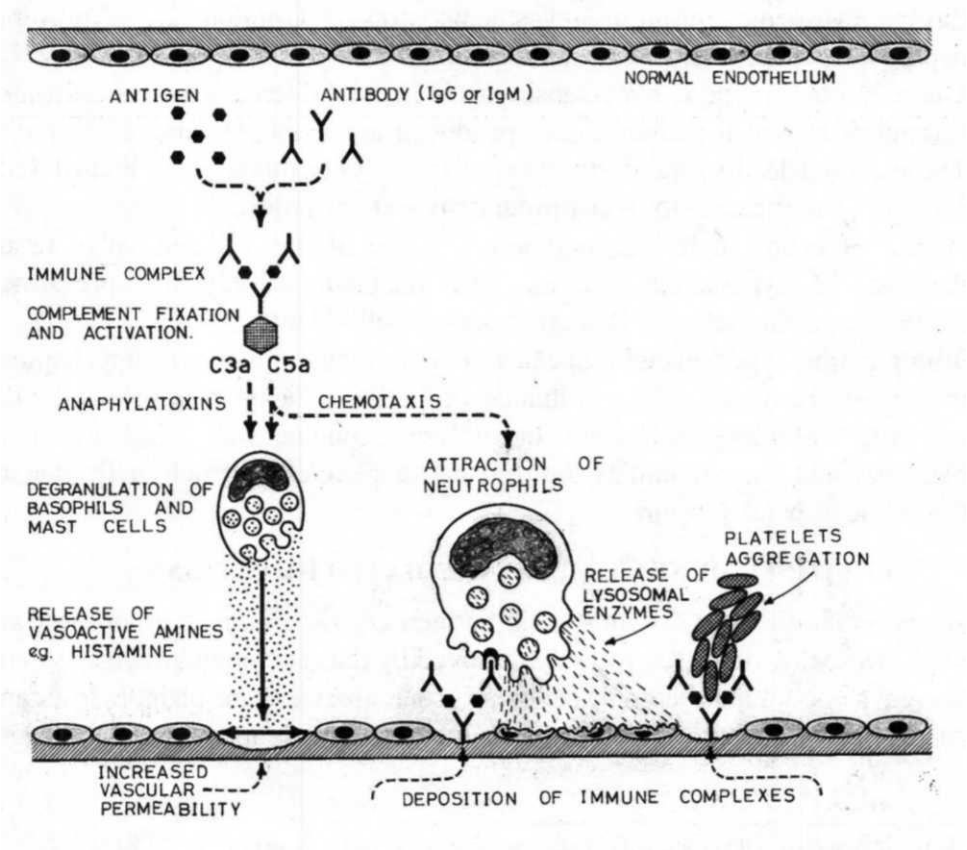
Mechanism of Tissue Injury (Fig. 39):

Irnmune complexes trigger a variety of mflammatory processes:

1- Immune complexes activate the complement, with the release of the anaphylatoxins C3a and C5a which stimulate degranulation of basophils and mast cells with release of vasoactive amines (e.g., histamine). These increase vascular permeability and help deposition of complexes on the basement membrane.

2- Neutrophils are attracted to the site by irnmune complexes and by C5a. On trying to phagocytose the complexes neutrophils release lysosomal enzymes which damage tissues and intensify the inflammatory process.

3- Platelets are aggregated with two consequences; they release vasoactive amines and form microthrombi which can lead to local ischaemia.



(Fig. 39): Mechanism of tissue injury due to immune complex deposition. Type III hypersensitivity reactions.

Clinical Conditions due to Immune Complexes:

Diseases that are produced by immune complexes are those in which the antigen persists without being eliminated as in: **a-** Repeated exposure to extrinsic antigens, **b-** Injection of large amounts of antigen, **c-** Persistent infections, **d-** Autoimmunity to self components.

Clinical examples include:

1- Arthus reaction:

This is a local immune complex deposition phenomenon which occurs on repeated injections of the same antigen subcutaneously. After 2 or 3 injections, a local reaction occurs in the form of oedema, erythema and necrosis. Immune complexes are deposited in the small blood vessels and trigger the above mentioned inflammatory reactions leading to vasculitis, microthrombi formation, vascular occlusion and necrosis. These reactions may occur in diabetics receiving repeated injections of insulin subcutaneously.

2- Serum sickness:

It is a systemic immune complex phenomenon that occurs after injection of relatively large doses of foreign serum. The antigen is slowly cleared from the circulation while antibody production begins. Simultaneous presence of antigen and antibody leads to production of soluble immune complexes which may circulate or are deposited in various sites causing the manifestations of the disease, which appear around 10 days after the injection. These are fever, urticaria, arthralgia, lymphadenopathy, splenomegaly, glomerulonephritis and albuminuria.

The disease is self limited and the patient gradually recovers as the antigen is eliminated. Corticosteroids help rapid recovery. The condition occurs after treatment with antidiphtheritic serum, penicillin or sulphonamides.

3- **Post-streptococcal glomerulonephritis** and glomerulitis associated with infective endocarditis.

4- Certain infections in which antigen is persistently produced as in hepatitis B surface antigen, HIV particles in AIDS and mycobacterial antigens in leprosy.

5- Hypersensitive pneumonitis (farmer lung) due to deposition of immune complexes in the lungs after repeated inhalation of dust, mould spores or fragments of bird's excreta.

6- Autoimmune disease: Endogenous antigen antibody complexes are involved in the pathogenesis of several autoimmune diseases e.g. SLE and rheumatoid arthritis.

Management of Immune Complex Disorders:

1- Withhold allergenic drugs.

2- Use immunosuppressive agents: corticosteroids and cyclosporine. 3-Remove the offending complexes by plasmapheresis or exchange transfusion.

Detection of Immune Complexes:

In tissues by staining tissue biopsies with fluorescein labelled anti-C3 or anti-IgG or anti-IgM.

In serum by:

a- Measuring the levels of C3, which may decrease.

b-Precipitation of irnmune complexes and determination of the amount of IgG in the precipitate, **c-** Estimation of binding of immune complexes to Clq, rheumatoid factor, or Raji cells*.

d- Sera containing immune complexes may form cryoprecipitate at 4°C.

TYPE IV: T CELL MEDIATED, DELAYED TYPE HYPERSENSITIVITY (DTH) REACTIONS

Delayed hypersensitivity reactions are initiated by sensitized (memory) T cells reacting with specific antigen. The response is delayed, i.e. it starts hours or days after contact with the antigen and often lasts for days.

Pathogenesis:

T cells cause tissue injury either by triggering DTH reactions by TH1 cells or by directly killing target cells by CD8 T cells. Both cells secrete cytokines (IFN-y and TNF) that attract lymphocytes, activate macrophages and induce inflammation that leads to tissue damage. The tissue injury results from the products of activated macrophages such as hydrolytic enzymes, reactive oxygen intermediates, nitric oxide and proinflammatory cytokines (TNF, IL-1, IL-6). Chronic DTH reactions often produce fibrosis as a result of the secretion of cytokines and growth factors by the macrophages.

* A human lymphoblastoid cell line that binds immune complexes through C3 receptors.

Conditions due to Delayed Hypersensitivity Reactions:

1- **Tuberculin-Type Hypersensitivity:** Delayed hypersensitivity to antigens of microorganisms occurs in many infectious diseases and has been used as an aid in diagnosis. It is typified by the tuberculin reaction:

When PPD* is injected intradermally in a sensitized person, i.e. previously exposed to tuberculosis; a local indurated area appears at the site of injection after 48-72 hours due to accumulation of macrophages and lymphocytes. Similar reactions are observed in diseases in which cell mediated immunity plays a role, e.g. brucellin test in brucellosis; lepromin test in leprosy; Frei's test in lymphogranuloma venereum; candidin test in Candida infections; as well as several viral and parasitic infections.

2- **Granulomatous Lesions:** In several chronic diseases specially those due to intracellular organisms that resist destruction by macrophages e.g. TB, leprosy, and schistosomiasis; the persistent antigens in tissues, especially in macrophages, stimulate a chronic local delayed hypersensitivity reaction. Continuous release of cytokines leads to accumulation of large numbers of macrophages which give rise to epitheloidal and giant cells and granuloma formation in an attempt by the host to isolate such resistant pathogens.

In some diseases e.g. secondary tuberculosis, the cells in the center of the granuloma die leading to caseation and cavitation which is associated with general toxaemia.

3- **Contact Dermatitis:** This occurs due to contact of the skin with chemical substances or drugs e.g. poison ivy, picryle chloride, nickel salts (as in nickel jewellery), hair dyes, neomycin skin ointment, cosmetics, soaps. These substances enter the skin in small molecules (they are hatpens) then they attach to body proteins (carriers) and become immunogenic. A delayed hypersensitivity reaction to these substances will lead to an inflammatory reaction of the skin in the form of eczema, rash and vesicular eruptions.

