PRINCIPLES OF CELL & MOLECULAR BIOLOGY & BIOINFORMATICS

(DIBO1)

(PG DIPLOMA)

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Lesson1.1.1

MORPHOLOGY AND ULTRASTRUCTURE OF BACTERIA

Objective

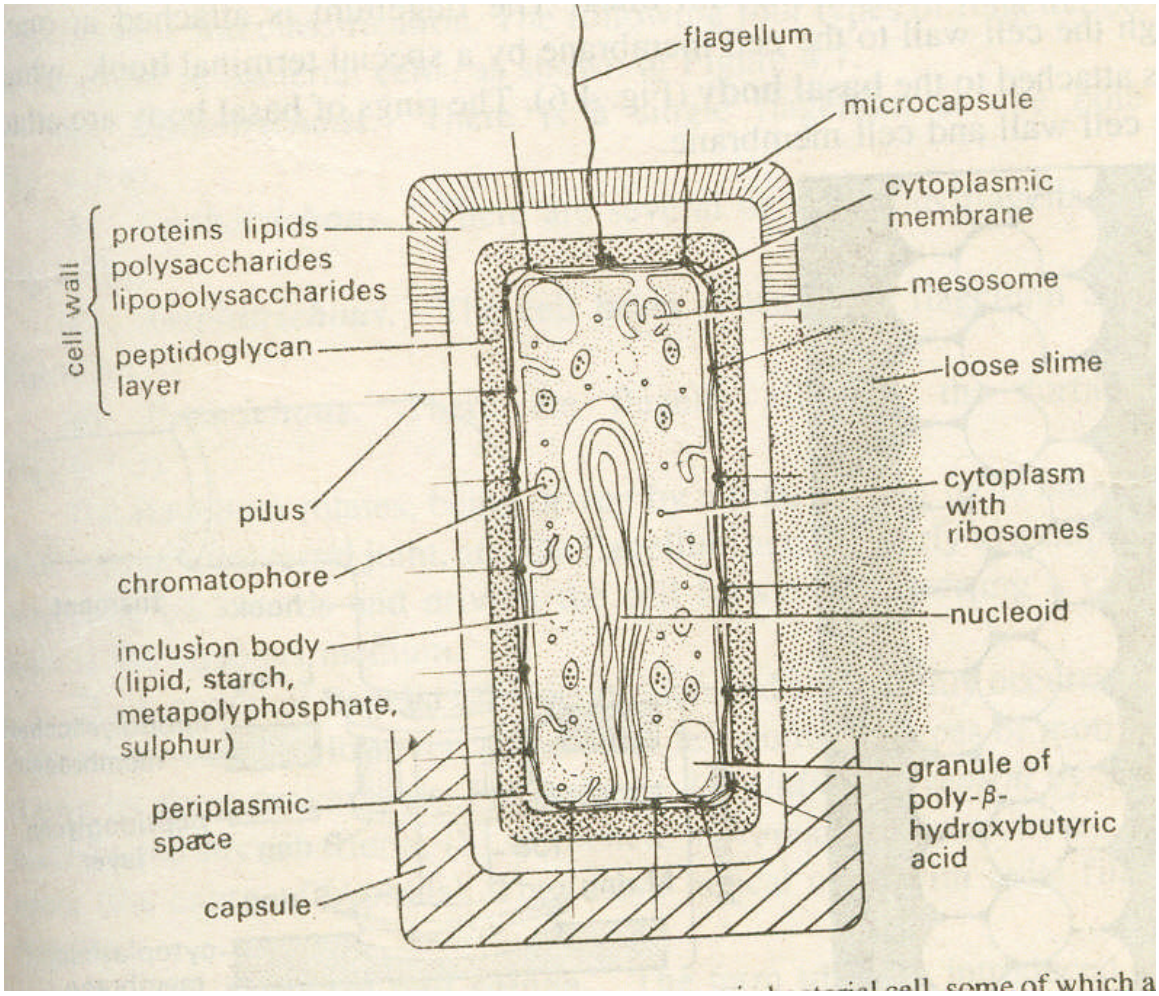
Bacterial cell is a typical prokaryotic cell .In the present chapter the structure and morphology of bacteria were clearly explained

Objective

1.1.1.1 Introduction

1.1.1.2 Morphology of bacterial cell

Size shape, structure and arrangement, all these characteristic features constitute the morphology.



1)Size

Bacteria are very small, most being approximately 0.5 to 1.0 um in diameter. The surface area / volume ratio of bacteria is high compared to the same ratio for larger organisms of similar shape. This would be the advantageous factor, that there bacteria no need of circulatory system to distribute nutrients, only little cytoplasmic movement is enough. And disadvantages of high surface area / volume is that it limits the size of bacteria to microscopic dimensions.

2)Shape & Arrangement

The shape of bacteria is governed by its rigid cell wall, various cell shapes occur among the bacteria. Generally bacteria are unicellular. However, in some cases, cell divisions and cross-wall formation are not followed by the separation of daughter cells, it leads to the appearance of multicellular structures, the shape of these structures depend upon the planes of cell division.

Typical bacterial cells are spherical, i.e., Cocci – (singular-Coccus)

Straight rod ⟶ Bacilli – (singular – Bacillus)

Generally most bacterial species have cells that are of a fairly constant and characteristic shape. But some cells exhibit a variety of shapes, i.e., pleomorphic in nature.

Bacterical cells are usually arranged in a manner characteristic of their particular species. Cocci appear in several characteristic arrangements, depending on the plane of division.

i. Diplococci – Cells divide in one plane and remain attached to form chains.

ii. Streptococci – Cells divide in one plane and remain attached to form chains.

iii. Staphylococci – Cells divide in three planes, in an irregular pattern, producing bunches of cocci.

iv. Tetra cocci (or) Tetrads – Cells divide in 2 planes and characteristically form groups of 4 cells.

v. Sarcinae – Cells divide in 3 planes in a regular pattern, producing a cnboidal arrangement of cells.

Bacilli mostly occur singly, and in some cases it may appear in pains or chains.

i. Diplobacilli – Bacilli in pairs.

Ii Streptobacilli – Bacilli are arranged in chains.

Ex: Bacilles subtilis

iii. Trichomes - These forms are similar to chains but have a much larger area of contact between adjacent cells.

Ex: Beggiata, Saprospira

In Corynebacterium diphtheriae, the cells are lived side by side like match sticks. This type of arrangement is called palisade arrangement.

In Streptomyces species, long, branched, multinucleate filaments are present. These filaments are called hyphae which collectively form mycelium.

In Curved shaped bacteria, the curve with a twist, bacteria with less than one complete twist (or) turn have vibroid shape, whereas those with one or more complete turns have a helical shape.

Spiral shaped bacteria are also seen. Spirilla are rigid helical bacteria. Ex: Spirillum volutans.

Spirochetes are helical bacteria but these are highly flexible. Ex: Treponema pallidum.

In addition to common bacterial shapes, the following types also pecur.

Pear shaped cells - Ex: Pasteuria

Lobed spheres - Ex: Sulfolobus

Rods with squared - Ex: Bacillus authracis

Stacks of coins

Rods with helically - Ex: Seliberia

Sculptured surface

3)Motility

In Bacteria, Motility is by flagellum.

a)Flagella

Bacterial flagella are hair-like, helical appendages that protrude through cell wall and are responsible for swimming motility. These are relatively long projections extending outward from the cytoplasmic membrane. The flagella is 0.01 to 0.02 um in diameter. Their location on the cell varies depending on the bacterial species.

Structure

A typical flagellum is composed of 3 parts.

i. Basal body → Associated with cytoplasmic membrane and cell wall.

ii. Hook

iii. Filament

Some G –ve bacteria have a sheath surrounding the flagellum. This sheath is continuous with the outer membrane of the Gram-ve cell wall.

Bacterial flagellum is composed of many subunits of protein called Flagellin. The filament of the flagellum is attached to the cell by book and basal body. Basal body has a set of rings that attach to the cytoplasmic membrane and a rod that posses through the rings to anchor the flagellum to the cell.

In Gram-ve bacteria, the basal body has 2 set of rings, with each set containing 2 rings. The two rings that attach cytoplasmic membrane are designated of the cell envelope and designated as L and P.

In G+ve bacteria, only one set of rings are present, and this set attached to the cytoplasmic membrane. These are designated as S and M.

The hook structures attaches the filament of the bacterial flagellum to the rod of the basal body. The flagellum is assembled in a series of steps involving the expression of over 40 genes. The basal body is assembled 1st followed by the hook, and finally the filament. The filament is made by exporting flagellin proteins, the subunit structures of the flagellum, through a central hollow-core of the growing flagellum. The flagellin molecules add to the tip of the flagellum, thus allowing it to grow longer. This is called a self assembly process because each flagellin molecule adds to the existing structure without the aid of an enzyme. The process slows as the flagellum reaches its full length. After flagellum membrane near the basal body. Only after completion of this process the flagellum will be functional. Immediately it regenerated up to the predetermined fixed length.

The structure of the bacterial flagellum allows it to spin like a propeller, with the basal body acting like a motor to rotate the flagellum, and thereby to propel bacterial cell. Rotation of bacterial flagellum requires energy, which is supplied by the proton gradient across the cytoplasmic membrane. Approximately 256 protons must cross the cytoplasmic membrane to power a single rotation of flagellum. The flagellum can rotate at speeds of upto 1200 revolutions perminute. Thus bacterial cell move at speeds at 100 um/second (0.0002 mile/ hour). Consider a bacterial cell has 24nm length, a rapidly swimming bacterial cell can move 50 times its body length / second – it is the twice as fast as a cheetah.

Structural variations are present in various types of bacteria. In vibriocholera, there is a lipo polysaccharide sheath surrounding the flagella. In the Spirochete, Trepouema pallidum, flagella surrounded by protein. The flagella in spirochetes do not extend from the cell but rather are attached to both ends of the cell, so that they form a central axial filament that wraps around the cell with in the periplasm. The rotation of periplasmic axial filament propels the cell by propagating a helical wane length of the cell so that it moves with a corkscrew – motion.

Basing on the arrangement and number of flagella on a cell, 4 types of flagellar arrangement are seen in bacteria. These are the useful criteria in identification and classification of bacteria.

If the flagella located at one or both ends of the bacterium, it is called Polar arrangement and, if, flagella are located along the sides of bacterium, it is called Lateral arrangement.

The four types of flagellation patterns are as follows:

i. Monotrichous : A single flagellum at one pole of the cell.

Ex: Psendomonas aeruginosa

ii. Lophotrichous : Several (or) numerous flagella at one pole.

Ex: psendomonas fluorescens

iii. Amphitrichous : The cell bears atleast one flagellum at each pole.

Ex: Aquaspirillum serpens

iv. Peritrichous : Plagella all over the surface of the cell.

Ex: Salmonella typhi

Gliding motility

In some bacteria, e.g., cytophaga species, are motile only when they are in contact with a solid surface. As they exhibit a sinuous, flexing motion, this kind of movement is comparatively slow, only a few um per second. The mechanism of gliding motility is unknown.

Chemotaxis

The bacterial hagellum provides the bacterium with a mechanism for swimming toward or away from chemical stimuli, this behaviour is called chemotaxis. By controlling the duration and direction in which their flagella rotate, bacteria move towards some chemicals, those are chemo attractants and some move away from chemicals, those are chemo repellents. Chemosensors or receptors in the periplasm or the cytoplasmic membrane can bind to these chemicals and send a singnal to the flagella.

Bacterial movement towards a chemical is called Positive chemotaxis and that away from a chemical is called Negative chemotaxis. Bacteria have a memory system that allows them to compare concentrations of chemical attractants or chemical repellents periodically as they swim through the environment. This is called temporal sensing. As motile bacteria move through the environment, they compare the present concentration of chemical attractants and chemical repellents with previous environment. This memory system is based on a complex system of interactions of receptors,

predominantly in cytoplasmic membrane, with Chemoattractant or Chemorepellent.

Tactic responses are not restricted to chemical gradients. The bacterium, Aquaspirillum magnetotacticum exhibits directed swimming in response to the earth's magnetic field (or) to local magnetic fields (magnets placed near the culture). This phenomena is called Magnetotaxis.

This is attributed to a chain of magnetite inclusions, i.e., magnetosomes within the cell, which allows the cell to become oriented as a magnetic dipole. Magnetotaxis may serve to direct the cells downward in aquatic environments toward oxygen-deficient areas more favourable for growth.

Phototaxis

Some bacteria detect and respond to differences in light intensity, a phenomenon called phototaxis.

Ex: Phototrophic bacteria show positive phototaxis towards increasing light intensities and are repelled by decreasing light intensities.

Some bacteria form gas vacuoles that enable them to respond to light. The formation of gas vacuoles by aquatic bacteria provides a mechanism for adjusting the buoyancy of the cell. The gas vacuoles are filled with buoyant hydrogen gas. This allows bacteria with gas vacuoles to adjust their height in water column. Many aquatic cyanobacteria, for example use their gas vacuoles to move up and down in the water column, depending on the light irradiation levels, to achieve optimal conditions for carrying out their photosynthetic metabolism.

Ex: Microcystis

4)Pili and fimbriae

Pili or fimbriae are short, thin, straight hair-like projections that emanate from the surface of some bacteria and are involved in attachement process. There are several types of pili or fimbriae

associated with bacterial surface, each serving a different function. Pilus (singular pili) refer only to attachment between the mating bacterial cells, and the term fimbria refer to all attachment processes.

Pili are phosphate – carbohydrate – protein complexes containing a single type of peptide subunit called pilin.

The F pilus (fertility pilus) is involved in bacterial mating and is found exclusively on the cells that donate DNA. I pilus is a hollow cylinder, which forms a bridge between cell receptor sites for some bacteriophages. The bacteriophage attach to the pili and subsequently transfer their genetic information to the bacterial cell. Pili also has an ability to recognize membrane of eukarytic that facilitate adhesion by allowing bacteria to attach to and colonize host cells; sometimes leads to disease in host organism.

Ex: Neisseria gonorhoeae attach to the surfaces of cells of human genitourinary tract via its pili where it initiates colonization and the subsequent disease process.

In some Gram +ve bacteria, tubular pericellular non-prosthecae, which are rigid appendages are seen. These are called spinae, made up of a protein, Spinin. These help the bacteria to adjust to environmental conditions like, salinity, pH, temperatures, etc.

5)Capsule

Some bacterial cells are surrounded by a viscous substance forming a covering layer or envelope around the cell wall. If this layer is visualized by light microscopy using special staining methods. It is termed as a capsule. If the layer is too thin to be seen by light microscopy, it is termed as microcapsule. Capsules appear to be amorphous gelatinous areas surrounding a cell by light microscopy. By electron microscopy, it is revealed that capsules consists of a mesh or network of fine strands.

Bacterial capsules are composed of polysaccharides. Capsules composed of homopoly saccharides, are usually syntherized outside the cell from disaccharides by exocellular enzymes.

For ex: the capsule in S.mutans is synthesizd from sucrose, polysaccharide is glucan (a polymer of glucose).

Some capsules are hetero polysaccharide capsules, usually synthesized from sugar precursors that are activated with in the cells

attached to a lipid carrier molecule, transported across the cytoplasmic membrane and polymerized outside the cell.

A few capsules are polypeptides.

In Bacillus authracis, the capsule is composed of a polymer of glutamic acid. This peptide is unusual one because the glutamic acid is a rare D-optical isomer rather than the usual L-isomer which is commonly found in nature.

Functions of a capsule

- → They may provide protection against temporary drying by binding water molecules.
- → They may block the attachment of bacteriophages.
- → They may be antiphagocytic i.e., inhibits the engulfment of pathogenic bacteria by WBC.
- → They may promote attachment of bacteria to surfaces.

  Ex: Streptococcus mutans, which adheres to smooth surface of teeth and responsible for dental caries.

6)Sheaths

Some species of bacteria, particularly those from fresh water and marine environments, forms chains or trichomes that are enclosed by a hollow tube called sheath. Sometimes these sheaths become impregnated with ferric or manganese hydroxides, which strengthen them.

Ex: Sphaerotilus natans (sheathed bacteria)

Prosthecae and stalks

Prosthecae (singular prostheca) are semirigid extensious of the cell wall and cytoplasmic membrane and have a diameter that is always less than that of cell. These are characteristic for number of aerobic bacteria of fresh water and marine environments. Prosthecae increase surface area of cells for nutrient absorption. Some

prosthecae bacteria may form a new cell (bud) at the end of prosthecae, others have an adhesive substance at the end of prostheca which help in attachment to surfaces.

Ex: Caulobacter (genera) have a single prostheca.

Stell a and Ancalomicrobium have several prosthecae.

7)Cell wall

The cell wall of the bacterial cell is strong and firm but flexible external structure that surrounds most bacterial cells. The cell wall establishes the shape of the cell.

Cell wall is a rigid structure which is useful to present the cell from expanding and eventually bursting because of uptake of water, since most of the bacteria live in hypotomic environments, i.e., the environments having a lower osmotic pressure than exist in bacterial cells. The rigidity of the wall gives the protection against high pressures and adverse physical conditions.

There are two basic types of cell wall structures, Grame-ve cell wall that occur in Gram-ve bacteria, Gram +ve that occur in Gram +ve bacteria. The differences in cell wall structures form an important basis for differentiating the bacterial genera and is widely used in classification and identification. The cell wall constitutes a significant portion of the dry weight of the cell, depending on the species and cultured conditions, it may account for as much as 10 to 40%. Bacterial cell walls are usually essential for bacterial growth and division.

The cell wall of almost every bacterial cell contains peptidoglycan, also known as Murein (or) Mucopeptide, which is largely responsible for protecting the cell against osmotic shock. This peptidoglycan layer occurs only in bacteria and it is not found in any archeal or eukaryotic cells. There are two parts to the peptidoglycan molecule, a peptide portion, which is composed of aminoacids connected by peptide linkages and a glycan (sugar) portion. The glycan portion, which forms the backbone of the molecule, is composed of alternately repeating units of he aminosugars, N-acetyl muramic acid and N-acetyl glucosamine linked each other by β (1$\rightarrow$4)

glycosidic bonds. Short peptide chains with four (tetrapeptides) aminoacids are attached to the N-acetyl muramic acids.

Some of the aminoacids occurring in the peptide portion of the molecule are relatively unusual in biological system. These include D-aminoacids and diaminopimelic acids (DAP) which occur in peptidoglycan but not in proteins. The tetrapeptide usually includes L-alanine, D-glutamic acid, either L-lysine (or) diaminopimelic acid, and D-alanine. The tetrapeptide chains are interlinked by peptide bridges between the carboxyl group of an amino acid in one tetrapeptide chain and the amino group of an aminoacid in another tetrapeptide chain. This cross linkage can occur between tetrapeptides in different chains, as well as directly between adjacent tetrapeptides, so that the peptidoglycan forms a strong, multified layered sheet. Infact, the peptidoglycan may be viewed as one cross-linked molecule, or Sacculus (latin sacculus, meaning "little sac") that entirely surrounds the bacterial cell.

Though the basic structure of peptidoglycan is same in both Gram the and Gram-Ve bacterial cell wall, some differences are there in composition and structure of the cell wall.

Gram +ve bacterial cell wall

The Gram +ve cell wall has a peptidoglycan layer that is relatively thick (40 um) and comprises approximately 90% of cell wall. This thick peptidoglycan layer which is considerably hydrated account for the staining reaction observed in gram stain procedure. The cell walls of most gram +ve bacteria also have teichoic acids which are acidic amionic polysaccharides. Teichoic acid contain a carbohydrate such as glucase, phosphate and alcohol (either glycerol or ribitol). The teichoic acids are bounded to the peptidoglycan, making them an integral part of the Gram +ve cell wall structure.

Teichoic acids can bind protons there by maintaining the cell wall at a relatively low pH. This low pH prevents autolysis from degrading cell wall. Teichoic acids also bind to cations such as Ca^{2+} and Mg^{2+} and act as receptor sites for some viruses.

When phosphate concentrations are low, Gram the bacteria replace the phosphate-rich teichoic acids of the cell wall with teichuronic acids. This enables them to conserve phosphate that is essential for ATP, DNA and other cellular components. Teichuronic acids are polysaccharide chains to uronic acids and N-

acetylglucosamine, which fulfill the cells requirement for an acidic, anionic polysaccharide in the cell wall.

Members of the genus Nycobacterium, include M.tuberculosis and M.leprae, have waxy lipids called mycolic acids as a part of their cell wall structure. The mycolic acids contribute to the characteristic acid-fast staining reactions of mycobacteria and playan important role in the survival of these bacteria.

Gram-ve bacterial cell wall

The Gram-ve cell wall is far more complex than its gram the counterpart. The peptidoglycan layer of the Gram-ve cell wall is very thin (2 um) and often consists of only 10% or less of the cell wall. Teichoic acids do not occur in Gram-ve cell wall. Lipoproteins (lipids linked to protein molecules) are bonded to the peptidoglycan forming an integral part of the Gram-ve cell wall.

Additionally, there are layers of lipopolysaccharide (lipids linked to carbohydrate molecules) phospholipids and proteins outside the peptidoglycan layer. Though these layers sometimes are considered part of the cell wall, it is now more complex structure called the cell envelope of the Gram-ve bacterial cell.

8)Outer membrane

The cell envelope of Gram-ve cell extends outward from the cytoplasmic membrane to a second membrane – the outer membrane. The outer membrane is lipid bilayer containing phospholipids, proteins, lipoproteins, and tipopolysaccharides. Unlike the cytoplasmic membrane it is relatively permeable to most small molecules. Electron microscopic analysers of E. Coli and Salmonella have indicated that cytoplasmic membrane and outer membrane, of these Gram-ve rod shaped bacteria, may be jained (or) fused at many points around the cell. These so called adhesion sites (or) Bayer junctions (discovered by Manfred Bayer0 are purportedly sites at which some material, such as excreted polysaccharide, is moved from the inside of the cell; where it is 1st synthesized to the outside of the cell. On the inner surface of the outer membrane in many Gram –ve bacteria is a lipoprotein that anchors or bridges the outer membrane to the peptidoglycan layer. The lipoprotein molecule contain fatty acids, which associate with hydrophobic portion of the outer numbrane. The protein position of some of the lipoprotein molecules (about 30%) is bonded to the back bone of the peptidoglycan layer.

The outer membrane contains lipopolysaccharides (LPS), which are not found in the cytoplasmic membranes. LPS is often called endotoxin because when this molecule is introduced into animals it causes fever and can lead to shock and death. It is called Endotoxin because it is present with in the cell. LPS is a complexmolecule, composed of 3 distinct regions. This inner most portion is a lipid called lipid A, which anchors the lipid to the hydropholic portion of the outer membrane. Lipid-A consists of N-acetyl glucosamine disaccharide linked via ester and amide bonds to unusual fatty acids such as β-hydroxymyristic caproic and lauric acids. The toxic portion of LPS lies in lipid A.

The polysaccharide portion of LPS, which is external to lipid A, consists of core polysaccharide and a repeat poly saccharide called the O-antigen or O-polysaccharide. The core polysaccharide is fairly constant for most Gram –ve bacteria and contain many type of sugars (Glucose, galactose, etc). The composition of sugars and their arrangement, varies from one Gram –ve bacterium to another.

Functionally, the outer membrane of the Gram –ve bacterial cell is a coarse molecular sieve. The permeability of the outer membrane to nutrients is due in part of the outer membrane proteins (DMP), collectively called parins. The porins, usually in aggregates of three, form cross membrane channels through which some molecules can diffuse, the outer membrane also consists of non-proteins, especially Omp. The outer membrane is more restrictive than the cytoplasmic membrane to certain substances. It is less permeable than (M to hydropholic (non polar)) molecules and amphipathic molecules (molecules that have both polar and non polar ends) such as phospholipids. For this reason, Gram –ve bacteria is less sensitive than Gram +ve bacteria to some antibiotics, because outer membrane prevents the drugs from reaching their trargets in the cytoplasm.

Fig.

9)Periplasm

The region between the cytoplasmic and outer membrane is known as the periplasm, also called periplsmic space or periplasmic gel. This is an important region in Gram –ve bacteria, where diverse chemical reactions occur including oxidation – reduction reactions, osmotic regulation and solute transport, protein secretion and hydrolytic activities. Several proteins are found in the periplasmic

region. These proteins includes, Binding proteins – which facilitate the transport of substances into the cell by delivering substances to carriers that are bound to the cytoplasmic membrane.

Chemoreceptors – These are proteins, that bind to substances and direct the cells movement towards or away from those substances Hydrolytic enzymes – These are responsible for breaking of large molecules so that smaller products can be transported across the cytoplasmic membrane where they are metabolized to produce ATP and cellular constituents.

1.1.1.3 Cell wall of Archeabacteria

The archea do not contain peptidoglycan in their cell wall. Some archea has walls composed of pseudopeptidoglycan, which resembles the peptidoglycan of bacteria but contains N-acetyltalosaminouronic acid instead of N-acetyl muramic acid and L-amino acids instead of D-aminoacids in bacterial cell wall. Also the bonding between the carbohydrates in psendopeptidoglycan is β 1→3 linkages instead of β 1→4.

Cell walls with pseudo peptidoglycan occur in some methanogenic bacteria and extremely halophilic (high salt requiring bacteria) bacteria. Other archea have cell walls composed of proteins, containing polysaccharides (or) made up with 3 different chemical compositions. In Thermoplasma spp the cell wall is completely absent.

Though there is no unifying structural composition, archeal cell walls can protect the cytoplasmic membranes even in hot, acidic and saline environments in which many archea live.

Halo bacterium contain glycoprotein in their walls. These glycoproteins with a high abundance of negatively charged (acidic) amino acids. The cell walls of halo bacterium are stabilized by the interaction between its acidic amino acids and the high abundance of positively charged sodium ions (Na^+) in the very saline environments in which organism lives.

Comparison of Gram +ve and Gram –ve bacterial cell walls

	Gram +ve wall	Gram –ve wall
Peptidoglyean	Always present, occurs as a thick layer 20-80% of the cell wall composition	Always present, occur as a thin layer. 5-15% of the cell wall composition
Peptidoglycan	Most contain lysine	All contain

tetrapeptide		Diaminopimelic acid
Peptidoglycan cross linkage	Generally pentapeptide, for ex., entirely glycine	Direct bonding of DAP of one chain to the terminal D-alamine of another chain
Teichoic aicd	Present	Absent
Teichuronic acid	Present	Absent
Lipoproteins	Absent	Present
LPS (Lipoply saccharide)	Absent	Present
Outer membrane	Absent	Present
Periplasmic space	Absent	Present
Surface antigen	Teichoic acids are the principle surface antigens	LPS are the principle surface antigens
Cell wall composition	Peptidoglycan – 20-80% orurs proteins, polysaccharids, teichoic acics	Peptidoglycans – 5-15% phospholipids – 35% proteins – 16% LPS – 50%
No. of layers	Single layered	Multi – layered

1.1.1.4 Gram staining

The development of the gram stain procedure by the Danish physician Hans Christian Gram. This technique is a basic differential staining technique to differentiate between Gram +ve and Gram –ve cells.

The thick peptidoglycan of Gram +ve cell accounts for the staining reaction account for Gram stain procedure.

Procedure

→ Preparation of smear on slide and is heat –fixed

→ The slide stain with primary stain i.e., crystal violet.

→ After rinsed with water, iodine solution is applied.

→ This inturn, is rinsed off with water.

→ Then, 95% ethyl alcohol is applied and then cleaned / washed with water.

→ Then applieda conunter stain i.e., saffranin or cosin red.

The primary stain (crystal violet) passes across the wall freely and is firmly attached to the cell structures by the addition of mordant grams iodine. The decolorizing agent, alcohol, dehydrates the wall, causing it to shrink and trap the primary stain-iodine complex (Crystal violet – Iodine complex). The Gram +ve bacteria retain the primary stain and appear in blue colour.

The Gram –ve bacteria have thin layer, the wall is thin to retain the crystal violet iodine complex after applying alcohol. Gram –ve cell have high lipid content, which dissolve when treated with alcohol. Gram +ve bacteria has less lipid content and peptidoglycan retain the iodine – primary stain color decolourizing agent alcohol action also.

Acid – Fast technique

Bacteria of the genus mycobacterium possess waxy materials and mycolic acids in the cell wall. It is very difficult to stain by ordinary methods. Heat must be applied to fore the dye to enter inside through cytoplasm. Once stained, the cells are not easily decolourised, even with alcohol solution containing 5% acid. Hence these are called Acid-fast or Acid-resistant.

Air dried, Heat fixed smears are heated while being stained with Liehl – Neelsons Carbol Fuchsin. The cells become bright red. Smears rinsed with acid alcohol decolourizer and then counter stained with methylene blue and observed under microscope. A red coloured cells are the mycobacterial cells.

M. tuberculosis decolourised by 20% H_2SO_4.

M. leprae decolourised by 5% H_2SO_4.

1.1.1.5 Cytoplasmic membrane

Immediately beneath the cell wall is the cytoplasmic membrane. This structure is approximately 7.5 um (0.0075 um) thick. Cytoplasmic membrane of the bacterial cells contain phospholipids, which are the molecules containing 2 functional groups.

i. a phosphate group

ii. a fatty acid – which joined together by glycerol.

Two fatty acids and one phosphate are bonded to glycerol to form the phospholipid. The phosphate group is negatively charged and hence is a hydrophilic group. The fatty acid portion is non polar & therefore hydropholic within aqueous environment. Phospholipid molecules tend to aggregate spontaneously such that their hydrophobic positions face one another and their hydrophilic portions are exposed to water. This arrangement results in a phospholipid bilayer, which is the basic structure of all bacterial cytoplasmic membranes. The hydrophilic portions of the cytoplasmic membrane occur at the outer and inner surfaces, those in direct contact with the exterior of the cell and the cytoplasm respectively. The hydrophobic portions occur within the internal matrix of the cytoplasmic membrane.

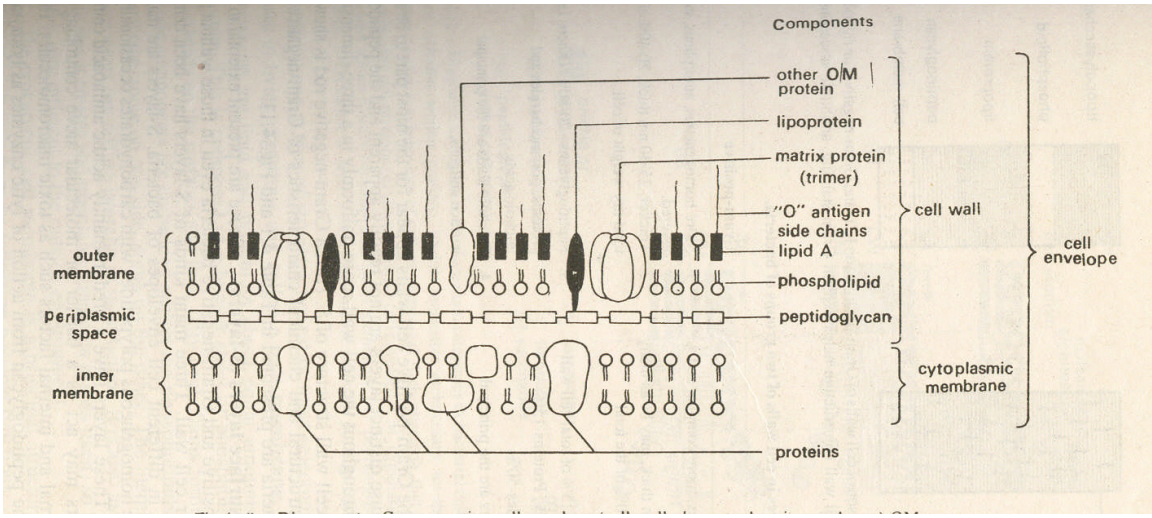
Phospholipid has hydrophobic and hydrophilic portions cotributes to the ability of the cytoplasmic membrane to regulate selectively the flow of material into and out of the cell.

Typically, the fatty acids that occur in cytoplasmic membrane of bacterial cells are unbranahed and contain 16-18 `e' – atom. The actual fatty acids in bacterial cyto-plasmic membrane vary depending on species and environmental conditions.

Proteins are interspersed in the matrix of the bacterial cytoplasmic membrane as described by fluid mosaic model. Bacterial cytoplasmic membrane tend to contain a higher portion of proteins than eukaryotic cytoplasmic membranes.

Some of these proteins are involved in ATP-generating metabolism that occurs at the cytoplasmic membranes of bacterial cells and within the mitochondria and chloroplast membranes of eukaryotic cells.

Generally the cytoplsmic membrane of bacterial cells do not contain sterols. The bacterial genus Mycoplasma, however, has sterols that it acquires from the cytoplasmic membrane of eukaryotic cells. Mycoplasma differ from other bacteria in that it lacks the cell wall, but these are considered true bacteria because they lack membrane bound nuclei. Polyene antibiotics are effective against mycoplasma because they interact with sterols and disrupt the integrity of the cytoplasmic membranes.



1.1.1.6 Cytoplasmic membrane of archeobacteria

Cytoplasmic membrane of Archea differ from bacteria. Structurally, they do not contain phospholipids, and in some cases, archae bacterial cytoplasmic membrane is monolayer (archeal diglycerol tetra ether) rather than bilipid structures.

The lipids of archeal cytoplasmic membranes are branched and linked to glycerol by ether bonds, whereas the cytoplasmic membranes of bacterial and eukaryotic cells contain straight chain fatty acids linked to glycerol by ester bonds. Branched fatty acids increase the fluidity of the cytoplasmic membrane. Many glycerol molecules in the cytoplasmic membrane of archea lack a phosphate group, that is, phospholipids are not structural lipids of archeal cytoplasmic –membrane.

In archeal members, copyarol is bonded to two unbranched hydrocarbons and the remaining hydroxyl group of glycerol is unbounded. This unbound hydroxyl group makes glycerol some what hydrophilic, in some cases, this hydroxyl group is replaced by more polar groups. Due to these replacements the polar lipids in archeal cytoplasmic membrane may be sulfolipids, glycolipids. And in some cases, isoprenoid hydrocarbon also founded.

Many archea have lipid bilayers composed of glycerol diether lipids, which are analogous to the lipid bilayers of bacteria and eukaryotes. The cytoplasmic membrane of some archea have monolayers composed of glycerol tetra ether lipids. These monolayers

still have hydrophilic portions (glycerol) at the eytoplasm and external interfaces an internal hydrophobic portion (hydrocarbons), but monolayers are more resistant to disruption by heat than bilipid layers.