4- **Autoimmune Diseases and Graft Rejection** are due in part to delayed hypersensitivity reactions.

5- **Insulin Dependent Diabetes Mellitus** is due to type IV hypersensitivity in which T cells invade the pancreatic islets and specifically destroy the insulin secreting beta cells.

* PPD is the purified protein derivative of tubercle bacilli used in the tuberculin test.

TRANSPLANTATION IMMUNOLOGY

The practice of grafting tissues or organs from one individual to another or from one place to another in the same individual is known as transplantation. This surgical procedure is very helpful as it enables the replacement of a diseased organ by another healthy one. The main obstacle in organ transplantation is the occurrence of graft rejection, which is due to the presence of tissue antigens that differ from one individual to the other, thus stimulating an immune response that causes the rejection. These antigens are known as human leucocyte antigens HLA (or histocompatibility antigens) and are genetically determined by a set of genes called the major histocompatibility gene complex (MHC). MHC-I and MHC-II molecules are the main antigenic targets in allograft rejection.

The Major Histocompatibility Gene Complex (MHC)

In humans, the MHC or HLA genes occupy a portion of the short arm of chromosome 6. These genes are found in regions A, B, C and D (Fig. 38). The genes in each region are clustered and located in one or more loci. Regions A, B, and C code for class I MHC antigenic surface molecules namely HLA-A, HLA-B and HLA-C. More than 147 HLA-A, 200 HLA-B, and 90 HLA-C genes have been recognized. This leads to the extensive polymorphism that exists for their molecular products.

The D region contains 3 loci; DR, DQ and DP which code for the antigenic specificities of class II MHC surface molecules namely HLA-DR, HLA-DQ, and HLA-DP. Class II genes are highly polymorphic; there are approximately 500 total class II gene products.

MHC genes are inherited one from each parent. Thus a heterozygous human inherits one paternal and one maternal haplotype, each containing three class I (A,B & C) and three class II (DP, DQ & DR). A heterozygous individual will inherit a maximum of 6 class I and 6 class II specificities.

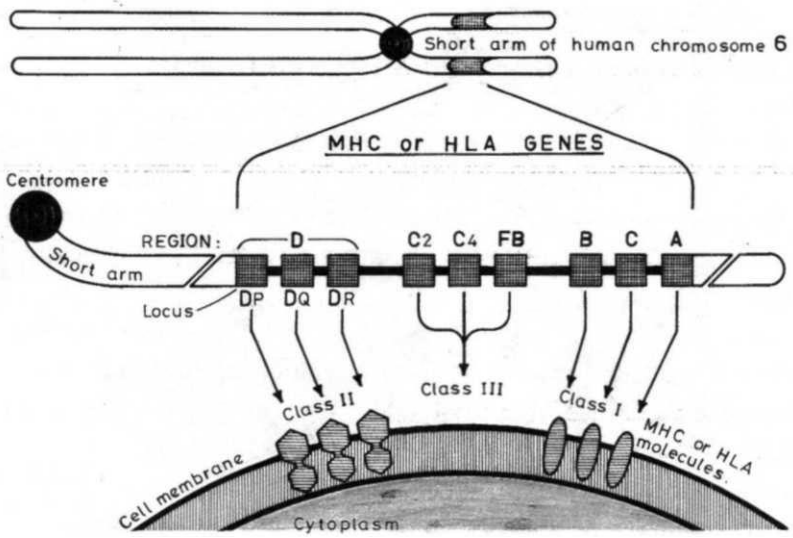
A region between D and B codes for certain complement components (C2, C4, factor B) and for 2 cytokines TNF-a and p. It is not concerned with graft rejection and is called class III MHC.

The Major Histocompatibility (MHC) Antigens or HLA

Class I MHC antigens are membrane glycoproteins on the surface of all nucleated cells and platelets. MHC class I molecule consists of a heavy polypeptide chain (a) which is non-covalently bound to a light **P** chain called P2-microglobulin (which is encoded on chromosome 15). The heavy chain is highly polymorphic and is similar to an immunoglobulin molecule; it has hypervariable regions in its N-terminal, which contain the antigen binding groove. There are several antigenic types

for HLA-A, HLA-B, and HLA-C which are determined by cytotoxicity tests (HLA typing) using the host's leucocytes, specific monoclonal antibodies and complement.

Class II MHC antigens are glycoproteins found chiefly on the surface of specialized antigen



(Fig. 40): The major histocompatibility gene complex and the corresponding MHC class I and II molecules on cell surface.

Endogenously processed cytosolic peptides in virus infected cells or tumour cells are transported to the surface of the cells, where they bind to MHC-I molecules to be recognized by cytotoxic T cells which then kill these cells. In other words, TC cells (CTLs) are only triggered or activated when they recognize both antigen and class I MHC molecules in close association.

presenting cells (macrophages, dendritic cells, Langerhans cells, B cells) and activated T cells. They include HLA-DP, HLA-DQ and HLA-DR. MHC class II molecules consist of 2 polypeptide chains (a and (3) that are non-covalently bound. They have a hypervariable region that provides the polymorphism and contains the peptide antigen binding groove.

Helper T cells will recognize foreign antigen (exogenously processed) bound to MHC II molecules on the surface of APCs. In other words, TH cells will not be activated unless they recognize the antigen peptide in **association** with class II MHC molecules. T cells recognition of peptides is restricted by the morphology of the MHC molecules not just the class, this is called MHC restriction.

There are several antigenic types for HLA-DP, HLA-DQ and HLA-DR. These antigens are determined by the mixed lymphocyte reaction or cytotoxicity tests.

Minor Histocompatibility Antigens: These are antigens that differ between the donor and the recipient. They can induce weak or slower rejection reactions than do MHC antigens. Most of these antigens are proteins that are presented to host T cells in association with self MHC molecules on host APCs.

Similarities and Differences between MHC class I and II:-Similarities:

1- Function: Both, **(a)** are critical cell interaction molecules. They bind peptides and present them at the surface to T cell receptors, **(b)** are strong transplantation antigens, **(c)** MHC molecules bind only one peptide at a time, and all the peptides that bind to a particular MHC molecule share common structural motifs.
2- Structure: Both have **(a)** two polypeptide chains, **(b)** a trans-membrane region and a cytoplasmic region **(c)** a single peptide binding groove, **(d)** a polymorphic region (which includes the peptide-binding region) and a constant region.
3- Both show genetic polymorphism, with multiple alleles in the population.
4- Genes encoding for MHC from both parents are all expressed.

Differences:

1- MHC-I is expressed on all nucleated cells; MHC-II is mainly expressed on APC and activated T cells.
2- Cells expressing MHC-I + peptide interact with CD8 TC cells; cells expressing MHC-II + peptide interact with CD4 TH cells.
3- MHC-I molecules bind peptides of 8-9 amino acids derived from endogenous cytosolic antigens; MHC-II molecules bind peptides of 12-25 amino acids or more derived from exogenous vesicular antigens (p. 65)
4- MHC-II molecule is coded for entirely within MHC on chromosome 6; but the light chain p_2-microglobulin of MHC-I, is coded for outside MHC on chromosome 15. (Fig. 44 & table p. 143)

Types of Grafts:

Autograft is the transfer of an individual's own tissues from place to place e.g. skin grafts. These are regularly accepted. **Isograft** is the transfer of tissues between genetically identical individuals, e.g. identical twins. These usually are accepted permanently. **Allograft** (homograft) is a graft between genetically different members of the same species, e.g. from one human to another. Rejection may occur if the donor and recipient are not properly matched. **Xenograft** (heterograft) is the transfer of tissues between different species. This is always rejected by an immunocompetent recipient. ***The foetus is an allograft that is not rejected (See p. 144).***

Mechanisms of Graft Rejection (Fig. 37):

Allograft (donor) MHC molecules are presented for recognition by the T cells of the recipient in two ways:

Donor MHC may be presented on donor APCs to recipient T cells (the direct pathway), or donor MHC may be picked up by host APCs that enter the graft or reside in draining lymphoid organs, and be processed and presented to T cells as peptides associated with self MHC molecules (the indirect pathway).

— Both TH and TC cells are activated.
- TC cells destroy graft cells by direct contact.
- TH cells secrete cytokines that attract and activate macrophages, NK cells and polymorphs leading to cellular infiltration and destruction of the graft, i.e. type IV hypersensitivity reaction.

~ B cells recognize foreign antigens on the graft and produce antibodies which bind to graft cells and:
- activate complement causing cell lysis.
- enhance phagocytosis, i.e. opsonization.
- lead to ADCC by macrophages, NK cells and polymorphs.

} i.e. type II hypersensitivity reactions.

— In addition, immune complex deposition on the vessel walls (e.g. in renal grafts) induce platelet aggregation, and microthrombi formation leading to ischaemia and necrosis of the graft, i.e. type III hypersensitivity reactions.