Many archea live in extreme environments wher unusual physiologically specialized CM's are needed for survival, for ex., sulfolobus, that live in high temperature for upto 90^{o}c and at pH-2 have cytoplasmic membranes that contains long chain, branched hydrocarbons twice the length of the fattyacids in the cytoplasmic. Membrane of bacteria. The diversity in structures of cytoplasmic membrane of archea make them resistant to conditions that would disrupt. The function of a normal bilipid layer, thereby enabling them to remain as semipermeable barriers in extreme habitats.

Cytoplasmic membrane is an extremely important functional structure, and damage to it by physical (or) chemical agents can result in the death of the cell.

1.1.1.7 Membranous intrusions

Bacterial cells do not contain membrane enclosed organ cells corresponding to mitochondria and chloroplast of eukaryotic cells. However, bacteria may have specialized invaginations of the cytoplasmic membrane that can increase their surface area for certain functions. Many bacteria especially, Gram +ve bacteria possess membrane – invaginations in the form of systems of convoluted tubules and vesicles termed mesosomes.

The mesosomes which penetrate deeply into cytoplasm are located near the middle of the cell and seem to be attached to cells nuclear material. They are thought to be involved in DNA replication and cell division & called Central Mesosomes. Whereas peripheral mesosomes show only a shallow penetration into the cytoplasm and are not restricted to a central location, and are not associated with nuclear material, they seem to be involved in export of exocellular enzymes such as penicillinase. Extensive intracellular membrane systems occur in methane-oxidizing bacterial and also in phototrophic bacteria. In phototrophic bacteria the intracellular membrane are the site of photosynthetic apparatus of the cell, the in foldings provide a large surface area to accumulate a high content of light absorbing pigments.

1.1.1.8 Cytoplasm

The cell material bounded by the cytoplasmic membrane may be divided into

- → The cytoplasmic area, - granular in appearance & rich in macro-molecular RNA – protein bodies known as Ribosomes, on which proteins are synthesized.
- → The chromatin area rich in DNA
- → The fluid portion with dissolved materials

Ribosomes

Bacterial & archea cells have 70s ribosomes composed of 30s & 50s. The 30s subunit contain about 21 proteins and a 165 r RNA molecule having approximately 1,540 nucleotides. The archea are not sensitive to chloramphenicol, streptomycin, kanamycin, erythromycin, tetracycline and various other antibiotics that digrupt protein synthesis at the 70s ribosomes of bacterial cells.

The differences in antibiotic sensitively reflects differences in the composition of ribosomal proteins of bacterial & archeal cells. Ribosomes of archeal cells are sensitive of diptheria toxin & anisomycin where as the ribosomes of bacterial cells are not the 70s ribosomes of archeal cells resemble the 80s ribosomes of eukanyolic cells, both being sensitive to diptheria toxin & anisomycin.

Cytoplasmic inclusions & vacuoles

Concentrated deposits of certain substances are found in cytoplasm of bacteria.

Volutin granuler

There are also known as metachro matic. Granules which are composed of polyphosphates. They stain an inteme reddish purple color with dilute methylene blue & can observed by light microscopy. By electron microscopy they appear as round, dark areas. Volutin serves as a reserve source of phosphate.

Poly -β-hydroxybutyrate (PHB) granules

These are often found in aerobic bacteria. These can serve as a reserve carbon and energy source. The PHB can be stained with liquid soluble dyes such as Nile blue. In some bacteria, intracellular

globules of elemenal sulfur occur. These may accmulate in certain bacteria gorwing in environments rich in hydrogen sulfide.

Nuclear material

Most of the genetic information of bacterial and archeal cells is usually contained within a single circular bacterial chromosome. Bacterial chromosomes are typically circular DNA macromolecules except in few cases such as Streptomyces and Borrellia spp, where it is linear and Rhodobacter sphaeroides, which has two separate chromosomes. In E. Coli, there are about 4.7×10^6 nucleotides base pairs are present in chromosomes. Mycoplasma genitalism, typically have fewer nucleotides, others such as Myxobacteria, have great no. of nucleotides.

The bacterial chromosome occupies a region within the cell referred to as the nucleotide region. Some sequences of the DNA are associated (or) complexed to the cytoplasmic membrane but the nucleotide region is not separated from the rest of the cell within a membrane – bound organelle. The bacterial chromosome sometimes referred to as `naked DNA'. The DNA of the bacterial chromosome is highly twisted, i.e., negatively supercoiled. If the bacterial chromosome were not supercoiled it would expand to about 1 millimeter in linear length, which is far longer than the bacterial cell. As a supercoiled molecule, the bacterial chromosome is condensed so that it occupies only a fraction of a bacterial cell, which is one micrometer long. Bacterial and archeal chromosomes lack the same basic proteins called Histones, that are responsible for the coiling of the DNA in eukaryotic chromosomes. Several histone-like proteins are found in association with the DNA of archeal and bacterial chromosomes.

Reproduction of a bacterial cell requires the replication of the bacterial chromosome so that each daughter cell receives a complete bacterial chromosome. Hence cell division must be synchronized with replication of the bacterial chromosome is a prerequisite for cell division. Bacteria normally reproduce by binary fission, a process in which a cell divides produce two equalsized daughter cells. In binary fission, the inward movement of the cytoplasmic membrane and cell wall, septurn formation pinches off and separates the two complete bacterial chromosomes, providing each of the progeny cells with a complete set of genetic information.

Plasmids

In addition to bacterial chromosome, bacteria and archea may contain one or more small, circular macromolecules of DNA capable of self replication known as `plasmids'. Plasmids contain a limited amount of specific genetic information that supplements the genetic information contained in bacterial chromosome. Bacteria may contain none (or) more different plasmids.

1.1.1.9 Spores

Certain microorganisms produce specialized resistant structures, called spores to enhance their survival potential. Spores typically are involved in reproduction dispersal or the ability of the organism to withstand adverse environmental conditions. The spores involved in reproduction are metabolically quite active, whereas those involved in dispersal (or) survival of microorganism are often metabolically dormant.

Spores are of two types.

i. Endospore : Spore formed within the cell.

ii. Exospore : Spore formed external to the cell.

Endospore

The bacterial endospore is a heat resistant spare formed within the cells of a few bacterial genera that is formed under adverse conditions and subsequently germinates to form a vegetative cell.

Endospore is a complex multilayered structure containing peptidoglycan with in its complex spore coat and calcium dipicolinate with in its core. Endospores can survive exposure to high temperature and in boiling water for extended periods, because of the calcium dipicolinate. The preference of calcium dipicolinate and presence of water are involved in conferring heat resistance on the endospore. Only a few genera form endospores, and most important types are Bacillus & Clostridium both are Gm +ve rods.

Endospore formation

Endospores are formed when conditions are unfavourable for continued growth of the bacterium, sporulation, that is the formation of spores can be initiated under conditions of starvation. Sporulation is an irreversible process.

During sporulation process, there is an invaginnation of the cytoplasmic membrane with in the cells to establish the site of endospore

Formation. A copy of bacterial chromosome is incorporated into the endospore, and various layers of endospore are synthesized around bacterial DNA. Substances are synthesized that are specifically related to the spore include, Dipicolinic acid, responsible for heat resistance. Polypeptide exclusively with single aminoacid such as cystine.

The complete formation of spores involves the synthesis of two layers and formation of cortex. Once endospore is formed with in cell it can be released by the lysis of parent cell. Endospores must have been dormant for thousands of years.

Endospore germination

Under favourable conditions like availability of nutrients and water and optimum temperature, the endospores can germinate and give rize to an active vegetative cell of the bacterium. During germination, the spore swells, breades out the spore coat and elongates. The striking feature in spore germination is the speed with which metabolism shifts from a state of dormancy to the high activity levels, that characterize a germinating spore. This shift can occur within minutes. During germination, ATP generation and protein synthesis can take place for 15 minutes by utilizing energy and substances. Principally phosphoglycerate, that are contained within the spore, after spore germination the organism can has normal vegetative growth.

Exospores

Cells of methane-oxidizing genus methylosimes forms exospores, i.e., spores external to the vegetative cell, by fudding at one end of the cell. These are dessication and heat resistant, but unlike endospores, they do not contain Dipicolinate.

Comidiospores and Sporangiospores

Actinomycetes forms branching hyphae, spores develop singly as in chains, forms the tips of these hyphae by cross well formation septation. If spores are contained in an enclosing sac (sporangium), they are termed as sporangiospores, if not are enclosed in sac are

called conidiospores or conidia. The spores do not have high heat resistance as endospores, but they can survive long periods of drying.

1.1.1.10 Cysts

Cysts are dormant, thick-walled, dessication resistant forms that develop from vegetative cell in adverse conditions. Under favourable conditions they germinate into vegetative cells. Cysts differ with endospores in chemical composition and also do not have high heat resistance compared to endospores.

Example for cyst is complex type cyst produced by Azatobacter genus. Several other bacteria can differentiate into cyst-like forms, but seem to lack structural similarity as that of Azatobacter cysts.

1.1.1.11 Model Questions

1)Write in detail abou the ultra structure of bacteria

2)Write a detailed account on bacterial spores

1. 1.1. Microbiology → Michael J. Pelczar, JR.
   E.C.S. Chan
   Noel R. Krieg
2. Microbiology → Prescott
   L.M. Harley
   J.P. Klein, D.A

1.12 Reference books

Lesson 1.1.2

MORPHOLOGY & ULTRASTRUCTURE OF FUNGI

Objective

Objective

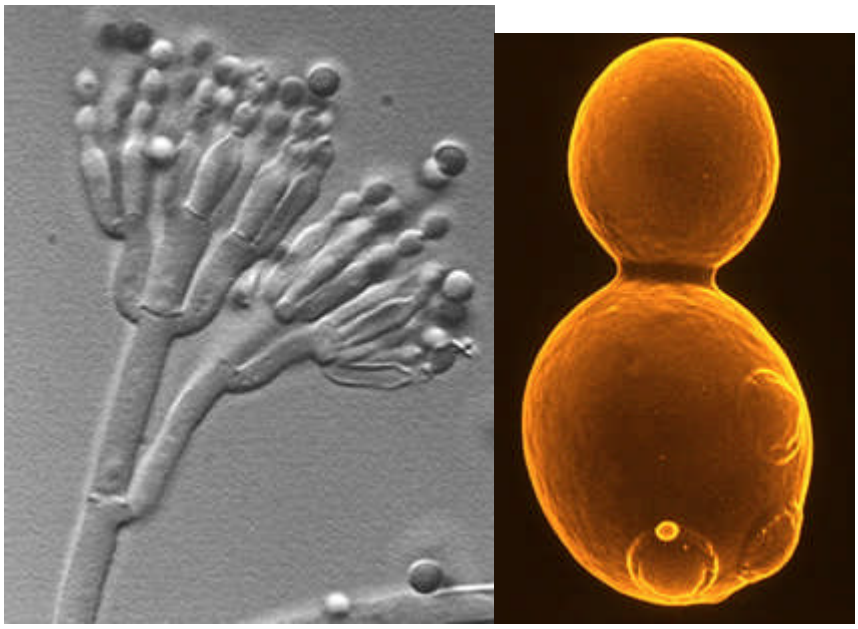
This chapter gives the detail descry[ption of fugal classification characters ,structure and morphology. The general features of yeast were also discussed .

1.1.2.1 Introduction

Fungi are achlorophyllous, saprophytic or parasitic, with unicellular or more typically filamentous vegetative structures. The cell walls of fungi are composed of chitin or other polysaccharides. Fungi are evolved from protozoa by the evolution of chitinous walls in the tropic phage: this necessptated a shift from phagotrophy to absorptive nutrition. The fungi are ehterotrophic organisms, that is they require organic compounds for nutrition. When they feed on dead organic matter, they are known as saprophytes. Saprophytes decompose complex plant and animal remains, breaking them down into simpler chemical substances that are returned to soil, there by increasing its fertility. Saprophytic fungi are also important in industrial fermentations, for example, the brewing of beer, the making of wine and production of antibiotics such as penicillin. The leavening of dough and the ripening of some cheeses also depend on fungal activity. As parasites (When living in or on another organism), fungi cause diseases in plants, humans and other animals.

1.1.2.2 Morphology and Ultrastructure

The thallus or body of a fungus may consists of a single cell as the yeasts, more typically, the thallus consists of filaments, 5 to 10 um across, which are commonly branched. The yeast cell or mold filament is surrounded by a true cell wall. In slime molds, the cell wall is absent, the thallus consisting of a nacked amoeboid mass of protoplasm. Same fungi are dimorphic, that is, they exist in two forms. Some pathogenic fungi of humans and other animals have a unicellular and yeast-like form in their host, but, when growing saprophytically in soil or on a laboratory medium, they have a filamentous mold from.



The opposite dimorphison occur in some plant pathogens.

Ex. Taphrina, causes peach leaf curl, the mycelial form occurs in the host and the unicellular yeast-like form occurs in laboratory culture. The fungal colony may be a mass of yeast cells or it may be a filamentous mat of mold.

Generally, yeast cells are larger than most bacteria. Yeasts vary considerably in size, ranging from 1 to 5 um in width and from 5 to 3 um (or) more in length. They are commonly egg-shaped but some are elangated and some spherieal. Each species has a characteristic shape. Yeasts have no flagella or other organelles of locomotion.

The thallus of a mold consists of essentially of two parts, the mycelium and the spores. The mycelium is a complex of several filaments called hyphae. New hyphae generally arise from a spore which on germination put out a germ tube. These germ tubes elongate and branch to form hyphae. Each hypha is about 5 to 10 um wide, as compared with a bacterial wall usually 1 um in diameter. Hyphae are composed of an outer tube-like wall surrounding cavity

the lumen, which is filled or lived by protoplasm. Between the protoplasm and the wall is the plasmalemma, a double layered membrane, surrounding the protoplasm. The hyphal wall consists of microfibrils composed for the most part of hemicellulose orchitin, true cellulose occurs only in the walls of lower fungi. Wall matrix material in which the microfibrits are embedded consists of proteins, lipids and other substances.

Growth of a hypha is distal, near the tip. The major region of elongation takes place in the region just behind the tip. The young hypha may become divided into cells, by cross walls which are formed by centripetal invagination from the existing cell wall. These cross walls constrict the plasmalemma and grow inward to form generally an incomplete septum that has a central pore which allows for protoplasmic streaming. Even nuclei may integrate from cell to cell in the hypha.

Hyphae occur in 3 forms.

1. Nonseptate or coenocytic . These hyphae have no septa.
2. Septate with uninucleate cells.
3. Septate with multinucleate cell → Each cell has more than one nuclens in each compartment.

Mycelia can be either vegetative or reproductive. Some hyphae of the vegetative mycelium penetrate into medium in order to obtain nutrients. Soluble nutrients are absorbed through the walls. Reproductive mycelia are responsible for spore production and usually extends from the medium into the air. The mycelium of a mold may be loosely wover network or it may be an organized compact structure as in mushrooms.

Propagation of fungi is by spores, and reproduction normally is by both sexual and asexual means. The life cycle of fungus is simple in some forms, but it is complex in many forms. The life cycles of most fungi involves asexual reproduction by fragmentation of hyphae or budding of fungal cells and formation of various asexual spores that permit disposal of the fungus and less frequent phase of sexual reproduction.

Based on the means of reproduction, including nature of life cyce, reproductive structures, and reproductive spores, fungi are classified into many divisions.

1.1.2.3 CLASSIFICATION OF FUNGI

The primary taxonomic groupinys are based on the sexual reproductive spores. To a lesser extent, classification of Fungi relies on morphological characteristics of the vegetative cells. Most classical approaches to fungal classification are based largely on observations of the morphology of the reproductive forms but physiological, biochemical and genetic characteristics are included in some modern classification systems. Physiological features are particularly important in the classification of yeasts, which are primarily, the unicellular fungi. As with other eukaryotic microorganisms the fungi are diverse, and many faxanomic groups traditionally studied by mycologists, such as slime molds, are actually associated with other groups of microorganisms.

Taxonomy of the fungi follows the recommendations of the committee on International Rules of Botanical Nomenelature. Accordingly, the various taxa have the suffix endings as follows:

Divisions - -mycota

Subdivisions - -mycotina

Classes - -mycetes

Subclasses - -mycetidae

Orders - -ales

Families - -aceae

Gevera and species have no standard –suffix endings.

Many classifications are available, but the classification given by Alexopoulos and Mime (1979) is followed by many mycologists, which was updates after Alexopoulos.

The Kingdom Myceteae (Fungi) is divided into 3 divisions.

I. DIVISION – I – GYMNOMYCOTA – Phagotrophic, Thallus lack cell wall

a. Subdivision - Acrasiogymnomycotina

i. Class – I - Aerasiomycetes (Cellular, slime molds)

ii. Class – II - Myxomycetes (Cellular, slime molds)

1. Subclass – I - Ceratio myxomycetidae

2. Subclass – II - Myxogastromycetidae

3. Subclass – III - Stemonito mycetidae

II. DIVISION – II – MASTIGOMYCOTA (Flagellated lower gungi)

Acquatic fungi producing motile,
Flagellated cells. Asexual reproduction
By zoospores. Centrioles functioning
During nuclear division.

a. Subdivision –I - Haplomastigomycotina

i. Class – I - Chytridiomycetes – Aquatic, Whiplash type flagellum. Ex: Allomyces Macrogymes

ii. Class – II - Hypochytridiomycetes – Aquatic, tinsel type flagella. Ex:Rhizomyces arbuscula

iii. Class-III - Plasmodiophoromycetes – Endoparasitic slime molds. Vegetative stage is plasmodium.