Types of Graft Rejection:

Depending on the speed of rejection, there are 3 types:

1- **Hyperacute rejection:** This type occurs minutes or hours after transplantation. It is a rare type of rejection that occurs in individuals with preformed antibodies either due to blood groups incompatibility or previous sensitization by blood transfusion, pregnancy or previous transplantation.

2- **Acute rejection:** This type occurs within days or weeks after transplantation. It is mainly T cell mediated.

3- **Chronic or late rejection:** It occurs slowly over a period of months or years. It may be cell mediated, antibody mediated or both.

Graft Versus Host (GVH) Reaction:

This condition occurs when an immunologically competent graft, e.g. bone marrow, is transplanted into an immunologically suppressed recipient (host). The grafted cells survive and react against the host cells i.e. instead of reaction of host against the graft, the reverse occurs. GVH reaction is characterized by fever, pancytopenia,weight loss, rash, diarrhoea, hepatosplenomegaly and death.

Prevention of Graft Rejection

A- Proper Choice of Donors:

1- **ABO blood group** compatibility must be fulfilled.

2- **Tissue Typing for Histocompatibility Antigen (HLA typing):-**

a- Serologic typing (lymphocytotoxicity tests): MHC class I as well as class II proteins can be detected by reacting lymphocytes from donor and recipient with a panel of antisera to the different histocompatibility antigens. These antisera may be obtained from multiparous women or monoclonal antibodies prepared to the different HLA antigens.

Lymphocytes of donor and recipient are reacted with the different antisera in the presence of complement. Lysis of lymphocytes indicates that they contain the antigen. Hence similarity of donor and recipient transplantation antigens is indicated by similarity in the specific antisera with which the 2 sets of cells react (or do not react).

b- Mixed lymphocyte reaction (MLR): It was previously used to determine class II MHC proteins. However, it is more useful in bone marrow transplantation. Class II proteins (DR loci) are the most important in tissue typing and can be detected by MLR. Currently, they can be detennined by serologic typing as MLR is more time consuming.

In the one way mixed lymphocyte reaction, a mixed lymphocytes culture is set up in which the donor cells are killed by X irradiation or mitomycin C to prevent their proliferation and mixed with live responder lymphocytes from the recipient. The mixture is incubated in cell culture to permit DNA synthesis which is measured by the incorporation of tritiated thymidine. The greater the amount of DNA synthesis in the responder cells, the more foreign are the class II MHC proteins of the donor cells and the more the disparity indicating an unsatisfactory match between donor and recipient.

c- Molecular HLA typing (genotyping of transplant epitopes) :-

These methods are based on amplification of DNA segments on chromosome 6 carrying the genes for HLA class I or II by PCR. The amplified product may be further identified by DNA probes using RFLPs* or by sequencing where the sequences are compared in donor and recipient.

The higher the degree of matching between donor and recipient, the better the chance of survival of the graft. The best results (nearlyl00%) are between identical twins; otherwise a reasonable degree of matching is aimed at.

3- **Cross-Matching** is used to test the recipient's serum for the presence of preformed antibodies against the donor's HLA antigens.

Restriction fragment length polymorphism.

B- Postoperative Immunosuppressive Therapy

Immunosuppressive therapy is used to prevent the graft rejection, by non-specifically interfering with the induction or expression of the immune response. However, such immunologically suppressed individuals are susceptible to infections and are more prone to develop lymphoreticular cancer. The following measures are in use:

1- Anti-CD3 monoclonal antibodies (OKT3) and anti-CD25 (anti-IL-2Ra). These deplete or inhibit T cells functions. They are widely used in postoperative prophylaxis and to reverse acute graft rejection.

2- Cyclosporin A is an antibiotic produced by a fungus. It prevents T cell activation and blocks the accompanying cytokine production.

3- FK-506 or tacrolimus and rapamycin are immuno-suppressive drugs that have a similar action to cyclosporine.

4- Anti-inflammatory corticosteroids are used to maintain therapy and are given in big doses at the time of a rejection crisis.

5- Anti-mitotic drugs e.g. azathioprine and methotrexate inhibit DNA synthesis and block the growth of T cells.

6- MMF (mycophenolate mofetil) is an anti-mitotic drug that specifically blocks lymphocytes maturation and proliferation with relatively few toxic side effects. It is routinely used in combination with cyclosporine to prevent acute graft rejection.

7- FTY20 (Fingolimod) causes sequestration of lymphocytes in the lymph nodes and prevents their migration to the site of the graft.

8- Abatacept (CTLA4-Ig) and Belatacept (LEA29Y), block B7-CD28 costimulatory signal and anti-CD40L antibodies; all block T cell activation and proliferation.

9- FK778 is immunosuppressive antiprolifative. It blocks T and B cell proliferation.

C- Antigen specific immunosuppression by induction of **tolerance** to the graft antigen is under trial.

Significance of MHC:

1. Organ transplantation and graft rejection.

2. MHC is essential for antigen presentation. TC cells are activated when they recognize antigen presented in the groove of MHC-I molecule. TH cells are activated when they recognize antigen presented in the groove of MHC-II molecule.
3. Disease association: It is found that the presence of certain HLA antigens is often associated with particular disease, e.g. HLA-B27 with ankylosing spondylitis, Reiter's disease and post-gonococcal arthritis, DR2 with multiple sclerosis, B8 with myasthenia gravis, DR4 with rheumatoid arthritis, and DR5 with Hashimoto's thyroiditis.
4. Paternity testing and forensic investigations.

CHAPTER 22

TOLERANCE AND AUTOIMMUNE DISEASES TOLERANCE

Tolerance is specific immunologic unresponsiveness, i.e. the absence of specific irnmune responses to a particular antigen in a fully immunocompetent person. Unresponsiveness to self antigens is known as autotolerance. It occurs through:-

I- T Cell Tolerance

1- Central T cell tolerance = Clonal deletion: In general, the irnmune system does not respond to self antigens. This state of autotolerance is acquired during embryonic life as a result of the deletion or killing (by apoptosis) of self reactive T cell precursors in the thymus. The process of central tolerance eliminates T cells with high affinity receptors for abundant and disseminated self antigens. The process is known as "negative selection".

2- Peripheral T cell tolerance = Clonal anergy: Peripheral tolerance is induced when mature T cells recognize self antigens in peripheral tissues, leading to functional inactivation (anergy), deletion or active suppression of self reactive T cells (that escape deletion in the thymus):-

a- Clonal anergy occurs when these cells recognize antigens without adequate levels of the "costimulatory signals" that are needed for full T cell activation. This may be due to insufficient production of IL-2 or improper interactions between cell surface proteins such as CD28 or CD40L on T cells and B7 or CD40, respectively, on B cells or other APCs.

In some cases, T cells that encounter self antigens may begin to express a molecule called CTLA-4, which interacts with B7 and delivers inhibitory signals to T cells. Several experiments show that the inactivating receptor, CTLA-4, is constantly functioning to keep autoreactive T cells in check.

b- Deletion; repeated activation of mature T cells by self antigens triggers a pathway of apoptosis that results in deletion of autoreactive lymphocytes.

c- Suppression of self reactive T cells by regulatory T (Treg) cells.

II- B Cell Tolerance:

1- Central B cell tolerance: When immature B cells interact with self antigens in the bone marrow, the B cells are killed (negative selection or clonal deletion) or they change their receptor specificity i.e receptor editing.

2- Peripheral B cell tolerance (clonal anergy); mature B cells that encounter high concentrations of self antigens in peripheral lymphoid tissues become anergic, and can not again respond to that self antigen. This is due to absence of T cell help (because helper T cells are tolerant to that self antigen). However,

tolerance in B cells is less complete than in T cell, which explains why most autoimmune diseases are mediated by antibodies.

Induction of tolerance to antigens may be used as a therapeutic approach for preventing harmful immune responses e.g. in prevention of graft rejection, in treatment of autoimmune and allergic diseases and other conditions. The antigen is administered in a special way that will lead to induction of tolerance, and it is called a **tolerogen.**

Factors Influencing the Induction of Tolerance:-

1- Immunologic maturity of the host; e.g. neonates are immunologically immature and will accept allografts that would be rejected by mature hosts.

2- Structure and dose of antigen; **a)** Simple molecules induce tolerance more readily than complex ones, **b)** Very high and very low doses of antigen may result in tolerance, **c)** Purified polysaccharides or amino acid co-polymers injected in large doses result in "immune paralysis", **d)** The continuous presence of antigen helps to maintain tolerance.

3- T cells become tolerant more readily and remain tolerant longer than B cells.

4- Administration of immunosuppressive drugs enhances tolerance as in transplantation.

5- Administration of cross reacting antigens tends to terminate tolerance.