Ex: Plasmodiophora brassiea

b. Subdivision-II - Diplomastigomycotina – Zoospores biflagellate

i. Class – I - Oomycetes, Ex. Saprolegnia ferax

III. DIVISION – III – AMASTIGOMYCOTA (terrestrial fungi)

Flagella absent

a. Subdivision – I - Zygomycotina

i. Class – I - Zygomycetes

ii. Class – II - Trichomycetes

b. Subdivision – II - Ascomycotina

i. Class – I - Ascomycetes

1. Subclass – 1 - Hemiascomycetidae

2. Subclass – 2 - Plectomycetidae

3. Subclass – 3 - Hymenoascomycetidae

4. Subclass – 4 - Laboulbeniomycetidae

5. Subclass – 5 - Loculoascomycetidae

c. Subdivision – III - Basidiomycotina

i. Class – I - Basidiomycetes

1. Subclass – 1 - Holobasidionycetidae

2. Subclass – 2 - Phragmobasidiomycetidae

3. Subclass – 3 - Teliomycetidae

d. Subdivision – IV - Denteromycotina

i. Class – I - Denteromycetes

1. Subclass – 1 - Blastomycetidae

2. Subclass – 2 - Coelomycetidae

3. Subclass – 3 - Hyphomycetidae

There is no universally accepted scheme of classification at this time also. The classification proposed by Ainsworth (1966, 71, 73) is also accepted by many mycologists.

Fungi are divided into two divisions distinguished by the presence or absence of a plasmodium or pseudoplasmodium.

KINGDOM - MYCOTA (fungi)

DIVISION I – MYXOMYCOTA (slime molds) – Fungi with plasmodium (or) Pseudoplasmodium

Class-I - Aerasiomycetes (Cellular slime molds)

Class-II - Hydromyxomycetes (Net slime molds)

Class-III - Myxomycetes (True slime molds)

Class – IV – Plasmodio phoromycetes (Endoparasitic slime molds)

DIVISION II - EUMYCOTA (True fungi) – Fungi with a cell wall and Usually filamentous.

Subdivision I - Mastigomycotina – Motile cells – Zoospores are Present.

Class 1 - Chytridiomycetes - Wiplash flagellum

Class 2 - Hypochytridiomycetes - Tinsel flagellum

Class 3 - Oomycetes - Biflagella

Subdivision II – Lygomycotina – Mycelial, aseptate, zygospores

Class 1 - Zygomycetes

Class 2 - Trichomycetes

Subdivision III - Ascomycotina - Ascospores

Class 1 - Hemiascomycetes

Class 2 - Loculoascomycetes

Class 3 - Plectomycetes

Class 4 - Laboulbeniomycetes

Class 5 - Pyrenomycetes

Class 6 - Discomycetes

Subdivision IV - Basidio mycotina – Basidiospores

Class 1 - Teliomycetes

Class 2 - Hymenomycetes

Class 3 - Gasteromycetes

Subdivision V - Deuteromycotina (or) Fungi imperfecti

Class 1 - Blastomycetes

Class 1 - Hyphomycetes

Class 1 - Coelomycetes

Some 63,500 species of fungi have been described with another 13,500 in association with algae as lichens.

1.1.2.4 GROWTH & REPRODUCTION

Filamentous fungi grow as long filaments by apieal extension. The diameter of the filament is fairly constant, usually between 2-10 mm while the length of the filament can be very long. Many fungal filaments are meters in length and some may extend for several miles. Hyphal extension is highly polarized, which contributes to the efficiency of nutrient absorption and rate of growth.

A filamentous fungus can be viewed as a cytoplasmic mass that moves inside tubes (hyphae) bounded by cell walls, which it builds gradually as it moves forward. Cytoplasm moves constantly towards the growing hyphal apices, leaving highly vacuolated or moribund hyphae behind. This allows a high rate of movement and spreading of the active cytoplasm, which is essential for acquiring the oxganic resources necessary for rapid growth. The morphology of mycelium is

determined by mechanisms that regulate the polarity and the direction of growth of hyphae and the frequency with which they branch. While continuing apieal growth, hyphae may develop highly organized multicellular structures also, such as fruiting bodies in which certain cells perform meiosis and produce sexual spores.

Many of these specialized structures rise into the air. The areal structures are apparently all designed to lift reproductive hyphae into the air for better spore dispersal. Some of these areal structures are very large, such as mushrooms.

The mycelia of basidiomycetes typically form clamp connections between cells. The clamp cell connections are generally indicative of a divaryotic mycelium. Additionally, the mycelia of many basidimycetes are characterized by special cross walls between connecting cells, known as Dolipore septa. The dolipore septum has a central pore surrounded by a barrel shaped swelling of cross wall. Effectively, the clamp cell connections and the dolipore septa permit enhanced chemical communication through the mycelia of the organism.

Filamentous fungi exhibit various reproductive strategies, typically involving in, Sexual reproduction, Asexual reproduction.

Asexual Reproduction

- This is also called somatic or vegetative reproduction.
- Does not involve the union of nuclei, sex cells or sex organs.
- It may be accomplished by

    1. Fission of somatic cells yielding two similar daughter cells.
    2. Budding of somatic cells or spores, each bud is a small out growth of the parent cell developing into a new individual.
    3. Fragmentation or disjoining of hyphal cells, each fragment becoming a new organism,
    4. Spore formation.

Asexual spores, whose function is to disseminate the species, are produced in large numbers. There are many kinds of asexual spores found among various groups.

Sporangiospores

- These are single – celled spores
- Formed within saes called sporangia (singular-sporangium), at the end of special hyphae called.

Sporangiophores

- Aplanospores are non-motile sporangiospores.
- Zoospores are motile sporangiospores – Motility is due to the presence of flagella.

Conidiospores (or) Conidia

- Two types of conidia are preset
- Small, single- celled conidia are called Microconidia
- Large, multicelled conidia are called Macroconidia
- Conidia are formed at the tip or side of a hypha.

OIDIA (Singular – Oidium) or ARTHROSPORES

- Single – celled spores formed by disjoining of hyphal cells.

  Ex. Mucor rouxii

CHLAMYDOSPORES

- thick-walled, single – celled spores.
- Highly resistant to adverse conditions.
- Formed from cells of the vegetative hypha.

BLASTOSPORES

- These are the spores formed by budding.

- These fungal spores can be dispersed from the fungal hyphae and latter germinate to form new mycelia.

1.1.2.5 SEXUAL REPRODUCTION

Sexual reproduction is carried out by fusion of the compatible nuclei of two parental cells. The process of sexual reproduction begins with the joining of 2 cells and fusion of their protoplasts, i.e., plasmogamy, thus enabling the two haploid nuclei of two mating types to fuse together, i.e., Karyogamy to form a diploid nucleus. This is followed by meiosis, which again reduces the no. of chromosomes to the haploid number. The sex organelles of fungi, if they are present, are called Ganutangia. They may form differentiated sex cells, gametes or may instead contain one or more gamete nuclei. If the male and female gametangia are morphologically different, the male gametaugium is called. Antheridium and the female gametangium is called Oogonium.

The various methods of sexual reproduction by which compatible nuclei are brought together in plasmogamy are:

1)Gametic Copulation – Fusion of naked gametes, one (or) both of which are motile.

2)GAMETE – GAMETANGIAL COPULATION

- Two gametangia come into contact but do not fuse.
- The male nucleus migrates through a pore of fertilization tube into the female gametangium.

3)GAMETANGIAL COPULATION

Two gametangia or their protoplasts fuse and give rise to a zygote that develops into a resting spore.

4)SOMATIC COPULATION

Fusion of somatic (or) vegetative cells is called somatic copulation.

5)SPERMATIZATION

- Union of a special male structure called spermatium, with a female receptive structure.
- The spermatium empties its contents into the latter, during plasmogamy.

Sexual spores which are produced by the fusion of two nuclei, occur less frequently, later, and in small numbers than do asexual spores. Several types of sexual spores are present.

a)Ascospores

- Single – celled spores.
- Produced in a sae called an ascus.
- There are usually 8 ascospores in each ascus.

b)Basidiospores

- Also single-celled spores.
- Borne on a club-shaped structure called a basidium.
- A basidium assumes the shape characteristic of that species and generally produces 4 tapering projectious, the sterigmata.
- The 4 nuclei produced after nuclear fission from meiosis, move toward sterigmata and form the basidiospores.

c)Zygospores

- These are large, thick – walled spores
- Formed when the tips of two sexually compatible hyphae or ganutangia, of certain fungi, fuse together.

d)Oospores

- There are formed within a special female structure, the Ooganium.

- Pertilization of the eggs, or Oospheres by female gametes formed in an antheridium give rise to Oospores.

- There are one or more oosphees in each oogonium.

Asexual and sexual spores may be surrounded by highly organized protective structures called fruiting bodies. Asexual fruiting bodies have names such as acerrulus & pyenidium.

Although a single fungus may produce asexual and sexual spores by several methods at different times and under different conditions the spores are sufficiently constant in their structures and in the method by which they are produced to be used in identification and classification.

1.1.2.6 The 4 important groups of Fungi

a)Zygomycetes

➔ These are filamentous fungi.

➔ These have coenocytic mycelia, i.e., mycelia that lack septa and hence contain multiple nuclei within the cytoplasm.

➔ This group is characterized by the formation of a zygospore, a sexual spore that results from the fasion of ganutangia,

➔ For sexual reproduction, some species require gametangia of (zygote formation) two different mating types (heterothallic) & others require only one type (homothallic).

➔ This group also produces asexual sporangiospores within a sporangium.

➔ Many of them or plant or animal pathogens.

➔ Some species are obligately associated with arthropods and normally grow within the guts of these animals, where they attach to the chitinous lining of the gut by means of a specialized structure known as a holdfast.

Ex: Rhizopus. Stoloniger → common bread mold.

Rhizopus oryzae → used for the production of oriental foods

such as tempch.

Some Rhizopus species are important cause of food spoilage.

b)Ascomycetes

- These are also called Sac-like fungi.
- Produce sexual spores within a specialized sac-like structure known as the ascus during their life cycles.
- They produce a specific non-of ascospores within the ascus.
- During sexual reproduction, the ascomycetes normally exhibit a short lived dikaryotic stage between the time of fusion of gametes, plasmogamy, and the time of fusion of the two nuclei-karyogamy.
- The mycelia of Ascomycetes is composed of septate hyphae, and the cell walls of the hyphae of most ascomycetes contain chitin.
- Asexual reproduction in the ascomycetes may be carried out by fission, fragmentation of hyphae, formation of chlamydospores and production of conidia.
- Basing on the structure of the ascocarp, the enascomycetes are divided as
- Plectomycates → in which ascocarp has no special opening.

  Pyrenomycetes → in which ascocarp is shaped like a flask.

  Discomycetes → in which ascocarp is cub-shaped.
- Among these, pyrenomycetes are important in scientific investigations.

  Ex: Neurospara – useful in genetic studies

Some other examples are

Clavieeps purpures → cause ergot of rye.

Produces various alkaloid biochemicals.

Taphrina deformans → cause peach leaf curl

c)Basidiomycetes

- ➔ Most complex fungi
- ➔ Include the smuts, rusts, jelly fungi, shelf fungi, stink horns, bird's nest fungi, puffballs and mushrooms.
- ➔ Produce sexual spores known as basidiospores on the surfaces of specialized spore producing structures, known as Basidia.
- ➔ Also known as the Club fungi because of typical shape of the basidia.
- ➔ Aphyllophorales which include cantharellscs, coral fungi, tooth fungi and pore fungi.
- ➔ Majority of these are saprophytic – growing on dead and living plant material.
- ➔ These are responsible for causing Brown Rot and White Rots in Woods.
- ➔ Mushrooms are the pruiting bodies (basidiocarps) of basidiomycetes, which occur as a part of the life cycle of basidiomycetes.
- ➔ Some mushrooms are edible but some are extremely poisonous.

Ex: Russula & Amantia spp – Poinsonous

Amantia caps → called death caps.

Agaricus sps. From the family Agaricaceae

Produce white to brown mushrooms

Agaricus bisporus – grown commercially for human

Consumption.

- Many no. of sps of rust and smut fungi are the most serious plant pathogens.
- oher 20,000 sps of rust fungi

  & 1000 sps of smut fungi are present.
- Rusts and smuts are characterized by the production of a resting spore known as teliospore, which is thick-walled and binucleate.
- Rust fungi require two unrelated hosts to complete its life cycle.
- These are important plant pathogens causing a high damage to agriculture.
- These include – Pueeinia, Uromyces, Cronartium spp. Responsible for rest in cereals, beans, etc.
- Smut fungi are also important plant pathogens.
- Members of the genus Ustilago cause smut of corn, wheat & other plants.

d)Deuteromycetes

- Also known as Fungi Imperfeeti.
- Sexual forms of reproduction are not yet detected.
- There are about 15000 species present in fungi imperfecti.
- These are classified largely on the basis of morphological structure of the vegetative phage and asexual spores produced.
- These include many important filamentous fungi

  Ex: Penicillium

  Yeasts (candida)

Aspergillus

- All these are useful in antibiotic productions and in preparation of various foods.

- The genera include

  Alternaria, Arthobotrys, Aspergillus, Aureobasidium, Gotrichum, Helminthosporium, Penicillium and Trichoderma.

1.1.2.6.7 CULTIVATION OF FUNGI

Molds and yeasts can be studies by the same general cultural methods used for bacteria. Nearly all of them grow aerobically on normal bacteriological media at a temperature ranging from 20 to 30^{o}c. To isolate the fungi, the media is acidic (pH 5.6) and sugar concentrations must be high, then, at these conditions bacteria will not grow and only fungi can grow.

1.1.2.8 Comparative Physiology of Fungi and Bacteria

1.1.2.9 Molds and their association with other organisms

There are some interesting partnerships in nature involving a mold and some other organisms. In some of these associations the partners are dependent on each other and cannot survive alone. In others, they individually can survive independently.

a)Lichens

Lichens are composite organisms composed of fungi and algae. Each contributing the benefit of both. The algae synthesized carbohydrates by photosynthesis and obtain other nutrients from fungi. The fungi depend on algae for organic carbon.

b)Fungi & Nematodes

Nematodes are small round worms predominantly pathogenic for plants. Some may be parasitic in humans and other animals. Certain fungi that thrive on a diet of nematodes, various techniques are employed by fungi to capture even large size nematodes also, the important technique being the use of a loop of fungal hyphae. Upon contact by nematodes, the loop constricts around the body of worm and holds it firmly penetrating the prey with haustoria, and special branches that penetrate a host cell, which will digest the nematodes body. Ex: Arthrobotrys conoides.

c)Fungi as Parasites of Insects

Fungi associate with insects and responsible for infection of insects. Some appear as epidemics when they destroy large number of hosts. The fungi entomophthora has been the cause of mild epidemics among common house flies, crickets and grasshoppers. Such epidemics are self-limiting and can only rarely be used effectively to destroy insect pests, e.g., pathogenic fungi which attach aplid infestations of citrus orchards have been used in attempts to develop biological control methods for insect pests.

d)Mycorrhizas

A mycorrhiza is an infected root system arising from the rootlets of a seed plant. The word mycorrhiza is a Greek term meaning "fungus-root". These associations are usually beneficial to the host plant as well as the symbiout. Mycorrhizas enhance mineral absorption by the green plants. Truffles are subterranean fruiting bodies of certain ascomycetes which grow in association with oak trees. These fungal mycelium help in nutrient and water uptake by the plant, and these fungi get growth substances from plant. Mycorrhizal fungi are associated with many plants.

Rhizoctonia and Basidiomycete bletus, and other groups of fungi are involved in mycorrhizal association. Vesicular Arbuscular Mycorrhiza (VAM) is the most common of Mycorrhizal form and fungal partner belongs to Zygomycetes. The fungi will form Vesicles and Arbuscules inside the plant cell.

1.1.2.10 General Characters of Yeast

Sub-class – Hemiascomycatidae

Order - Endomycetales

Family – Saccharomycetaceae

Genus – Saccharomyces

Species – Cerevisiae

1)Introduction

- The sub-class Hemiascomycetidae includes the simplest ascomycetes which some mycologists consider primitive and others degenerate.
- They have very simple structure.
- Sexual reproduction takes place by the conjugation of two cells (gametangia) which in some species are alike (Eremascus) and in others unlike (Dipodascus).
- The fusion cell may be directly transformed into an ascus or grows out to produce an ascus.
- The chief characteristics of this class are:

i. The mycelium either poorly developed or lacking

ii. Absence of ascogenous hyphae.

iii. Complete absence of ascocarps.

iv. Direct development of asci without the intervention of ascogenous hyphae.

v. Asci borne directly on the mycelium or formed from a specialized ascogenous cell.

2)Classification

The sub-class includes about 250 species assigned to nearly 50 genera which are placed under the following two orders.

1. Order Taphrinales

➔ characterized by the presence of a true mycelium.

- ➔ Asci are developed from special, binucleate ascogenous cells derived from the binucleate mycelium and not from the zygote.
- ➔ The asci lie in a palisade-like layer parallel to one another but without any enclosing sheath or peridium.
- ➔ No fruit body recognized.
- ➔ Some mycologists recognize only a single family Taphrinaceae with a single genus Ascomyces (= Taphrina) in this order.
- ➔ Some others like Kramer (1958) include family Protomycetaceae in addition.
- ➔ They make about 6 genera with 125 species in this order.
- ➔ The best known species of this order is Ascomyces (= Taphrina) deformans.
- ➔ It causes a digease of the peach plant known as Peach leaf curl.