AUTOIMMUNE DISEASES

Autoimmune diseases occur due to breakdown of the mechanisms that maintain autotolerance. When this occurs, auto-antibodies and self reactive T cells are produced, resulting in tissue damage by several mechanisms.

Etiology of Autoimmune Diseases; the major factors that contribute to the development of autoimmunity are genetic susceptibility and environmental triggers, such as infections or trauma.

1- Genetic predisposition: There is a familial incidence of autoimmune diseases. Most of them appear to be associated with certain MHC genes specially MHC-II genes. For example, rheumatoid arthritis is associated with DR4, thyroiditis with DR5, multiple sclerosis with DR2, SLE with DR2/DR3, type I diabetes with DR3/DR4, ankylosing spondylitis with B27.

2- Infectious microbes may produce peptide antigens that are similar to and cross react with, self antigens this is called **"molecular mimicry".** An immune response to these antigens will result in an immune attack against self antigens, e.g. antibodies formed against M protein of *Strept. pyogenes* cross react with cardiac myosin leading to rheumatic fever.

Infections of particular tissues may enhance expression of costimulators

on tissue APCs leading to activation of T cells that are not specific for the infectious pathogen. This is called **bystander activation.**

It is noted that several microbial diseases are associated with development of autoimmune sequeal; e.g. Rubella with type 1 diabetes, influenza and Campylobacter with Guillain-Barre' syndrome.

3- Alteration of self antigens or the appearance of new antigens under the effect of drugs, chemicals or viral infections.

4- Release of sequestrated antigens: Certain tissues, e.g. eye lens, the sperms and the C.N.S., are "immunologically privileged sites" i.e. are sequestrated so that their antigens have no access to the immune system. When such antigens enter the circulation accidentally e.g. after infection or trauma, they will stimulate antibody and/or cell mediated immunity leading to endophthalmitis, aspermatogenesis or encephalitis.

5- Hormonal influences play a role in some diseases e.g. SLE affects women 10 times more than men.

Mechanisms of Tissue Damage in Autoimmune Diseases: The disease may be **organ** specific, e.g. Hashimoto thyroiditis, Grave's disease or pernicious anaemia or **systemic** as in SLE or rheumatoid arthritis (RA). The tissue damage that occurs can be due to any of the hypersensitivity reactions except type I:

- Cytotoxic reactions (type II) as occurs in autoimmune haemolytic anaemias, Hashimoto thyroiditis and Grave's disease. In the latter disease, antibodies to the surface of the cell stimulate rather than destroy.
- Immune complex deposition (type III) as in SLE and rheumatoid arthritis.
- DTH reactions (type IV) due to autoreactive T cells as in ulcerative colitis, celiac disease, multiple sclerosis and type 1 diabetes.

Laboratory Diagnosis

1- There is elevated serum immunoglobulins.
2- Autoantibodies can be detected in the serum, e.g. anti-nuclear, anti-smooth muscles and anti-mitochondrial antibodies and rheumatoid factor.
3- Testing for antibodies specific to the particular antigen, involved in organ specific diseases e.g. anti-thyroid antibodies.
4- Complement levels may be decreased.
5- Immune complexes may be detected in serum or organ biopsy.

Management varies depending on the disease

- Anti-inflammatory drugs e.g. aspirin, corticosteroids, etc...
- Immunosuppressive antimetabolites e.g. azathioprine and methotrexate.
- Plasmapheresis to remove the offending immune complexes and antibodies.
- Anti-TNF and Abatacept (CTLA4-Ig) that blocks B7-CD28 costimulatory signal are approved for treatment of RA.
- Induction of tolerance.

CHAPTER 23

IMMUNODEFICIENCY DISORDERS

Irnmunodeficiency diseases are caused by congenital (primary) or acquired (secondary) defects in lymphocytes, phagocytes and other mediators of the acquired (adaptive) or innate immunity. The presenting symptoms of these disorders are persistent and recurrent infections which are severe and are caused by unusual organisms, of low pathogenicity or part of the normal microbiota (opportunistic).

PRIMARY IMMUNODEFICIENCIES

These result from total or partial decrease in numbers of one or more of the cells of the irnmune system or their functions. These are usually due to congenital defects in the development of such cells.

Defects in Acquired Immunity:

I- T-Cell Deficiency:

1- **DiGeorge's Syndrome** (congenital thymic hypoplasia) is due to faulty development of the third and fourth pharyngeal pouches during embryogenesis, resulting in absence or hypoplasia of the thymus and parathyroid glands. Since T cell maturation occurs in the thymus; such infants will be born with severe T cell deficiency. It is due to translocation of a large part of chromosome 22.

Manifestations:

The first presentation may be neonatal hypocalcaemic tetany due to hypoparathyroidism followed by recurrent viral, fungal (candida) and protozoal infections very early in life and may be associated with cardiac and facial anomalies (fish mouth and flat face).

Such children should not receive living vaccines since even attenuated organisms can cause infections.

Immunologic findings:

1- Lymphopenia and decreased T cell numbers.

2- Decreased T cell functions:

- Decreased response to phytohaemagglutinin (PHA).
- Impaired delayed hypersensitivity reactions.
- Impaired ability to reject allografts.

-Levels of circulating antibodies to T cell dependent antigens may be low due to low numbers of TH cells.

Treatment: Administration of calcium and vitamin D to treat hypocalcaemia. The condition can be reversed by implanting foetal thymic tissues or administration of thymic hormones. However, such treatment might not be necessary since T cell functions improve with age and is often normal by the age of 5.

II- B Cell Deficiency:

1- X-Linked Infantile (Bruton's) Agammaglobulinaemia:

It affects male infants. The condition manifests by repeated pyogenic bacterial infections 6-9 months after birth (i.e. after maternal immunity disappears). Cell mediated immunity is normal and viral infections, e.g. measles, are readily brought under control.

It is due to block in maturation of B cells due to mutation or deletion of the gene encoding the enzyme B cell tyrosine kinase.

Immunologic findings:

- No circulating B cells.
- Patients lack all classes of immunoglobulins.
- Failure of response to antigenic stimuli e.g. the child remains Schick positive after DTP vaccination.
- Lymph node biopsy after antigenic stimuli shows absence of plasma cells.
- Hypoplasia of the tonsils.

Treatment:

- Avoidance of infection.
- Antibiotic therapy when indicated.
- Gamma globulin injections every 3-4 weeks to maintain adequate concentration of serum immunoglobulins.
- Specific hyperimmune serum when indicated, e.g. in varicella-zoster virus infection.

2- Selective Immunoglobulin Deficiency:

In which there is a deficiency of one or two of the immunoglobulins while levels of the others are normal or elevated.

Selective IgA Deficiency is the most common condition in which there are low or normal numbers of mature B cells that fail to secrete IgA due to an unknown genetic lesion. It is characterized by recurrent respiratory infections, diarrhoea, and associated with autoimmune disease, malignancy and allergy. However, some individuals may be asymptomatic.

Immunologic findings:

- Low levels of serum IgA less than 10 mg/dL.
- Normal or low numbers of IgA - bearing B cells.
- Normal levels of serum IgG and IgM.

Treatment:

- Antibiotics to treat infections.
- Avoid the use of gamma globulins since IgA in these preparations may be recognized as foreign. Antibodies to IgA are formed leading to hypersensitivity reactions.

3- **X-linked Hyper-IgM Syndrome** is characterized by defective B cell heavy chain class switching, resulting in IgM being the major serum antibody. It is due to failure of expression of functional CD40L on TH cells -as a result of mutation of the CD40L gene- leading to failure of class switching and failure of T cell dependent activation of B cells and macrophages leading to severe deficiency of CMI against intracellular microbes. However, it is a rare syndrome.

4- Transient Hypogammaglobulinaemia of Infancy: In which there is delayed onset of IgG synthesis. There is increased susceptibility to bacterial infections starting the sixth month of life. The condition usually resolves by the 16-30 months. It is treated by antibiotics and sometimes gamma globulins.

5- **Common Variable Hypogammaglobulinaemia.**

Ill- Combined T and B Cells Deficiency:

Combined immunodeficiency diseases are variable in cause and severity.

1- Severe Combined Immunodeficiency (SCID):

This may be due to failure of differentiation of stem cells into T and B cells. However, the explanation of this failure is not known.

Both humoral and cell mediated immunity are deficient. These infants suffer from all kinds of infections; viral, bacterial, fungal and protozoal. Death occurs early in childhood (before 1 year). The disease may be inherited in an X-linked recessive or autosomal recessive manner.

Immunologic findings:

a- Hypoplasia of the thymus as shown by chest x-ray.

b- Lymphopenia and decreased T cell numbers and functions.

- Impaired response to PHA.
- Impaired delayed hypersensitivity reactions.

c- Decreased B cell number and functions:

- Serum immunoglobulins are absent or decreased.
- No response to antigenic stimuli.

Treatment:

Patients may be made completely normal by grafting with histocompatible bone marrow from a sibling. Incompatible grafts will lead to a fatal "graft versus host" (GVH) reaction.

- Gamma globulins may be administered.
- Antibiotics are used for treatment of infections.
- Gene therapy proved successful in treatment of some types of SCID. However, few treated patients developed leukaemia.

2- **Ataxia-Telangiectasia** is a combined immunodeficiency with ataxia and dilated small vessels of the sclera of the eye. It develops at 1 -2 years of age. It is autosomal recessive and is associated with selective IgA deficiency

3- **Wiskott-Aldrich Syndrome:** Immunodeficiency with eczema, recurrent infections and thrombocytopenia. It is an X-linked recessive disease. Patients are prone to the development of malignant lymphoma.

4- **SCID with Adenine Deaminase Deficiency (ADA).**

5- **Bare Lymphocyte Syndrome:** There is deficient expression of class II MHC molecules with or without class I expression.

Bone marrow transplantation is currently the treatment of choice for various immunodeficiency diseases and has been successful in the treatment of SCID with ADA deficiency, Wiskott-Aldrich syndrome, bare lymphocyte syndrome and leucocyte adhesion deficiency (LAD).

Gene therapy remains a distant goal for most human immunodeficiencies at present.

Defects in Innate Immunity:

IV- Phagocytic Cell Defects: These may be due to:

a- Primary defects in neutrophil number which are rare as most cases are secondary to treatment with cytotoxic drugs.

b- Genetic defects in the different steps of phagocytic cell actions leading to elimination of the pathogen. Defective migration and adhesion as in Leucocyte Adhesion Deficiency syndrome (LAD), defective phagocytosis and lysosomal fusion as in Chediak Higashi Syndrome (CHS), and defective respiratory burst for intracellular killing as in Chronic Granulomatous Disease (CGD).

1- Leucocyte Adhesion Deficiency (LAD) is due to a defect in the *f*>-subunit (CD 18) of integrins (one of the adhesion molecules), preventing their expression. The **P** subunit is common to three integrins LFA-1, complement receptors CR3 (Mac-1) and CR4. These are glycoproteins found on granulocytes, monocytes and lymphocytes and are important in conveying to the leucocyte the surface properties of adhesion and motility. Therefore, adhesion and migration of all white cells to the site of inflammation are impaired predisposing such patients to recurrent soft-tissue infections without pus formation and impaired wound healing. Newborns have delayed separation of umbilical cord. Lymphocyte functions are also affected due to lack of LFA-1 expression. Bone marrow transplantation is the treatment of choice.

2- Chediak Higashi Syndrome (CHS) is an autosomal recessive disease. It is characterized by abnormal giant granules which are defective in degranulation and in fusion of lysosomes with phagosomes and decrease in proteolytic enzymes. This leads to diminished intracellular killing of pathogens leading to severe recurrent bacterial and fungal infections. It is associated with albinism and bleeding tendencies. **NK cell** functions are impaired, probably because of an abnormality in the cytoplasmic granules that store proteins mediating cytolysis.

3- **Chronic Granulomatous Disease (CGD):** The commonest type is sex linked due to a defect in NADPH oxidase enzyme and failure to generate superoxide anion, and other 0_2 radicals needed for intracellular killing of phagocytosed organisms. This results in severe recurrent bacterial and fungal infections. It is treated with antibiotics and IFN-y. Gene therapy by transfer of the correct gene into the bone marrow stem cells is under trial with encouraging results.

V- Complement Deficiencies:

These are rare conditions in which certain components of the complement system are absent. These patients suffer from increased incidence of infections and autoimmune diseases, for example:

1- Deficiency in C2 and C4 is associated with a syndrome resembling SLE possibly due to failure of the complement-dependent mechanisms to eliminate immune complexes.

2- Deficiency in C3, is associated with recurrent pyogenic infections while deficiency in C5-C8 increased susceptibility to disseminated *N.*

meningitidis and *N. gonorrhoeae* since complement mediated lysis of neisseria is a major control mechanism.

3- Deficiency in factor B and properdin of the alternate pathway is associated with increased neisseria infections.

4- Deficiency of complement regulating proteins e.g. CI inhibitor leads to overuse of CI, C4 or C2 which causes oedema at mucosal surfaces i.e. hereditary angioedema.

5- Deficiency of complement receptors, see LAD mentioned above.

SECONDARY IMMUNODEFICIENCY

Immune responsiveness can be depressed non-specifically by many factors:

1- Infections:

a- Viral: It has been noticed that a child with measles reverts from tuberculin positive to tuberculin negative i.e. transient depression of cell mediated immunity. Human immunodeficiency virus (HIV) causes acquired irnmune deficiency syndrome (AIDS).

b- Bacterial, e.g. tuberculosis and leprosy.

c- Fungal (coccidioidomycosis).

d- Parasitic (schistosomiasis).

The immunodeficiency induced by infections is usually mild and transient except for AIDS, (see Vol. II p. 134)

2- Malignancies, e.g. leukaemias and myelomas.

3- Autoimmune disease, e.g. SLE.

4- Other conditions which are chronic, debilitating, or lead to loss of proteins, e.g. diabetes, burns, uraemia, malnutrition and old age.

5- Prolonged procedures in hospitals, e.g.:

- Urinary catheterization causes a focus of infection and pyelonephritis.
- Intravenous cannulation causes a focus of infection and septicaemia.
- Artificial prosthesis, e.g. artificial valves, or artificial hip are local sites of immunodeficiency.

6- Drugs e.g.

a- Immunosuppressive drugs used intentionally as after kidney or heart graft or in treatment of some autoimmune diseases, e.g. corticosteroids or antimetabolites.

b- Cytotoxic drugs used in cancer therapy.

c- Antibiotics which may lead to agranulocytosis or which on prolonged use may lead to disturbed microbiota and colonization with unmanageable serious infections.

ASSESSMENT OF IMMUNE COMPETENCE

I- Assessment of T cell Competence:

a- Enumeration of T cells:

1- Enumeration of total T cells by using monoclonal antibodies to CD3 conjugated to fluorescent dyes. The cells are counted by fluorescent microscopy or flow cytometry (FCM).

2- Enumeration of T cell subsets using monoclonal antibodies to CD4 or CD8, or monoclonal antibodies to other T cell surface markers.

b- Evaluation of T cell Functions:

1- Delayed Hypersensitivity Skin Tests:

These are skin tests that demonstrate the presence of delayed-type hypersensitivity to universal antigens. Most normal persons respond with delayed-type reactions (induration after 48 hrs) when injected intradermally with several of the following antigens: PPD, candidin, mumps, streptokinase, streptodornase, trichophytin, due to previous exposure. Absence of reactions to several of these skin tests suggest impairment of cell mediated immunity or T cell functions.

2- Lymphoblast Transformation:

Normally functioning T cells transform to large blast cells with great increase in DNA synthesis when exposed to the mitogen phyto-haemagglutinin (PHA) or concanavalin A (plant extracts that stimulate T cells). The degree of mitosis is measured by the degree of incorporation of tritiated thymidine added to the reaction medium.

3- Assessment of Cytokine production and cytokine receptors.

4- Lymph Node Biopsy to study T cell areas.

II- Assessment of B cell Competence

a- Enumeration of B cells:

1- By immunofluorescent techniques using fluorescein labelled poly-clonal anti-human immunoglobulin or by flow cytometry.

2- Monoclonal antisera to specific heavy chain or light chain type may be used to evaluate subclasses of immunoglobulins.

b- Evaluation of B cell Functions:

1- Lymphocyte transformation: Normally functioning B cells can be stimulated to transform to large blast cells with great increase in DNA synthesis when exposed to lipopolysaccharides (LPS). This can be

measured by the degree of incorporation of tritiated thymidine added to the reaction medium.

2- Detenriination of the level of gamma globulins by protein electrophoresis. A decrease or increase in gamma globulins can point to an abnormality in antibody production.

3- Quantitation of the different types of immunoglobulins by different methods, e.g. radial immunodiffusion, ELISA,etc...

4- Active immunization may be used as an ***in*** *vivo* method to test for the ability to produce antibodies. For example, immunization with tetanus vaccine and measuring the level of anti-tetanus antibodies produced.

5- Lymph node biopsy to study B cell areas (lack of germinal centers).

III- <u>Assessment of Phagocytic Functions</u>:

1- Assessment of chemotaxis is done by testing the ability of phagocytic cells to migrate through membranes toward a chemotactic substance.

2- Assessment of ingestion of phagocytosis is done by direct visual counting of ingested particles, (e.g. heat killed yeast) using a microscope.

3- Assessment of ingestion and/or intracellular killing using nitroblue tetrazohum (NBT) reduction test. Normal phagocytic cells when stimulated, (e.g. by endotoxin) ingest the dye and reduce it (it changes from yellow to blue). Abnormality in intracellular killing abilities is associated with decreased NBT reduction.