Fig.

Order: Endomycetales (Saccharomycetates)

- ➔ Asci develop directly from the fusion cell (zygote) or sometimes parthenogenetically.
- ➔ The zygote is formed by the fusion of the protoplasts of two ganutangia or two ascospores or two haploid vegetative cells.
- ➔ The plasmogamy is immediately followed by karyogamy so that there is no dikaryophase in the life cycle.
- ➔ Martin (1961) recognizes 4 families.

1. Ascoideaceae (or) Dipodascaecae

2. Endomycetaceae
3. Spermophthoraceae, and
4. Saccharomycetaceae

➔ Of these families, the reduced or degenerate ascomycetes popularly called the Yeasts belong to the family, Saccharomycetaceae.

3)Salient features of the yeast

➔ The yeasts are unicellular, degenerate, non-mycelial saprobic fungi that form no spore fruit. They are found in nature in the liquid organic material in the soil, animal excreta, surface of ripe fruits and nectar of flowers.

➔ They are capable of fermenting carbohydrate solutions producing ethyl alcohol and carbondioxide.

➔ The thallus is a minute hyaline oval or spherical cell which is differentiated into a distinct cell wall enclosing the protoplast.

➔ The cell wall is thin, delicate and chitinous in nature.

➔ The cell protoplast consists of granular dense – cytoplasm, a minute nucleus, a large vacuole and mitochondria.

➔ Vegetative reproduction takes place chiefly by Budding. In some yeasts it takes place by the method of fission.

➔ Ascoscope formation is the usual method of reproduction. Their formation involves a sexual process. The enlarged, diploid, old yeast cells under adverse circumstances function as asci.

➔ The diploid nucleus of the aseus cell undergoes meiosic to form 4 haploid nuclei which are organized into non-motile meiospores known as ascospores. The mature ascus thus contains 4-8 ascospores.

➔ The liberated ascospores under favourable conditions germinate, each producing a small, haploid spherical yeast cell. It is the dwarf strain of yeast.

- ➔ The dwarf-strain yeast cells multiply by budding and constitute the haplophase in the life of Saccharomyces cerevisiae.
- ➔ Sexual reproduction by the formation of sex organs is absent. The simplified sexual process is represented by plasmogamy immediately followed by karyogamy and meiosis.
- ➔ Plasmogamy in Saccharomyces cerevisiae consists in the fusion of he two haploid dwarf strain yeast cells to farm the large strain diploid yeast cell which normally reproduces by budding.
- ➔ In Saccharomycodes lud wigii, the two haploid ascospores fase to form a diploid zygote. The zygote germinates to produce the sprout mycelium.
- ➔ The cells of the sprout mycelium bud off diploid yeast cells.
- ➔ The diploid vegetative cells function as asci and undergo meiosis to form the ascospores.
- ➔ The yeasts thus exhibit alternation of generations in their life cycles which is of 3 types.

    a. In Saccharomyces cerevisiae both the alternating generations are propagated by budding and thus are of equal importance.

        (Haplo – diplobiontic)

    b. In Saccharomyces ludwigii the haploid phase is short and represented by the haploid ascospores only. (Diplobiontic).

    c. In Schizosaccharomyces octosporus, the diploid phase is represented by the zygote only which immediately after its formation undergoes meiosis. (Haplobiontic).

4)Inter-relationships and Taxonomy of Yeasts

The yeasts in their life cycle normally do not form a mycelium. The usual and dominant growth form is a unicell. The unicellular thallus is very simple in its structures. Some mycologists imply that yeasts are the primitive ones because of their simple structure and their unicellular nature. In the thallus being a unicell, the yeasts

resemble bacteria. Like most bacteria, they are non-motile, lack chlorophyll and absorb food materials in a soluble form through the cell wall only by diffusion. The cell physiology of yeasts is basically, like that of bacteria.

The structure and methods of reproduction of yeasts resemble fungi closely. As the fungi, the yeast cell has a definite nucleus and possesses mitochondria and endoplasmic reticulum which are lacking in the bacterial cells. The occurrence of chains of yeast cells (psendomycilium) in a nutritive medium and the development of hyphal filaments in many species of yeasts under certain cultural conditions are indicative of the fact that the yeasts may be related to mycelial ascomycetes. The production of ascospores in sac-like asci by a process similar to that of the mycelial Ascomycetes is a further proof in support of this contention. It has been experimentally proved that yeast cells grown in the presence of chemicals such as penicillin and other suitable media may produce a true mycelium. Some produce ascospores by a process similar to the asco-mycelous molds. These observations indicate close relationships between the yeasts and ascomycelous molds. On the basis of above-metioned experimental evidence most of the mycologists consider yeasts as ascomycetous molds whose usual and dominant growth form is a budding unicell. It is connected with their habit of growing in sugary solutions. Many normally filamentous fungi can be induced to develop budding stages in media with high concentration of nutrients and by reducing the oxygen content of the culture medium. In Saccharomyces this habit has become permanent. On the basis of these experimental data, the yeasts are now considered reduced as degenerate forms.

The taxonomic position of yeasts, at present, thus is:

5)Alexopoulos (1962)

Division - Mycola

Sub-division - Enmycotina

Class - Ascomycetes

Sub - class - Hemiascomycetidae

Order - Endomycetales

Family - Saccharomycetaceae

Genus - Saccharomyces

Species - Cerevisial

6)Aexopoulos and Mims (1979)

Super kingdom - Eukaryonta

Kingdom - Mycetae

Division - Amastigomycotina

Sub-division - Ascomycotina

Class - Ascomycetes

Subclass - Hemiascomycetidae

Order - Endomycetales

Genus - Saccharomyces

Species - Cerevisiae

7)Economic Importance of Yeasts

The importance of yeasts to man lies in their ability to ferment carbohydrates hence the name Saccharomyces (Gr. Saccharon meaning sugar and mykes meaning fungi) is applied to them. During respiration the yeast cell oxidizes sugar to form a simple organic acid and releases energy. When the supply of free oxygen is restricted the organic acid is split into CO_2 and alcohol. These products are of no use to the yeast plant but are of immense value to the baking and brewing industries. In baking, CO_2 is an industrial product and the alcohol is waste. The former is responsible for raising of dough and giving spongy texture to the bread. The alcohol produced during the baking process is driven off as a by-product. In the brewery, on the other hand, alcohol is a valuable product. Commercial manufacture of ethyl alcohol is a large industry. Carbondioxide, which is a by-product in the process, can be compressed into solid form.

In view of their industrial importance the yeasts have been hybridized and certain domesticated strains, varieties and species

have been developed by selection and breeding. These are superior to the wild yeasts industrially as they differ in their ability to synthesize vitamins and to ferment sugars. Some strains are better from the standpoint of nutrition or keeping qualities. The common among them are the brewer's yeast, bakers' yeast, distillers' yeast and the like.

The extensive use of yeast in brewing and baking industries has given great impetus to the establishment of another important industry. It is the preparation of yeast cake on commercial scale. Commercially the yeast cakes are prepared by pressing into cubes a large number of yeast cells together with starch which is an inert substance. The yeast cakes are doubly useful, in the home and in the baking industry. They may be eaten directly as health giving food. It was a fad sometimes ago. In the laboratory the yeast cakes are placed in a warm sugary solution to demonstrate the fermentation process. The yeast cells are activated. The tiny yeast cells are rich in protein. Some strains afford considerable fat. They are, therefore, mingled with other food-stuffs to increase their food value. Fresh yeast cells are excellent source of vitamins b and g and thus form an important source of these highly valued substances. Compressed yeast is also used as a source of vitamins and of enzymes useful in the manufacture of syrups and confectionery products. Ashby gosypii, a filamentous yeast, is used in the production of vitamin Rboflavin. Yeasts are also employed to impart flavour to the cocao beans. Besides the above-mentioned uses of yeasts, they are utilized in medicine, as a source of enzymes, in the production of alcohol and in many other ways.

The yeasts have a negative value as well. Some species are trouble some contaminants in brewing and wine making. They release unpleasant flavours or odours in their metabolic activities. Yeasts may spoil foods containing sugar especially fruits. They impart a yeasty flavour. A few species are parasitic on man. One of them causes a serious disease known as Cryptococcosis. Candida albicans is the cause of thrush. Some produce superficial infections of man.

1.1.2.11 Model Questions

1)write in detail abot the structure4 and morphology of Fungi and add a note on fungal classification

2)Describe the general features of yeast

1.1.2.12 Reference books

1. Microbiology → Michael J. Pelczar, JR.
E.C.S. Chan
Noel R. Krieg

2. Microbiology → Prescott
L.M. Harley
J.P. Klein, D.A

Lesson 1.1.3

MORPHOLOGY AND ULTRASTRUCTURE OF VIRUSES

Objective

1.1.3.1 introduction

1.1.3.2 Identification of human viruses

1.1.3.3 Brief out line discovery of viruses

1.1.3.4 Sociological significance of theory in the discovery of viruses

1.1.3.5 Definitions

1.1.3.6 Methods of study of viruses

1.1.3.7 Properties and morphology of viruses

1.1.3.8 Dose inoculum

1.1.3.9 Chemistry of viruses and the genomes and types

1.1.3.10 Study of sub viral agents

1.1.3.11 Model questions

1.1.3,12 Reference books

Objctive

The present chapter deals with structure classification and morphology ofviruses and different disease and their symptoms caused by the viruses

1.1.3.1 Introduction

In the early 18th century `Lady Montagu', wife of an English Ambassador to Turkey, observed that Turkish women inoculated their children against small pox. The children came down in mild attack and were immuned.

Later an English Doctor `Edward Jenner' was surprised by a girl's claim that she could not get small pox because she had cowpox. Jenner started inoeulating humans with the material from cowpox lesions. At that time he did not understand about Small pox.

In 19th century, harmful agents were grouped together and called them as viruses.

In 1884, porcelain filters were discovered by Charles Chamberlandt. TMV (Tobacco Mosaie Virus) was filtered through this filter.

In 1892, Iwanowsky filtered TMV virus – effected plant juice through porcelain filters. But the filters were effective in removing bacterial but not virus particles. The juice still contained TMV virus even after filteration. He named these viral particles as Toxins.

During the period 1898-1900, Beijerinck also performed his studies extensively on TMV. He also, was able to show the same results as that of Imanously. He name them as Filterable viruses. Viruses multiply only in the presence of a living cell but they cannot surgive a long time in dried stage.

At the same time, Frederick Loeffler and Frasch found that `Foot and Mouth disease' was also caused by filterable viruses.

1.1.3.2 Identification of human viruses

The 1st human virus identified in 1901 was the causative of yellow fever. It was wide spread in the tropical countries since the 15th century.

Later in 1880, Cuban Physician Carolas Juan finally proposed that a blood sucking insect mosquito was transmitting this disease. Dr. Jesse Lazear, member of Reeds Commission (A Commission to study the etiology of yellow fever was established by the U.S. Army by Colnol Walter Reed in 1899), sacrificed his life to demonstrate that yellow fever was transmitted by mosquitoes. He died of the disease due to experimental infection.

Mosquito control remains to this day as an important method for the control of yellow fever.

Though, human viruses were identified during the early decades of this century, it was slow because of the dangerous difficulties associated with viruses and bitter experiences with yellow fever virus. In mid 1700's, Influenza virus was identified. Until 1933, Human Influenza Virus was not isolated. Wilson Smith, Christopher Andrew's and Patrick Laid law identified Human Influenza and found a host suitable for it spropagation. They identified Influenza A virus and inoculated adult mice and chicken embryos. In chicken embryo, vast quantities of the virus was produced in the allautoic sac. Till today, chick embryos are used to produce influenza vaccine.

In 1908 Ellerman and Bang reported that Leukemia in chicken was transmitted through a filterable virus. In 1911, Peyton Rous reported that a virus is responsible for malignant muscle tumour in chicken.

Bacteria also could be attacked by viruses. In 1915 Twort isolated bacterial viruses which could attack and destroy micrococci and in testinal bacilli.

De Herelle independently isolated bacterial virus from patients with dysentery. When a viral suspension wees spread over a lawn of bacteria grown on agar medium, after incerbaction clear circular areas containing viruses and lysed bacterial cells was observed. He named these viral particles as Bacteriophages (Bacteria eating viruses).

The chemical nature of viruses was studied by Stanley in 1935. He crystallized TMV and found that its completely a protein molecule. He got Nobel Prize in 1946 in Chemistry.

A little later, Bawden and Pirie managed to separate TMV particles into Proteins and Nucleic acids. So by the end of 1930's, it was a little bit different task, because animal cell cultivation was a difficult work.

In 1931, Robert Koch discovered. Nutrient agar for cultivation of viruses. In the same year, Woodruff and Good Pastem Published a paper describing the use offertilized chicken egg as a nutrient for cultivating some viruses. The shell of chick embryo act as Petri dish, the nutrients include the chick enbryoand its contents.

In 1940 Electron microscopy and test tube cultivation of viruses which made possible for controlling certain viral diseases.

In 1952, Alfred Hershey, chase showed that Nucleic acids, but not proteins are the sources of hereditay information in virus. They examined the replication of Bacteriaphage T_2.

1.1.3.3 Brief outline and discovery of viruses

- The branch of biology or discipline i.e., virology is being quite young and H is very recently originated.
- The people that deal with viruses are call Virolgists.
- The viruses are otherwise called as ultra filterable agents or sub-microscopic agents.

- Viruses can't pass through even a very minute pouring filters. Hence these are called ultra filterable agents.

- These agents can't visualize through an ordinary light microscope. Hence we required a sophisticated instrument or fool to visualize such kind of submicroscopic agents, which is as Electron Microscope, which was 1st discovered by Knoll and Ruska but the agents nothing but the viruses have been seen under the electron microscope by Dr. Williams.

- These organisms are usually available in the sizes of ranges from 20 mm to 200 mm.

- The greatest contribution in the field of virology has been done by Dimitry Iwanowsky in the 18th Century.

- The role of Iwanously in the discovery of viruses is the invention of filterable agents but he is not considered about the thought of the presence of other creatures other than bacteria that are very minute.

- Later a Saientist, M.W. Beijerinck has proposed the concept of `Contagium vivum fluidum' in Greek language Contagium means infections. Vivum means viable.

- He concluded that the organisms can pass through even submicrospic filters or ultra filters.

- He confirmed the aspects of viruses, especially, the discovery of Tobacco Mosaic Virus (TMV).

- As the tobacco plants are being plated in the field, they may sub with each other frequently due to wind currents or air currents. Hence automatically, the infections sap may be transmitted to the healthy plant and can be infected.

- Another modern development that was taking place in the Virology is the discovery of crystallization process.

- As it is necessary to purify the viruses in their original forms, we need the crystallization indeed.

- To a void the contamination of other microbial population, the crystallization can be done by using alcohol (or) Acetone.

1.1.3.4 Sociological significance of theory in the discovery of viruses

The cultivation and possession of `Tulip' flowers became crazy in 16th and 17th centuries in the countries like France and Holland.

- As due to the occurrence of brokens or striations on the petals of the flowers gave the beautiful appearance which is being like by female population.

- In the initial stage it was known that the brokens (or) Tulips are made only because of the characteristic feature but later it was concluded that the causative agent responsible for the Tulips in the petals of flowers is because of these ultrafilterable agents.

- The advantage associated with these infections is that they may not accountable for the infection in human beings as these purely exhibit the most specificity.

1.1.3.5 Definitions

Viruses are obligate intracellular parasites which can only be viewed with the aid of an electron microscope. They vary in size from approximately 20-200nm. Inorder to peasist in the environment they must be capable of being passed from host to host and of infecting and replicating in subceptible host cells. A virus particle has thus been defined as a structure which has evolved to transfer nucleic acid from one cell to another. The nucleic acid found in the particle is either DNA or RNA, is single- or double-stranded and linear or segmented. In some cases the nucleic acid may be circular. The simplest of virus particles consists of a protein coat (some times made up of only one type

of protein which is repeated hundreds of times) which surrounds a strand of meleic acid. More complicated viruses have their nucleic acid surrounded by a protein coat which is further engulfed in a membrane structure, an envelope consisting of virally coded glycoproteins derived from one of several regions within the infected cell during the maturation of the virus particle. The genetic material of these complex viruses encodes for many dozens of virus specific proteins.

The complete fully virus is termed the virion. It may have glycoprotein envelope which has peplomers (projections) which form a `fringe' around the particle. The protein coat surrounding the nucleic acid is referred to as the capsid. The capsid is composed of morphological units or capsomer. The type of capsomer depends on the overall shape of the capsid, but in the case of icosahedral capsids. The capsomers are either peatamers or hexamers. Capsomers themselves consist of assembly units that comprise a set of structure units or protomers. Structure units are a collection of one or more non-identical protein subunits that together form the building block of a larger assembly complex (e.g, virus proteins VP_1, VP_2, VP_3 and VP_4 of picornaviruses). The combined nucleic acid protein complex which comprises the genome is termed the nucleocapsid, which is often enclosed in a core-within the virion.