IV- <u>Assessment of Complement</u>:

1- Measuring the quantity of the different components of the complement in the serum, e.g. C3, C4, etc... This can be done by radial immunodiffusion, RIA or ELISA.

2- Measuring the total haemolytic activity of the complement: The assay is based on the ability of the intact complement system to rupture sheep RBCs coated with their antibody.

CHAPTER 24 COMPARATIVE

Innate and Acquired Immunity

	Innate Immunity	Acquired Immunity
I- Components		
Physical and chemical barriers	Skin, mucosal epithelia, antimicrobial chemicals.	Lymphocytes in epithelia and antibodies secreted at epithelial surfaces
Blood proteins	Complement, CRP, MBL*.	Antibodies
Cells	Phagocytes (macrophage and neutrophils), NK cells.	Lymphocytes T and B
II- Characters		
Specificity	Non-specific Act against all antigens without previous exposure	Specific Act against a particular antigen
Presence	Since birth	After exposure to pathogen
Memory	None	Yes
Non-reactive to self	Yes	Yes
Onset of action	Prompt, immediate.	Delayed (need some time for recognition of antigens)
Efficiency	Less efficient.	More efficient. Improves with second exposure

TABLES

Note: Both innate and acquired mechanisms interact with one another through; antigen presentation and cytokine secretion by macrophages. T cells and NK cells activate one another. Antibodies secreted by B cells enhance phagocytosis by macrophages (opsonization) and ADCC by macrophages and NK cells.

•VIHI = mannose-binding lectins.

Lymphocyte Classes

Class	Function	Antigen receptor	Selected surface markers	Percent of total lymphocyt es		
				Blood	Lymph node	Spleen
T cells						
CD4 TH	Stimulate **B** cells. Activate NK cells & macrophages by secreted cytokines	ap hetero-dimer	$CD3^+$ $CD4^+$ CD8	50-60	50-60	50-60
CD8 TC (CTLs)	Kill virus infected cells, tumor cells and graft cells.	aP hetero-dimer	$CD3^+$ $CD8^+$ CD4"	20-25	15-20	10-15
B cells	Antibody production Humoral immunity	Surface antibody (IgM/IgD)	Fc receptors class **II** MHC CD19 CD21	10-15	20-25	40-45
Natural killer (NK) cells	Kill virus infected cells and tumor cells. ADCC.	Killer cell Ig-like receptor	Fc receptors for IgG (CD 16)	5-10	rare	5-10

N.B: In most tissues the ratio of CD4 to CD8 is about 2/1. Ig = immunoglobulin.

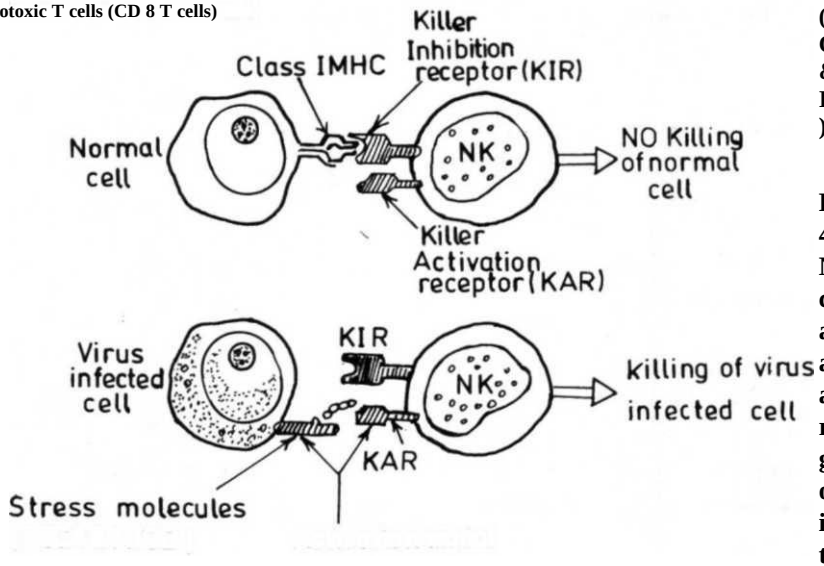
TH1 and TH2

	TH1	TH2
Interleukins produced	IFN-y, IL-2, TNF-p, IL-3, **GM**-CSF	IL-4, IL-5, IL-6, IL-13, TGFp IL-3, GM-CSF
Production enhanced by	IL-12 IFN-y	IL-4
Production inhibited by	IL-4, IL-10	IFN-y
Surface markers that are more prevalent on	CD26, CCR5, CXCR3	CCR3, CCR4
Main functions	Cell mediated immunity. Activation of macrophages, NK and TC. Stimulate production of some classes of antibody	Humoral immunity **B** cell differentiation and antibody production. Antibody class switching. Stimulate mast cells and eosinophils.

Effector Cells in Cell Mediated Immunity

Effector cell	CTLs*	NK cells	Macrophages
CD markers	TCR, CD3, CD8, CD2	CD 16, CD56, CD2	CD14
Antigen recognition	By specific TCR	ADCC: specific by IgG. By activation and inhibition receptors	Non-specific
MHC recognition required for killing	Yes, class I	No, MHC-I recognition inhibits killing	No
Effector molecules	Perforin, granzymes cytokines (TNF-(3, IFN-y)	Perforin, granzymes cytokines (TNF-**P,** IFN-y)	TNF-a, enzymes, nitric oxide, oxygen radicals
Activity enhanced by	IL-2	IL-12, IL-15, IL-2 and IFN type I	IFN-y
ADCC	No	Yes	Yes
Act in	Acquired Immunity	Innate and Acquired Immunity	Innate and Acquired Immunity
Action	Specific	Non-specific	Non-specific

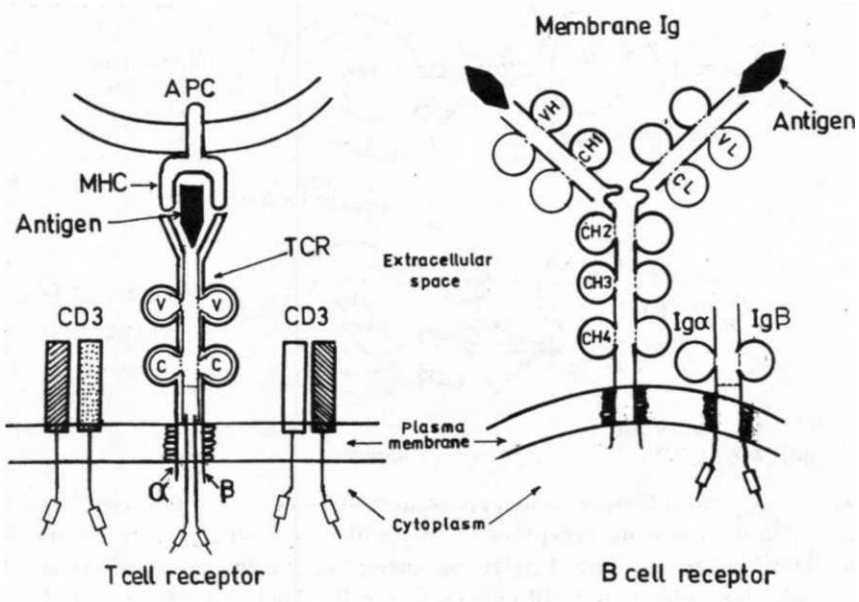
CTLs = Cytotoxic T cells (CD 8 T cells)



(MICA &MICB) (Fig. 41): NK cell activation and recognition of infected cells is regulated by a combination of killer activating receptors (KAR) and killer inhibitory receptors (KIR) on NK cells. KIR bind to self class I MHC on normal cells and inhibit NK cells killing action; that is why NK cells do not kill normal host cells. They kill cells with reduced or lost expression of MHC-I as virus infected cells and tumour cells. At the same time, activating signals are induced by binding of KAR to stress molecules (MICA and MICB) that are expressed on the surface of infected cells, thus the infected cells are killed.

T Cell and B cell Antigen Receptors

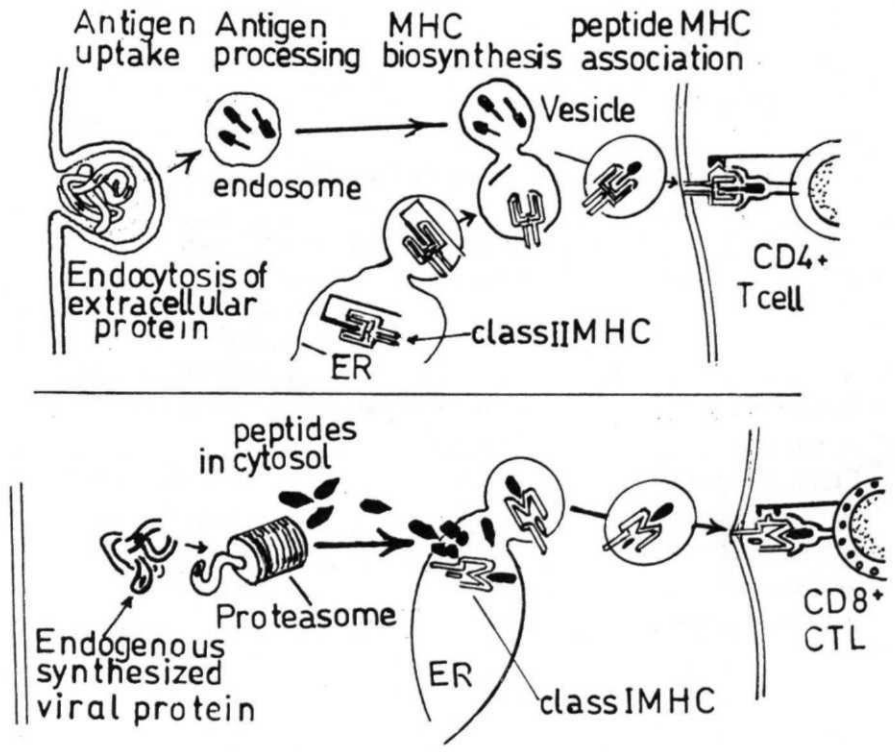
	T cell Antigen Receptor (TCR)	B cell Antigen Receptor (BCR)
Components	a and p chains	Heavy and light chains
Number of Ig domains	One V domain and one C domain in each chain	Heavy chain: one V domain and 3 or 4 C domains Light chain: one V domain and one C domain
Isotype/ lymphocyte	I (a/0)	2 (IgM and IgD)
Isotype switching	No	Yes
Number of combining sites to antigen molecule	1	2
Mobility	Rigid	Flexible (hinge region)
Antigen recognition	On APC associated with MHC	Direct
Nature of antigen that may be bound	Processed cell-bound peptide/MHC complex.	Unprocessed antigen, macromolecules (proteins, lipids, polysaccharides) and small chemicals.
Signal transduction molecules	CD3	Ig-a and Ig-B
Is secretion possible	No (it is a cell surface molecule)	Yes (as antibodies in blood and body fluids)



(Fig. 42): T cell antigen receptor (TCR) and B cell antigen receptor (BCR).

Antigen Processing Pathways

	Endogenous (cytosolic)	**Exogenous (vesicular)**
Types of cells in which antigen is processed then presented	All nucleated cells	Dendritic cells, Macrophages and B cells
Source of protein antigen	Mainly endogenously synthesized proteins in the cytosol e.g. viruses.	Extracellular proteins or microbes internalized in vesicular compartment
Site of processing	Cytoplasm	Vesicles
Enzymes responsible for peptide generation	Cytosolic proteasomes	Endosomal and lysosomal proteases
Site of peptide loading of MHC	Endoplasmic reticulum (ER)	Specialized vesicular compartment
Peptides bind to	MHC-I	MHC-II
Peptide/MHC complex is presented to	CD8 cytotoxic T cells	CD4 helper T cells
Outcome	Killing of presenting cells by CTLS	Secretion of cytokines by TH cells activating macrophages and other cells.



(Fig. 43): Pathways of antigen processing and presentation. The upper figure demonstrates the exogenous (vesicular) pathway of antigen processing and presentation by MHC-II to CD4 T cells. The lower figure demonstrates the endogenous (cytosolic) pathway of antigen processing and presentation by MHC-I to CD8 T cells. ER= endoplasmic reticulum

Antigens and Superantigens

	Antigens	Superantigens
Processing in APCs	Occurs	Does not occur
Presentation by MHC	Essential	Does not occur
Binding to MHC	into the peptide-binding groove	outside the peptide-binding groove
TCR bind by	variable regions of both a and P chains	variable region of p chain only
Interaction with and activation of T cells	Specific Few T cells only	Non-specific Many T cells
Cytokines released	The required levels	Very high harmful levels
Development of memory	Occurs	Does not occur
Outcome	Acquired immunity (beneficial to host)	Anergy (harmful to host)
Example	All ordinary antigens	*St. aureus* enterotoxin and TSST. *Str. pyogenes* pyrogenic toxin. Mouse mammary tumour virus (MMTV).

T Cell Dependent Antigens and T Cell Independent Antigens

	T cell dependent Ag	T cell independent Ag
Types of antigen	Peptide antigens	Non-peptide large polymers with repeating antigenic epitopes e.g. capsular polysaccharides
Antibody produced	All classes IgG, IgM, IgA, IgE	Only IgM
Class switching	Occurs	Does not occur
Development of memory cells	Occurs	Does not occur
Secondary immune response	Occurs	Does not occur

Properties of Immunoglobulins

Property	IgG	IgM	IgA	IgE	IgD
% in Serum	75	9	15	0.004	0.2
M.W. (X 1000)	160	900	170-400	190	180
Half life (days)	23	5	6	2.5	3
Molecular form	Monomer	Pentamer	Monomer or dimer	Monome r	Monome r
Subclass number	4	2	2	1	1
H-chain type	Y		a	e	5
J chain		+	+	–	–
Secretory piece	–	–	+	–	–
Complement activation	+	+ +	–	–	–
Crosses placenta	+	–	–	–	–
Mediates allergy	–	–	–	+	–
Opsonization	+	–	–	–	–
Binds to Fc receptors on phagocytes or NK cells (ADCC)	+				
Antigen receptor on B cells	–	+	–	–	+

Primary and Secondary Immune Response

Features	Primary response	Secondary response
Induction period	Long (7-10) days	Short (1-3) days
Phase for antigen recognition	Is needed	Is not needed
Antigen concentration needed to induce response	Relatively high (adjuvant may be needed)	Relatively low (adjuvant not necessary)
Antibody peak level	Low	High
Type of immunoglobulin	IgM	IgG (IgA, IgE under certain situations)
Persistence of antibody	Short time	Long time (months)
Antibody affinity	Variable to low	High (affinity maturation)
Memory cells	Absent	Present
Inducing agent	All immunogens	Protein antigen only

Pathways of Complement Activation

	Classic	Alternate	Lectin
Type of immunity	Acquired	Innate	Innate
Initiated by	Antigen-antibody complex	Microbial cell surface products (endotoxin, zymosan) binding to C3b	Mannose-binding lectin (MBL) binds to mannose residues on surface of microbe
Activation	All components starting Ci, C_4, C2, c_3 (then C5,6,7,8,9)	Starts with activation of **C3** (then C5,6,7,8,9) by-passing Ci, **C4,** C2	MBL associated protease activates **c4**, C2 then C **3** (then C5,6,7,8,9)
Properdin system* involved	No	Yes	No
Complement mediated defense mechanisms	Specific after antibody appears	Non-specific before antibody appears	Non-specific before antibody appears

***Properdin system includes; Factor B, Factor D and Properdin .**

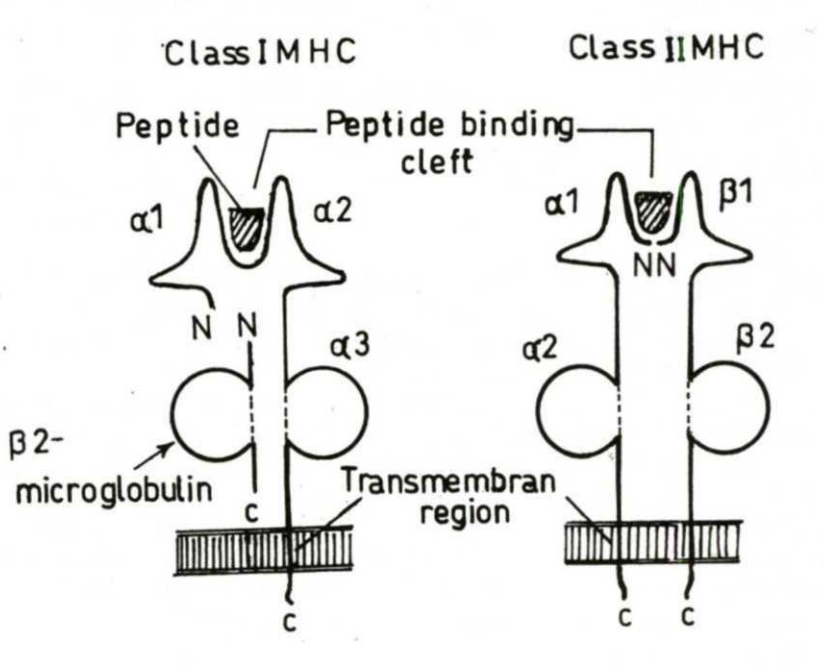
Active and Passive Immunity

	Active immunity	Passive immunity
Mediated by	Antibody and **T** cells	Antibody only
Onset of protective action	Slow onset	Immediate onset
Persistence of action	Long duration (years)	Short duration (weeks)
Development of memory	Occurs	Does not occur
Examples	Natural infection or vaccination.	- Passive immunization with antitoxin or gamma globulins. - Maternally-acquired antibodies.