1.1.3.6 Methods of study

While the electron microscope (EM) had been known for many years, the invention of the negative-staining technique in 1959 revolutionized studies on virus structure. In negative – contrast EM, virus particles are mixed with a heavy metal solution (e.g. Sodium phosphotungstate)

And dried out a support film. The stain provides an electron-opaque background against which the virus can be visualized. Observation under the electron microscope has thus allowed the definition of virus morphology at the 50-77A^o resolution level. In addition, negative staining of thin sections of infected cells has allowed definition of structures which appear during virus maturation & their interactions with cellular proteins. Immuno

electron microscopy is used to study those viruses which may be present in low concentrations or grow poorly in tissue culture (e.g., Norwalk virus). These clumps of virum are more readily observed. Electron cryomicroscopy reduces the risk of seeing artifaets as may be unavoidable by negative staining. High concentrations of virus are rapidly frozen in liquid ethane while on carbon grids. Electron micrographs can be digitized and three-dimensional reconstruction performed. Resolutions of 9^{o}A have been achieved using this method. X-ray diffraction of virus crystals is the ultimate in determining the ultra structure of vision morphology.

1.1.3.7 Propertees of the viruses – Morphology of viruses

1)Virus Symmetry

The capsids of various tend to have one of two symmetries helical or culooid. Helical symmetry can be loosely described as having a `spiral stair case' structure. The structure has an obvious axis down the center of the helix. The subunits are placed between the turns of the nucleic acid. Ex: Tobacco Mosaic Virus.

Animal viruses with a similar capsid structure include measles, rabies and influenza. Most animal viruses have spherical or cuboid symmetry. Obtaining a true sphere is not possible for such structures and hence subunits come together to produce a cuboid structure which is very close to being spherical. The `closed shell' capsid is usually based on the structure referred to as an icosahedron. A regular icosaheron, formed from assembly of identical subunits, consists of 20 equilateral triangular faces, 30 edges & 12 vertices & exhibits 2, -3, & -5 fold symmetry.

The minimum number of capsomers required to construct an icosahedron is 12, each composed of 5 identical subunits. Many viruses have more than 12. Ex: Adenovirus. In this,

projecting fibers are also present, which distinguishes this capsid from that of other viruses. The maturation & assembly of these structures is very complex; indeed, much of how it happens is unknown.

I cosa hedral structures are, however, usually formed via a complex but structured array of molecular-assembly procedures which eventually give rise to the mature capsid. These may be self-assembly processes or may involve virus non-structural proteins acting as scaffolding proteins which do not finish up in the nature capsid.

2)Virus Envelopes

Many viruses in addition to having a capsid also contain a virus-encoded envelope. Most enveloped viruses bud from a cellular membrane (plasma membrane, e.g., influenza virus, or nuclear membrane, e.g. herpes simplex virus). Whithin this virus lipid/protein bilayer are a number of inserted virus-encoded glycoproteins. The envelopes can be amorphous (e.g., the herpes virion). Or tightly bound to the capsid (e.g., HIV). Thus, the lipid of the envelope is derived from the cell, the glycoprotein being encoded by the virus.

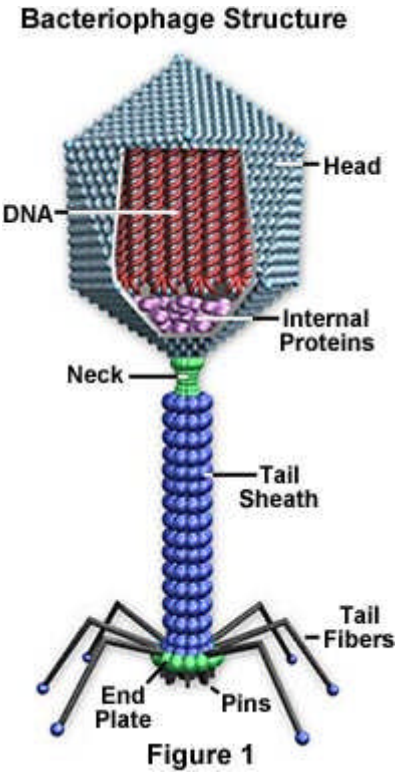


Figure 1

3)Properties of the viruses

- The organisms are available in both cellular and acellular forms.
- They measure around the various diameters but only in `nm'.
- The viruses may contain the genetic material either DNA (or) RNA (DNA e.g., Ademoirus, Hepatic virus) (RNA Polio virus, HIV).
- The majority of the viruses may possess the genetic material as DNA but the exception to this property is the presence of genetic material as RNA in most of the plant viruses and as well as the animal viruses.
- The exception to the plant viruses that contains the genetic material as DNA is CaMV (Cauliflower Mosaic Virus).
- The viruses can be attained in the form of crystals.

- The genetic material of the viruses can be well protected by the proteinaceous matrix or network or mesh-like structures namely capsids. The outer protection of the capsid is done with the help of capsomeres.
- The capsomeres that are rich in protein, carbohydrates and lipids are together referred to as peplomers.
- The virus may be either enveloped (or) non-enveloped.
- The non-enveloped viruses are otherwise referred to as Naked viruses.
- The viruses may available in different types according to origin or mode of construction.
- They exhibit in cuboidal shape, spherical shape, spiroidal, rod shaped, brick shaped virus and Icosahedral.
- Icosahedral associated with 12 vertices and 20 facets.
- The viruses are classified into two kinds based on their host specificity.

    i. Plant viruses

    ii. Animal viruses

- 4)**Plant viruses**

The viruses that are utilizing the plant material for their survivance and existence and complete their life cycle are called plant viruses.

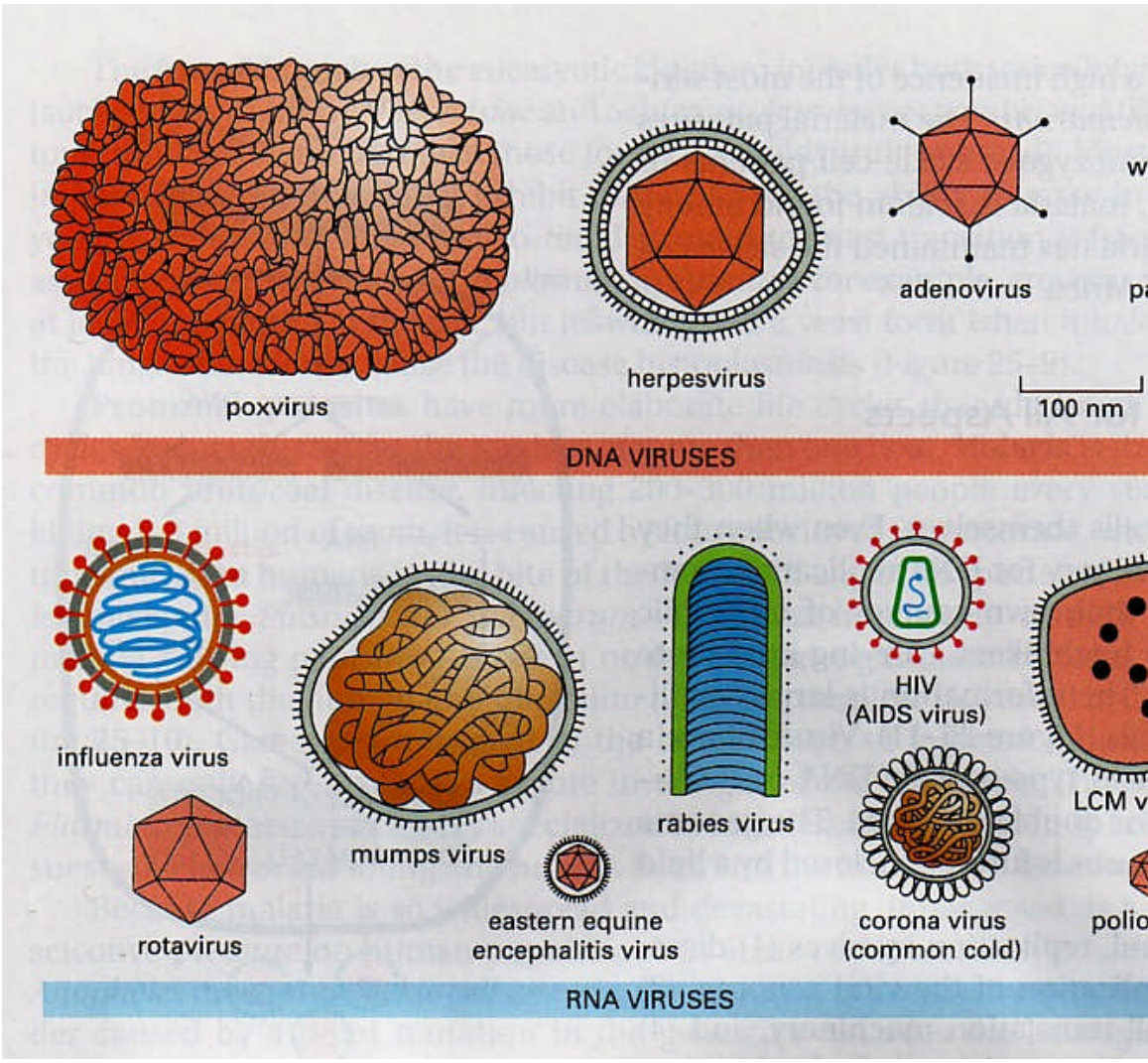
-5)Animal viruses

The regular metabolic activities of the virus and its life cycle has been takes place in the host of either animal and human beings.

➔ The diseases that are transmitted from animal to man or from animal – animal are called as zoonotic infections.

Ex: Rabies virus

➔ The routes of transmission and portal of entry and the dose of inoculum and the individual immune status, all these factors may profoundly influence the occurrence of disease.



➔

1.1.3.8 Dose of inoculum

The lower dose will decline the status of the infection where as the higher doses may enhance the disease progression.

1)Immune status of the individual

a)Immuno compromising person

The person who is suffering with lethal infection (near to death).

The pre disposing conditions for the occurrence of the infection are as follows.

i. Immuno compromising person (HIV patients or Hepatitis –B)

ii. Serve injury or trauma to the human body.

iii. Immuno suppressed patients (transplantation)

iv. Diabetes

v. Persons who are subjected to stress and B.P.

B)The contribution of various scientists in the field of (viruses) virology

S.No.	Scientist	Year	Discovery
1	Edward Jenner	1796	Discovery of Vaccine
2	Louis Pasteur	1880	Discovery of causative agent of Rabies disease
3	Edolf Meyer	1892	The discovery of submicroscopic agents (or) ultrafilterable agents
4	Dimitry Iwanowsky	1896	Invented the pathogenic nature of filter able agents
5	M.W.Beijerinek	1898	Proposed the concept of contagium vivum fluidum
6	F.de.Herelle	1916	Proposed concept of contagium vivum fluidum
7	F.Parker	1925	Successful cultivation of

			vaccinia virus
8	W.M.Stanley	1935	Discovery of crystalline (pure form) form of viruses
9	Knoll and Ruska	1935	Invention of Electron Microscope
10	Dr.Williams	1941-1945	The invention of electron microscope for the observation of viruses
11	Zinder and Leerberg	1952	Transduction (the transfer of genetic information from organism to organism by bacteriophate)
12	Hershey and chase	1952	Transformation process
13	Salk and Sobin	1957	Discovery of both Qral Vaccine and intravenous polio vaccine
14	A.Issac and J.Lindermann	1957	The discovery of viral growth cycle inhibitos namely interferous
15	Gallow	1984	Discovery of HIV virus

M.W. Beijerinck is the father of virology.

2)Morphology of viruses

- Study of shape & forms of the viral organisms is together called morphology.
- Morphology be broadly categorized into external and internal characters.
- External characters – size, shape, appearance & color.
- Internal characters - Anatomy (Ana; up; tomy; to cut)

  Physiology (study of function of the tissues)

- The morphological features of any organism is being dependent and may vary from organism to organism. It depends on a variety of features and number of factors may influence them, such as the internal organization of the cell and the constituents of the cell like proteins, carbohydrates and lipids; where as in case of viruses, the viral external morphology is being effected by the outer proteinaceous layers that is `capsid'.

- The viruses are available in different sizes ranging from minute to large extent but they are very minute than prokaryotes Ex: E.Coli

- The shape of the viruses is usually from spherical to rod like structures and sometimes cuboidal in shape. But some times the viruses may also exhibit the more complex shape, the best exemplified shape given by the pox virus i.e, brick shaped.

- The bullet shaped virus is also predominant which can be found in animals of both domestic and wild.

  Ex: Rabies virus.

- The linear shaped viruses are highly complexed ones.

3)Virus symmitry

a)Capsid

- This is the outer protein and well protects the internal constituents of the virus which is formed of the combination of glycoproteins, lipids & carbohydrates.

- It is not sustained that the capsid may reside the shape of the virus-some times a single cell may be infected by a group of viruses. Among them, they may alter or convert their capsid to the other organism. Hence, the organism may be benefiled by protecting its original nucleicacid. Hence, such kind of transfers of capsids from organism to

organism and its phenomenon is called as Transcapsidation.

- The genetic material of the organism may also show the impact on the shape of the virus, whether that genetic material is either DNA or RNA, whether it is single stranded or double stranded, whether it is left handed helix or right handed helix, whether it is segmented or unsegmanted.

- Another characteristic feature that determines the shape of the virus is symmetry. It can be defined as the design and arrangement of capsomer around the capsid.

4)Virus envelopes

a)Envelope

- The viruses may be either enveloped or coated or non-enveloped or naked.

- The envelope can be made up with proteins, carbohydrates, lipids but the major constituents of the envelope is lipid. Hence, the structural integrity of any enveloped virus is depends on the lipid content as it gives flexibility to the organism.

- The envelopes are usually measures about 10-15 nm in diameter.

- The envelope may also contain some projections which are made up purely with proteinaceous components. These are referred as peplomers (Ex. Adenovirus).

1.1.3.9 Chemistry of viruses & The genomes and types

- The chemical composition of the whole viral component is considered as chemistry of the viruses.

- The major ingredients and the components of viruses are nucleic acids, proteins, carbohydrates and lipids and other essential miscellaneous compounds.

1)Nucleic acids

- The entity of the pathogenicity of an organism is completely depends on the genetic material possessed by the organisms.

- The acidic property of the genetic material that is present in the nucleus of an organism is called nucleic acid and the perform of the nucleic acid is called as nuclein.

- The genetic material may be either DNA or RNA which have been classified on the basis of the location of sugar.

- The DNA is a self replicating molecule where as the RNA is dependent replicative molecule.

- Most of the viruses especially animal viruses contains the genetic material of equal priority whereas, in plant viruses, almost all the viruses contain the RNA as genetic material.

b)Proteins

- To protect the etiological nature of the organism, they must possess an external coat and as well as the other integral proteins which are being classified as the structural and regulatory proteins.

- Along with the synthesis of nucleic acids the viruses also having the ability to synthesize the proteins to form a mesh or network and sometimes the proteins of the viruses may contain the receptors or the external projections.

  They may act as pathogenic determinants of the organism.

- Most of the viral proteins are glycoproteins. During replication process, the viruses case able to synthesize the essential glycoproteins.

3)Carbohydrates

- The vital role of the carbohydrates is more in case of the enveloped viruses.

- As the enveloped viruses being composed of majorly by carbohydrates and tracer amounts of the proteins and lipids.

- The carbohydrate content that is present in virus is ribose sugar and deoxyribose sugar.

4)Lipids

The lipid content of the virus will give the flexibility and fragility to the viruses. Hence, eventhough the envelope is formed with the majority of the content of the carbohydrates the structural integrity of the virus is purely depends on the lipids.

5)Genomes

A complement of the genetic material of the organism is called as genome.

- There are 2 kinds of the genomes are available such as prokaryotic genome and eukaryotic genome when compared to the size and complexity of the virus.

- The complexity of the genome is purely depends on the no. of genes that are arranged in the genome of an organism and as well as on the basis of the introns or repetitive.

- The complexity of the prokaryotic genome is more than that of eukaryotic genome.

- In case of the RNA, there are two kinds of the genomes namely positive mRNA & negative mRNA. (or)

  Positive sense mRNA & negative sense mRNA.

- The RNA that does not depends on the host enzyme for the synthesis of essential components for the pathogenicity is called positive sense RNA, where as if they are dependent on the host enzymes, it is called negative sense mRNA.

1.1.3.10 Study of sub-viral agents

Sub-viral agents are those which cause the infection and are smaller than viruses.

Prions → Proteinaceous material is predominant

Viroids → Geutic material is predominant.

1)Viroids

In 1971, T.D. Diener, a plant pathologist, studied about potato tube disease. This was caused by virus. Later on he identified that RNA particle is causing this disease, but not the virus. He named these RNA particles as viroids.

This viroid is a single stranded RNA normally existing as a closed circle collapsed into a rod-like shape by intrastrand base pairing.

Viroids differ from viruses in 6 ways. They are:

- → Single stranded, RNA circle with low molecular weight.
- → Capsid and cover are absent.
- → They cannot synthesize proteins
- → Do not require any helper viruses.
- → Present in nucleus of infected cell.
- → They need special techniques to identity RNA (or) nucleotide sequence stridy.

The diseases caused by these viroids are:

- → Potato spindle tuber disease.

→ Exocortis disease of citrus plants.

→ Cucumber pale fruit disease.

→ Tomato apical stunt disease.

→ Chrysanthemum stunt disease.

→ Cadang-cadang.

→ The RNA of viroids consists of intrastrand base pairs.