Class I MHC and Class II MHC

	MHC I	MHC II
Nomenclature	HLA- A, B, C	HLA-DP, DQ, DR
Found on	all nucleated cells	dendritic cells, macrophages, B cells and activated T cells
Recognized by	CD8 TC (CTLs)	CD4 TH cells
Peptides that are bound	Endogenous Ag processed in cytosol	Exogenous Ag processed in vesicles
Bind peptides	of 8-9 amino acids	of 12-25 amino acids
Functions	Presentation of antigens to TC cells leading to elimination of abnormal or infected host cells.	Presentation of antigen to TH cells which secrete cytokines.
B2 **microglobulin**	Yes	No
Coded for	on chromosome 6 but light chain p2 microglobulin is on chromosome 15	entirely on chromosome 6.

Note that class I MHC molecules are formed on all nucleated cells, including those that have class II MHC molecules on their surface.



(Fig. 44): Class I MHC and Class II MHC molecules.

Classification of instruments and patient care items according to the risk of infection when used, and the recommended methods of sterilization or decontamination

Critical items; are instruments that enter sterile tissues, cavities or vascular systems. These include surgical instruments, needles, implants and catheters. These should be sterilized by autoclaving, ethylene oxide or plasma sterilizers.

Semi-critical items; are objects that come in contact with mucous membranes or non-intact skin. These include endoscopes and clinical thermometers. These should be decontaminated by high level disinfectants e.g. gluteraldehyde, chlorine-active substances, and hydrogen peroxide. These kill all microbial pathogens but not all bacterial spores.

Non-critical items, these are objects that contact intact skin only, but not mucous membrane. These include sphygmomanometers, bed linen or floors. These are decontaminated by intermediate level disinfectants, which kill all microbial pathogens but not bacterial spores e.g. isopropyl alcohol and iodophors. These items can also be disinfected by low level disinfectants e.g. quaternary ammonium compounds for disinfection of floors and food preparation areas. These kill most vegetative bacteria (except tubercle bacilli) and lipid-enveloped and medium-sized viruses such as HBV and HIV.

The Foetus as an Allograft (Immunologic Tolerance of the Foetus)

The foetus is a naturally occurring allograft that is not rejected. The foetus expresses paternally inherited antigens that are allogenic to the mother. Protection of the foetus against the maternal immune system involves several mechanisms including special molecular and barrier features of the placenta and local immunosuppression.

The anatomic location of the foetus is a critical factor in the absence of rejection. The **placenta** which is the region of local contact between mother and foetus is composed of **vascular trophoblast** exposed to maternal blood for nutrition exchange or **implantation site trophoblast** which infiltrates the uterine lining **(decidua)** for anchoring the placenta to the mother. Different explanations for the absence of rejection include:-

1- Trophoblast cells do not express paternal MHC molecules. They express only nonpolymorphic class IB molecules called HLA-G. These molecules protect trophoblast cells from maternal NK cells mediated lysis.

2-Trophoblast cells fail to act as APC as they lack costimulatory molecules. CTLA4 is expressed in foetal tissues at the matemal-foetal interface throughout gestation. It blocks costimulatory B7-CD28 signal.

3-A specialized subset of NK cells called uterine NK are the major type present in the implantation site. IFN-y produced by these cells is essential for decidual development.

4-Uterine decidua may be an **immunologically privileged site.** The barrier is not simply anatomical, but is likely created by functional inhibition:

a- Regulatory T (Treg) cells are expanded in the deciduas during pregnancy where they secrete the immunosuppressive TGF-P and IL-10. b- TH2 cytokines produced at the matemal-foetal interface are responsible for local suppression of TH1 responses to foetal antigens, c- The enzyme indolamine 2,3-dioxygenase (IDO) is present in the decidua. It metabolizes tryptophan keeping its levels low, which blocks maternal T cell responses to the foetus, d- Trophoblasts and deciduas are relatively resistant to complement mediated lysis because they express high levels of C3 and C4 inhibitors i.e. decay accelerating factor (DAF) and membrane cofactor protein (MCP).

e- Cells of the trophoblast and decidua express FasL and induce deletion of T cells that recognize paternal HLA antigens in the placenta.

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Abbreviations Used in the Text

ADCC	Antibody-dependent cell-mediated cytotoxicity.
AFP	Alpha-foeto-protein.
AIDS	Acquired immunodeficiency syndrome.
Amp"	Ampicillin resistance gene on plasmid
ANA	Antinuclear antibody.
ANF	Antinuclear factor.
APCs	Antigen-presenting cells.
ASO	Antistreptolysin O.
ATP	Adenosine triphosphate.
B27	HLA antigen with strong disease association.
BAF	B cell-activating factor.
BCG	Bacillus Calmette-Guerin.
BCDF	B cells differentiation factor.
BCGF	B cell growth factors.
C	Complement.
cAMP	Cyclic adenosine monophosphate.
CD	Cluster of differentiation.
CD3	Antigenic marker on T cell associated with T cell receptor.
CD4	Antigenic marker of helper T cells.
CD8	Antigenic marker of cytotoxic T cells.
CEA	Carcinoembryonic antigen.
CGD	Chronic granulomatous disease.
CHS	Chediak Higashi Syndrome
CH	Constant domain of H chain.
CL	Constant domain of L chain.
CMI	Cell-mediated immunity.
CMV	Cytomegalovirus.
Con A	Concanavalin A.
CRP	C-reactive protein.
CSF	Colony-stimulating factor.
CTLs	Cytotoxic T lymphocytes.
DIC	Disseminated intravascular coagulation.
DP	Human class II MHC allele.
DQ	Human class II MHC allele.
DR	D-related HLA locus in humans.
DTH	Delayed type hypersensitivity.
DTaP	Diphtheria and tetanus toxoid + acellular pertussis vaccine.
DTP	Diphtheria and tetanus toxoid combined with pertussis vaccine.
EB	Epstein-Barr.
EcoRl	***E.coli*** restriction endonuclease.
ELISA	Enzyme-linked immunosorbent assay.
F+	Cell carrying fertility plasmid.
F-	Cell does not carry fertility palsmid.
Fab	Antigen-binding fragment.
FAS	Factor of apoptotic signal.
Fc	Crystallizable fragment.
FCM	Flow cytometry.
GM-CSF	Granulocyte-macrophage colony-stimulating factor.
G6PD	Glucose-6-phosphate dehydrogenase.
GVH	Graft-versus-host.
HAT	Hypoxanthine, aminopterin, and thymidine.
HCG	Human chorionic gonadotrophins.
HIV	Human immunodeficiency virus.
HLA	Human leucocyte antigen.
ICAM	Intracellular adhesion molecules.
IFN	Interferon.
IL-1,IL-12	Interleukins 1-12.
J chain	Joining monomers of IgA and IgM.
Kbp	Kilo base pair.
LAD	Leucocytes Adhesion Deficiency.
LAK	Lymphokine-activated killer (cells).
LFA-1	Lymohocyte functional antigen.
LPS	Lipopolysaccharide.
MAC-1	Macrophage-1 glycoprotein.
MAF	Macrophage activating factor.
MBL	Mannose- binding lectins.
MHC	Major histocompatibility complex.
MIF	Migration inhibitory factor.
MLC	Mixed lymphocyte (leucocyte) culture.
NBT	Nitroblue tetrazolium.
NK	Natural killer (cells).
PAIs	Pathogenicity islands.
PEG	Polyethylene glycol.
PHA	Phytohaemagglutinin.
PMN	Polymorphonuclear neutrophil.
PPD	Purified protein derivative (tuberculin).
PWM	Pokeweed mitogen.
RFLP	Restriction fragment length polymorphism.
RIA	Radioimmunoassay.
RPR	Rapid plasma reagin.
SCID	Severe combined immunodeficiency.
SLE	Systemic lupus erythematosis.
SRS-A	Slow-reacting substance of anaphylaxis.
SAg	Superantigen.
TAA	Tumour associated antigens.
TAB	Typhoid paratyphoid A and B vaccine.
TATA	Tumour-associated transplantation antigen.
TCR	T cell receptor.
Tc	Cytotoxic T cells.
TGF	Transforming growth factor.
TH	Helper T cells.
TI	T independent antigen
TNF	Tumour necrosis factor.
TSA	Tumour specific antigens.
tra	Transfer promotion gene.
TSH	Thyroid stimulating hormone.
VDRL	Venereal Disease Research Laboratory.
VH	Variable domain of heavy chain.
VL	Variable domain of light chain.
Z.N.	Ziehl Neelsen stain.

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