→ Nucleotides are of 250-370 in RNA.

→ Susceptible to environmental condition because of absence of capsid (or) cover.

→ Vd is meant for viroid.

→ RNA of viroids do not act on mRNA in protein synthesis.

→ Replication occur in host cell only by using RNA dependent RNA polymerase.

→ Rolling circle mechanism seems to be in the replication of host cell.

→ In Potato Spindle Tuber Viruses (PSTV), there are 359 nucleotides and it is 130,000 daltons.

→ It is the smallest viroid among all the viroids that are known for plant diseases.

3)Cadang-Cadang

The disease was 1st found in the Philippines Country only and it is purely restricted to the limited area or specified area.

- The term cadang-cadang means dying.
- In the year 1937, an estimated no. of 30 million coconut trees are devasted.

- According to the plant pathologist, Agreos, this is highly an economically an important disease as the people of the Philippines are dependent on these trees for the food and lumbar and also the dried meet of coconut which is responsible for the or / extraction.

a)Causative agent (or) entity

The agent i.e., responsible for the disease is cadang-cadang viroid which contains the genetic material of RNA, cotaining 246 nucleotides. In case of the longer viroid, another one or more additional nucleotide will be added making 247 nucleotides.

b)Clinical Manifestations

The characteristic features of the disease can be represented as follows.

- The symptoms many not appear upto 8-15 yrs by the entry (or) inoculation of the organism takes place.

- After the appearance of 1st symptom, the plant may not die even upto 5-10 yrs.

  But it may affect the yield of coconut trees.

- The yellow colour spots may usually be seen on the leaves of the coconut tree.

- As the disease is progressively occurring the yellow colouration of the leaves is more and lead to the conversion of leaves into bronze colour.

- As the age of plant is growing the symptoms may also be enhanced and lead to the falling of leaves and appears in the shape of telephone poles.

c)Mode of disease transmission

- It is not clearly known that how the viroid is able to transmit from plant to plant but the viroid can be able to survive in the husk and embryo of the coconut.

- The viroids may also be seen in the seedlings of the plant.
- It is still under suspection whether the viroid is transmitted through the tools of the cultivation or not.

d)Prevention and control

- There is no treatment for this viroidal disease.
- No disease recistant cultivors are developed.
- Due to the advancements in the techniques like electrophorests, we can be able to diagnose the viroid in the given plant material.
- As a major source of the infection is seeds, they has to be treated either with heat or Bordeaux mixture.

2)Potato spindle tubes viroid

- The disease is highly predominant in the countries like United States, Russia, Canada and now-a-days, it is also found in India.
- The causative viroid of this disease PSTV. This is the viroid that has been 1st recognized. It contains a no. of 359 nucleotides in their genetic material of RNA i.e., single stranded.

a)Symptoms of the disease

- Presence of chlorotic spots led to the formation of greenish discoloration in the leaves.
- The plant usually appear as dwarf. The leaves are nuythin and become pale.
- The tubere are seen as an elongated structure.
- The leaves may be sometimes either twisted (or) coiled (or) wrinkled.

- The symptoms may usually appear within a short span of the entry of viroid.
- Due to the tissue culture methods and advanced techniques in the biology, the disease resistant cultivars are developed.

3)Prions

The other subviral agents which are highly proteinaceous in nature is referred to as `Prions'.

Around 1920, some of the Scientists studied about neurological diseases.

Cruetzfeld and Jacob, they observed an organism causing neurological diseases in live stocks and humans. So it was named as CJD disease. In 1982, Prusiner identified a proteinaceous substance in the live stock i.e., sheep and goat. He named that proteinaceous substance as prion.

(Prion = Proteinaceous infections particle).

It is a partly purified protein without nucleic acid. This protein in about 30-35 kilo Dalton. It is smaller than virus.

b)Conclusions given by prusiner

- They are resistance to heat
- They cannot be damaged by U.V. radiation.
- They cannot be denatured by enzymes.
- They can be damaged by phenols and urea.
- Prp proteinaceous particle found in the brain cells of human beings.
- They are present in the plasma membranes of brain cells.

- Due to disorganization of proteins and mutation in the brain cells, they are causing neurological disease in livestocks and humans.

- In 1987, he got Nobel Prize in Medicine.

- The infections that are caused by prions are referred to as "Transmissable Spongiform Enccphalopathies'

- The naming of these diseases is characterized by two features. They are:

i. As the infection is easily transmissible and the components are proteinaceous.

ii. As the organisms cause spongiform cephalitic region (or) cerebellar region, where it forms the vacuoles of 10-200 u, hence they are being named as Spongiform Encephalopathies.

Some of the diseases caused by prions are:

1. Scrapie

- In animals, the 1st encountered disease of neurogenerative is `Scrapie'.

- It is highly endemic in nature.

- The disease transmission rate is more.

- It is caused by the proteinaceous infections material (or) prion.

- It is the naturally occurring disease of sheep found in many parts of the world.

- More than 200 yrs ago, it was 1st described.

- It is organized in Spain and Spread through out Western Europe.

- In 19th Century, export of sheep from Britain help Serapie spread around the World.
- It affects mainly sheep (or) goat.
- Infected animals show severe (or), progressive neurological symptoms like abnormal gait, disorder of central nervous system, scraping against fences on posts.
- Australia & Newzealand has eliminated Scrapie by slaughtering infected sheep.
- Effected animals lose coordination of their movements and cannot walk.
- The natural mode of transmission between sheep is unclear.
- Symptoms are seen in sheep of less than 1 ½ year old.
- The 1st traces are detected in the tonsils, mesenteric lumph nodes and intestine of sheep.
- This suggests an oral route of infection. The infective agent is present in the membranes of embryo, but it has not been found to be present in milk or tissues of new born lambs.
- Neurological symptoms like nervous weakness, neck stiffness, coma and death may take place.
- The characteristic protein that is being responsible for serapic prpse (Prion Protein Scrapic).
- The incubation period of the organisms is more when compared to the other convention viruses.

2)Creutzfeldt – Jakob Disease (CJD)

- The humanized infection caused by prions is Creutzfeldt-Jakob Disease.

- The effected organs of the body is usually central nervous system.
- The rate of the infection depends on variety of factors like
    i. the immune status of the individual.
    ii. The dose of the inoculum.
    iii. The route of inoculum, etc.
- The route of both oral and parentoral are not recommendable, where as the intraocular route of prions may show the asverse effect.
- The incubation period may be from 2 months to 2 yrs.
- There are 3 variants of Creutz-feldt-Jakob's disease.

    Ideopathic – accountable for the infection through natural sources.

- Ramilial – occurrence of the infection through hereditary.
- Acquired – occurrence of the infection through accidental route.
- It is a slowly progressive disease of the Central Nervous System.

4)Satellite Viruses

Satellite viruses are small RNA molecules which are absolutely dependent on the presence of another virus for multiplication and existence. Viruses have their own parasites. Most satellites are associated with plant viruses, but a few are associated with bacteriophage (or) animal viruses. Neither DNA or RNA shows homology to the satellite viruses.

Ex: The dependo viruses which are satellites of adenovirus.

a)Properties of Satellite Viruses

- The genome is a single stranded (ss) RNA.
- Size of the genome is 500-2000 nucleotides.
- No nucleotide sequence similarity between the satellite and the helper virus genome.
- They cause disease symptoms in plants which are not seen with the helper virus alone.
- Replication of satellites interferes with the replication of the helper virus.

Satellite Viruses are of 3 types, namely

i. Plant satellite viruses

Best exemplified with Gemini and Canlimo viruses, where the genetic material is RNA.

ii. Bacterial viruses

The bacteriophages lke B_4 requires the bacteriophage of B_2 for the conduction of replication and able to cause of infection.

iii. Animal or Human viruses

The Hepatitis-D (RNA) particles are able to cause the infection only with the help of Hepatitic – B (DNA), even though they differ with the homology of the genetic material.

Satellite RNA's

The genetic material of the virus containing genome may acquire the protein-source from others or the duplication of the RNA is influenced by DNA is called Satellite RNA.

For ex: The genome greater than 0.7 kb is considered as a larger genome which is circular and acquire the protein source from the other viral genome.

- If the genomes that are greater than 0.7 kb is being circular and for the formation of (or) for the duplication of the RNA requires the DNA genome.

- The genome that is less than 0.7 kb is being linear and acquire the protein source from the other genomes (or) structural protein source genome.

- The genome that is less than 0.7 kb is being circular, it will alter its nature of RNA genetic material from the source of RNA dependent DNA polymerase.

  Ex: Retroviruses

Examples

One of the simplest examples is Satellite Tobacco Necrosis Virus (STNV). This satellite virus is totally dependent for its replication on simultaneous infection of tobacco cells by its infections helper virus, TNV. The icosahedral particles of STNV are considerably smaller than the particles of TNV, as is is defective RNA genome with 1200 nucleotides. They can synthesize nearly 400 proteins. The STNV single stranded RNA genome encodes its own protein coat and becomes preferentially encapsidated within this coat after replicating at the expense of its helper virus.

- With the help of Coliphage P_2, P_4 Satellite viruses infects E. Coli and replicates or lysogenizes E. Coli. A much more complicated satillite virus is coliphage P_4. This temperate ptrage infects & lysogenizes E. Coli by itself, but it cannot replicate and produce mature phage particles, without its non-defective helper virus, Coliphage P_2, another larger temperate coliphage. Several gene products of P_4 activate late gene transcription of its helper P_2. Some of the P_2 late gene products are capsid proteins and others active capsid protein synthesis by late genes of P_4. SV together with the capsid proteins of both viruses, assemble the head of P_4 phage. This mutual transaction of gene products from a helper and a satellite virus is referred to as transactivation.

1.1.3.12 Modelquestions

1)write a detailed account on classification ofviruses

1.1.3.13 Reference books

1. Microbiology → Michael J. Pelczar, JR.
E.C.S. Chan
Noel R. Krieg

2. Microbiology → Prescott
L.M. Harley
J.P. Klein, D.A

LESSON 1.1.4

Generalcharacters of different bacterial groups

Objective

1.1.4.`1 introduction

1.1.4.2 General characterstics of Rickettsia

1.1.4.3 General characterstics ofChlymydomonas

1.1.4.4 Archaeo bacteria

1.1.4.5 Methanogenic bacteria

1.1.4.6 Generalcharacters nbof methanogens

1.1.4.7 Mycoplasma

1.1.4.8 Photosynthetic bacteria

1.1.4.9 Sulfur bacteria

1.1.4.10 Model Questions

1.1.4.11 Reference books

Objective

The bacteria were grouped based in their specialcharacterstic features .In this chapter the different classes of bacteria and their character stics were explained.

Objective

1.1.4.1 Introduction

Rickettsias & chlamydias traditionally have been grouped together in this category because of some basic similarities. They are both non motile, small gram –ve bacteria that are obligate intracellular parasites within eukaryotic host cells.

Rickettsias are closely related to purple bacteria, because of this phylogenetic relationship, the genera Rickettsia, Rochalinaea, Ehrlichia, Cowdria, Neoriekettsia, Coxiella, Wolbachia, Rickettsiella are grouped.

1.1.4.2General features of rickettsias

- Rickettsias are parasitic (or) mutualistic bacteria.
- The parasitic forms are associated with endophelial cells of vertebrate hosts & the mutualistic forms are associated with cells of arthropod hosts.
- The majority of members of the riekettsias are small gram-ve rods & multiply within host cells.
- They are small for bacteria put larger than viruses.
- Within host cells, the rickettsias reproduce by binary fission.
- They are metabolically limited & are unable to generate sufficient ATP on their own to support cellular growth and reproduction.

- Hence they depend on host cells to supplement their cellular supply of ATP.

- The genes rickettsia is divided generally into three cellular.

1. The typhus group

Ex. Rickettsia prowazekii

Cause epidemic tyhus, its arthropod veetor is human body lice.

R. typhi

Cause endemic (or) murine typhue, arthropod vector is – fleas and lice. Vertebrate vector is – Rodents.

2. Spotted fever group

Ex: Rickettsia rickettsii – cause rocky mountain spotted fever, its arthropod vector is Ticks. Vertebrate vector is rodents.

Rickettsia japonica – cause Japanese spotted fever,

Asthropod vector – unknown

Vertebrate vectors – Rodents and dogs.

3. Scrub typhus group

Ex. Rochalinaea Quintana – causes trench fever,

Arthropod vector – Human body lice,

Vertebrate vector – humans

Coxiella burnetti – cause Q fever

Arthropod vector – ticks

Vertebrate vector – Cattle, sheep & goats.

- These three groups contain species that are pathogenic for humans and other vertebrate animals.

- Rickettsiae are difficult to study because of their requirement for intracellular growth.

- They can be propagated in embryonated eggs, in laboratory animals, & in tissue culture.

- Rochalinaea Quintana are the only rickettsia to be cultured in cell free medium.

- Most rickettsias grow in vertebrate host endothelial cells associated with small blood vessels and generally engulfed by phagocytic cells such as machophages & neutrophils.

- Most rickettsiees produces phospholipase A which allows then to escape from phagosomal vesicle in which they were engulfed & survive in cytoplasm.

- Coxiella burnctti, remain within the phagolysosome and have adapted growth in this acidic environment (pH 4.0).

- C.burnetti are unusual in their ability to form endospores also.

- Rickettsial species cause human diseases such as rocky mountain spotted fever and typhys fever.

- In most cases rickettsias are transmitted to humans by arthropod vectors.

- Weil Felix test is used to detect the rickettsial without specifying a particular species.

- In Weil-Felix test, the proteins 0 x 19 antigens are used to detect the rickettsia, because the rickettsia and proteins cells have same type of antigens which can stimulate the antibody production.

1.1.4.3 General features of chlamydias

- These are obligate gram – ve intracellular parasites.

- The chlamydias are metabolically limited and can reproduce only within compatible host cells.
- They lack the ability to synthesize ATP to support their growth and reproduction.
- Chlamydias depend on host cells for precursors and energy (aminoacids & nucleotides).
- Chlamydias have a complex biphasic life cycle.
- They have been some time referred as large viruses, but they are true bacteria.
- Chlamydias exists in two different developmental forms,
    1. Small (0.2 to 0.4 um), infections spore – like elementary bodies.
    2. Larger (0.6 to 1.5 um) non infection reticulate bodies that divide by fission.
- The elementary bodies are surrounded by rigid three layered walls and reticulate bodies are surrounded by thin flexible three layered walls.
- The chlamydial cell wall lack (or) contain traces of muramic acid and these are similar to that of Gram – ve cell walls.
- The reproductive cycle for chlamydias is characterized by the biphasic change of elementary bodies (EB's) to reticulate bodies (RB's) and RB's back to EB's.
- The elementary body (EB) is suited for survival in the environment much like a bacterial endospore.
- It is metabolically inactive and nonreproducing but highly infections for host cells.
- The elementary body (EB) attaches to the host cell membrane and penetrates the cell into a membrane bound vesicle where it differentiates into an RB.

- RBs are metabolically active and divide by binary fission, after several rounds of division they convert back to elementary bodies (EB's)

- Although the Chlamydial envelope leads the peptidoglycan (lack muramic acid in cell wall), chlamydiae species possess the penicillin binding proteins are sensitive to drugs that inhibit peptidoglycan synthesis.

- Three recognized species of chlamydias are present, & all of them cause disease in humans and animals.

- Chlamydia psittaci is mainly a pathogen of animals, especially birds but can cause serious pneumonia-like respiratory infections in humans.

- Chlamydia pneumoniae cause acute respiratory disease in humans.

- Chlamydia trachomatis is a human pathogen that cause diseases of the eye and genitourinary tract.

- Trachoma & conjunctivitis – eye diseases.

- Urethritis, cervicitis and lymphogranuloma

- Are genifourinary tract diseases,

- Chlamydia infectious are a major cause of sexually transmitted diseases.

1.1.4.4ARCHAEBACTERIA

The archaea comprise organisms that enolned as a separate domain, often retaining highly specialized phenotypic characteristics. Most archaea grow under extreme environmental conditions. Many archaea are extreme thermophiles & some can grow at temperatures over 100^{o}C. Yet, others are extreme halophiles, growing in such hostile environments as the Dead sea. Archae, more than bacteria, seem to have conserved primitive physiological and metabolic

traits, especially life at high temperatures and life without oxygen.

1)Cell Structure & Function

Archaea have several distinguishing features relative to their cell structures that permit them to live in extreme habitats and to function under conditions considered in hospitable to life.

2)Cytoplasmic membrane

It is a semi permeable membrane / barrier, which regulate the movement of substances into and out of the cell. Archaeal cytoplasmic membrane contains glyeeroldiether and diglycorol tetraether lipids.

3)Cell wall

The chemical composition of cell wall varies among various archaea pseudopeptidoglycan, `S-layer' of glycoprotein, methanochondroitin, sulfated heteropolysaeeharides are found in various bacteria. Cell wall gives protection against osmotic shock or physical damage.

4)Chromosome

Circular molecule that contains genome, histone-like proteins occur in association with the DNA and play a significant role in maintaining archaeal chromosome structure and gene expression.

5)Ribosomes

70 s type of ribosomes are present. Some archeal specific proteins are present. Translation of genetic information is carried by mRNA into proteins.

6)Moltility

Motility is by flagella. Flagella is made up of proteins. Sulfate oligosaccharides also accur in halophilic archea.

Archaeobacteria differ from each other in terms of morphology, chemical composition, metabolism and habitat. Three main categories are recognized.

1. Methanogens
2. Red extreme halophiles
3. Thermoacidophiles

1.1.4.5 Methanogenic bacteria

These are stringent anaerobes that share an ability to obtain energy for growth by oxidizing compounds such as hydrogen or formate, and utilize the electrons thus generated to reduce CO_2 with the formation of Nethane (CH_4) gas. Some genera can grow as autotrophs, using H_2 and CO_2 as sole sources of carbon and energy. Others require additional substances such as vitamins, acetate, aminoacids or organic sulfur compounds. Most species grow better in complex media than in organic media. Two unusual enzymes occur in almost all methanogens that have not been found in other bacteria. Coenzyme M, involved in methyl transfer reactions, and coenzyme F420, a flavin –like compound involved in an aerobic electron transport system of these bacteria.

The general of methane – producing bacteria are differentiated on the basis of morphology and gram reaction. Methanogens occur in various anaerobic habitats rich in organic matter which non methanogenic bacteria ferment to produce H_2 and CO_2. Such habitats include marshes, swamps, ponds & lake mud, marine sediments, the intestinal tract of humans and animals, the rumen of cattle and an aerobic sludge digesters in sewage-treatment plants.

Ex. Methanobacterium (Gram +ve)

Methanobrevibacter (Gram +ve)

Methanomicrobium (Gram –ve)

Methanogenium (Gram –ve)

Methanospirillum (Gram –ve)

Methanosareina (Gram +ve)

Methanococcus (Gram-ve)

1)Extreme Halophiles

These are chemoorganotrophic, aerobic bacteria requiring approximately 17 to 23% NaCl for good growth. They stain grame negative and range from rod or disc shaped cells (Halobacterium) to cocei (halococcus). They occur in salt lakes (eg. Dead sea and Great salt lake), industrial plants that produce salt by solar evaporation of sea water and salted proteinaceous materials such as salted fish. The colonies are a red to orange colour due to carotenoids which seem to protect the cells against the damaging effect of sunlight.

At high NaCl concentrations the cells resist dehydration by maintaining a high intracellular osmotic concentrations of KCl. Halobacterium cell walls are composed of protein subunits that are held together only in the presence of salt, this if the level of NaCl falls below about 10% the cell lyse. Halococcus cells are composed of a complex heteropolysaccharide that is stable even at low salt concentrations.

Halobacteria are aerobic. As in other aerobic organisms, an electron transport chain generates a proton motive force which in turn drives ATP synthesis. Halobacteria can generate ATP by fermenting amino acid arginine, this allows them to grow anaerobically. At low O_2 levels patches of purple pigment called bacteriorhodopsin are formed in the cell membrane. When cells containing bacteriorhodopsin are exposed to light, the pigment gets bleached. During this bleaching protons are extruded to the outside membrane, thus creating a proton motive force which in turn drives ATP synthesis.

2)Thermoacidophiles

These are aerobic gram –ve archaeobacteria. These are characterized by a remarkable ability to grow under highly acidic

conditions and high temperatures. Two genera are included in this group.

1. Thermoplasma
2. Sulfolobus

3)Thermoplasma

These are chemoorganotrophic organisms resembling mycoplasmas in lacking a cell wall and forming tiny "fried-egg" colonies. The cells are pleomorphic, ranging from spherical to filamentous. The optimum temperature for growth is 55 to 90^{o} C (Max-40^{o}C) and optimum pH is 2 (Max – 4 & min – 1). Cells undergo lysis at neutral pH. Thermoplasmas have been isolated from piles of burning coal refuse.

5)Sulfolobus

Cells of this genus are spherical or lobe – shaped. The cell wall mainly composed of proteins. Optimum temperature ranging from 70-87^{o}c. The optimum pH is 2 (max-4, min-1). Sulfolobus species are facultative autotrophic. They can grow as chemolithotrophs when supplied with elemented sulfur as an electron donor. Alternatively, they can grow as chemoorganotrophs in media containing organic substances. In nature, the organisms are predominate in acidic hot springs.

1.1.4.6 General characters ofMETHANOGENIC BACTERIA

The archaebacteria are stringent anaerobes that share an ability to obtain energy for growth by oxidizing compounds such as hydrogen or formate, so the electrons generated in this process (i.e., oxidation of hydrogen) is used to reduce CO_2 and form methane (CH_4) gas. These bacteria are called Methanogenic Bacteria.

Methane is produced during the anaerobic catabolism of organic substances.

It is estimated that 1-1.5% of the carbon liberated as atmospheric CO_2, by minerlisation of organic substances,

reaches atmosphere, first as CH_4, which is then converted to CO_2 by OH radicals. So methane in atmosphere is present at a rate of 1 ppm.

Methane is reduced in two ways

1. Priogenic sources
2. Abiogenic sources

The ecosystem where methane production occurs are the

- tundras and swampy regions (19.44%)
- Rice fields (25%)
- Sediments of lakes, ponds and paddy – 2.22%
- Sewage digestors and of the more than 10^9 minerals on earth produce 47.22% of methane.
- Altogether 58% of CH_4 is produced through biogenic sources involving methanogenic bacteria.
- Abiogenic sources, coal mines (25%), pipe leakages, burning of biomass and even from automobile industry, from volcanoes – total is 42%.
- Methane occurs in atmosphere at a rate of 1ppm. It is odourless, colourless and highly inflammable gas. Molecular weight is 16.04, boiling point is 161.61^oC.
- Methane is oxidized to CO_2 after 36 years of its existence as CH_4 as in atmosphere.
- About 70% of the total methane produced is derived from acetate and 30% from CO_2 and H_2O.
- There are about 10 genera and 27 species of methanogenic bacteria involving in methanogenesis.

Methanobacterium - Rod shaped

Methanococcus	-	Coccoid
Methanosarrina	-	Sarcina-like
Methanospirilla	-	Spirillae

The methanogens belongs to a special group of bacteria the Archaebacteria. They differ from other bacteria not only by their type of metabolism, but also by a number of characteristic features in the composition of their cell constituents.

1)Cell wall

They lack a typical peptidoglycan skeleton.

1. Methanococcus – has only protein envelope.
2. Methanospirillum – peptide sheath is found
3. Methanosareina – Consists of polysaccharides, the cell wall is composed of uronic acid, neutral sugars, amino sugars.

- Cell Wall is composed of pseudomurein, a substitution of N-acetyltalosaminuronic acid for N-acetyl muramic acid.
- Growth cannot be inhibited by penicillin.
- The cytoplasmic membrane of methanogens contain glycerol ethers with phytanyl (C_2O) and biphytanyl (C_4O), alkyl isoprenoids in place of the fatty acid, glycerol esters.
- Ribosomes are similar in size to those of eubacteria i.e., 70 S, but the base sequence of 16 S rRNA is quite different.
- Methanogene differ from E.Coli than do the cyanobacteria.
- This ribosomal translation is insensitive to antibiotics that inhibit protein synthesis in eubacteria.
- By these differences, the methanogenic bacteria are placed in archaebacteria.

2)Physiology

- Methanogens are strict anaerobes, exposure to air is lethal.
- Most of the methanogene use molecular hydrogen as H_2 donor and CO_2 as carbon source.

$$CO_2 + 3H_2 \longrightarrow CH_4 + H_2O$$

- Some can also use formate, methanol, acetate or methylamine as H_2 donor.
- In some anaerobic ecosystems, acetate is known to be the main substrate for Methane formation.

$$CH_3COOH \longrightarrow CH_4 + CO_2$$

- Different genera used different substrate for methane production.
- Spectrum of substrates is very narrow

	Substrate
Methanobacterium	$H_2 + CO_2 + HCOOH$
Methanobrevi bacteria	$H_2 + CO_2$/ HCOOH
Methano thermus	$H_2 + CO_2$
Methano coccus	
Methano spirillum	$H_2 + CO_2 + HCOOH$
Methanothrix	CH_3COOH

- Methanogenesis is not a pure culture fermentation. The methanogenic bacteria are the last link in an anaerobic nutritional chain that starts with polysaccharides, proteins, lipids.

There are three types of fermentative bacteria.

I. Bacteria that ferment cellulose to succinate, propionate, butyrate, lactate, acetate, alcohols, CO_2 & H_2O

Bacillus cereus Pseudomonas formicaus

Bacillus megaterium Clostridium cellobiparum

Ruminococcus havicoccus

II Acetogenic bacteria – Which ferment their primary products to acetate, formate, CO_2 and H_2.

These products are substrates for the methanogenic bacteria.

II. Methane producing bacteria occur in close association with hydrogen producing organisms. The excreted H_2 is dissolved in medium and it is assimilated by methanogenic bacterium. If the partial pressure of H_2 is increased, it can inhibit the metabolism of hydrogen producing organisms, so this is a mutual symlriosis (syntrophy).

- Methanogens are able to activate hydrogen and able to couple its oxidation to the reduction of CO_2, since they can synthesize all their cell substances using CO_2 as sole carbon source. Their mode of life is chemoautotrophic.

- All the CO_2 is also used as a `H' –acceptor in the generation of energy, with the production of CH_4, known as carbonate respiration.

$$4H_2 + CO_2 \longrightarrow CH_4 + 2H_2O$$

Some methanogens can also take CO to form methane with the intermediary formation of CO_2 and H_2O.

Methanogenesis occur in 3 stages.

I. Hydrolysis

Complex insoluble biodegradable organic substances are converted to soluble organic substances by cellulolytic and proteolytic enzymes.

II. Non-methanogenic fermentation

Soluble organic substances are converted to acetate, formate, CO_2, H_2 = - etc by anaerobic bacteria.

III. Methanogenic fermentation

All products of stage II are converted to methane.

3)Various chemical reactions involved are

1. Fermentation of glucose to acetate

   $C_6H_{12}O_6 + 4H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$

2. Fermentation of glucose to butyrate

   $C_6H_{12}O_6 + 2H_2O \longrightarrow C_4H_7O_2 + 2HCO_3 + 2H_2$

3. Fermentation of butyrate to acetate

   $C_4H_7O_2 + 2H_2O \longrightarrow 2CH_3COOH + H_2$

4. Fermentation of propionate to acetate

   $CH_3CH_2COOH + 3H_2O \rightarrow CH_3COOH + HCO_3 + H_2$

5. Acetogeresis from CO_2 and H_2

   $CO_2 + H_2 \rightarrow 2CH_3COOH \rightarrow 2CH_4 + 2H_2O$

6. Formation of CH_4 from acetate.

   $CH_3COOH + H_2O \longrightarrow CH_4 + CO_2 + H_2O$

7. Formation of methane from H_2 and CO_2

$$CO_2 + 4H_2 \longrightarrow CH_4 + 2H_2O$$

Fermentation of methanol and methylamines occur only in marine environments rich in sulfate reducers.

$$CH_3O_4 + H_2 \longrightarrow CH_4 + H_2O$$

4)Biochemistry of methane formation and energy gain

Biochemical conversion of H_2 and CO_2 to methane, and of acetate to methane and CO_2 involve a number of coenzymes and prosthetic groups that have been found so far in methanogens are:

1. Methanofuran
2. methanopterin
3. Coenzyme F_{420} (deazoriboflavine derivatine)
4. Coenzyme F_{430}
5. Coenzyme M
6. Component B.

Very little is yet known about enzymes in individual reactions.

- The enzyme methyl-coenzyme M methyl reductase is a multienzyme complex which contains, besides other proteins, F_{420}, F_{430} and dehydrogenase.
- The enzyme methyl transferase converts methanol directly to methyl coenzyme M, which is reduced with the liberation of methane.

$$CH_3 – S - CoM + H_2 \longrightarrow CH_4 + HS – CoM$$

- methanogenic bacteria regenerate ATP not by substrate phospherylation but by electron transport phosphorylation under anaerobic conditions (Anaerobic respiration).

1.1.4.7 MYCOPLASMAS

In 1989 E. Nocard and R.R. Roux discovered the organisms that were entirely different from other microbes while studying the pleural fluids of cattle suffering from bovine pleuro pneumonia. Organisms show different forms when they are cultivated on rich agar media containing about 20% animal serum such as spheroid, thin, branching filaments and many irregular forms. Similar pleomorphic forms are observed and isolated from other animals as sheep, goat, rats, humans. These similar form are also found as saprophytes in decaying organic matter. These are naned as pleuro-pneumonia – like organisms (PPLO's). PPLO's named by Borrel et al (1910) as Asterococcus mycoides (round satellite form, mold-like filaments). These organisms are commonly called mycoplasmas.

1)Characteristic features

Mycoplasmas are unicellular prokaryotic usually non-motile and form fried-egg shaped colonies. They are distinguished by their lack of cell wall, the outer boundary of cell being the cytoplasmic membrane. It contains sterols. The cells show plasticity. Because of the plasticity the organisms are pleomorphic, form different shapes ranging from spheres to branched filaments. They are filterable through bacteriological filters.

- ➔ The genetic machinery is the form of DNA, RNA and ribosomes.
- ➔ They are susceptible to lysis by osmotic shock caused by sudden dilution of the medium with water.
- ➔ They are resistant to penicillin action.
- ➔ But these mycoplasmas can be inhibited by tetracyclins, chloramphenicol, which act on metabolic, pathways.
- ➔ They vary in size from 300nm to 0.2 um in diameter.
- ➔ Mycoplasmas divide unevenly into very minute bodies called the elementary bodies or minimal reproductive units.
- ➔ Growth rate of mycoplasma is very rapid.

Mycoplasmas differ from the L-phage variants. L-phage variants develop from the other bacteria like streptobacillus. L-forms are fragite, cell wall defective forms that may occur spontaneously or as the result of continuous exposure to sublethal levels of penicillin. These also form fried egg-shaped colonies like mycoplasma. L-forms are derived from walled bacteria penicillin binding proteins and peptidoglycau precursors can be demonstrated in L-forms but not in mycoplasmas.

At present mycoplasmas are placed in the class mollicutes. It contains single order mycoplasmatales. Of the mycoplasmas, 3 families are present.

2)Mycoplasmataceae

Mycoplasmas are parasites of the mucous membrane and joints of humans and animals. They require cholesterol for their growth. These are pathogenic to animals.

Mycoplasma pneumonia cause atypical pneumonia

Ureaplasma → require urea for growth and cause urethritis in humans & pneumonia in cattle.

3)Acholeplasmataceae

These do not require cholesterol for growth.

Distributed in vertebrates, in sewage & soil and possibly on plants.

Pathogenicity is unknown.

4)Spiroplasmataceae

These organisms are helical and exhilit swimming motility.

Swimming motility is without flagella.

Single genus, spiroplasma, is pathogenic to citrus and other plants.

These can be isolated from plant fluids and plant surfaces.

1.1.4.9 PHOTOSYNTHETIC BACTERIA

These are also known as phototrophic bacteria. These are distinguished from other bacterial groups by their ability to use light energy to drive the synthesis of ATP by photosynthesis. Most of the organisms included in this group are autotrophs that are capable of fixing CO_2 into organic carbon molecules. Some of the phototrophic bacteria use H_2O as an electron donor and generate O_2 in this process. These bacteria belong to the oxygenic phototrophic bacteria. They possess two photosystems (photosystem I & II) that enable them to couple the generation of NADPH with the conversion of water to oxygen and convert light energy into cellular energy in the form of ATP. Other phototrophic bacteria which do not use water as an electron donor and do not produce oxygen, such bacteria can be classified as an anoxygenic bacteria, possessing only photosystem I and use a reverse electron flow to generate NADPH.

1)Types of Photosynthetic Bacteria

a) Anoxygenic Phototrophic Bacteria

These bacteria are gram –ve photosynthetic microorganisms that use an electron donor other than water during photosynthesis and consequently do not produce oxygen. Anoxygenic phototrophic bacteria possess diverse morphological, biochemical and physiological characteristics. These forms may be motile (or) non-motile, motility is by flagellar or gliding. Anoxygenic phototrophs have only photosystem I. The photosynthetic pigments are located within cytoplasmic membrane or within the specialized structures, include various types of intra cytoplasmic membranes, which can be vesicular, tubular or lamellar stacks. These are after extensions and are continuous with the cytoplasmic membrane. Some bacteria house their light-harvesting or antenna photopigments in vesicles called chlorosomes; these are centilshaped, non unit membrane bound structures that lie adjacent to the cytoplasmic membrane.

Ex. Chloroflexus and chlorobiaceae.

The photo pigments of anoxygenic phototrophic bacteria include bacteriochelorophylls and carotenoids. Bacteriochlorophylls a-g are present and the appearance of specific bacteriochlorophyll is characteristic of some of the groups. Carotenoids are more abundant than bacterio chlorophylls and show diversity and characteristic of certain species. Carotenoids impart billiant pigmentation to the cell. These bacteria carry anaerobic photosynthesis without using

water as an electron donor, but they utilize reducing sulfur compounds like hydrogen sulfide (H_2S), thiosulfate ($S_2O_3^{2-}$), H_2, or organic acids (malate, acetate, pyruvate) as electron donors. When sulfur compound is oxidized, the cells store sulfur either inside or outside cells, leads to form sulfur globules. Many species of anoxygenic phototrophic bacteria can grow chemotropically in the dark under aerobic or microaerophilic conditions.

As a result of the diversity of photopigments, sulfur utilization and metabolic capabilities, the anoxygenic phototrophic bacteria can be divided into 7 sub groups. They are:

1.1.4.9 types of sulfur bacteia

1) **Purple sulfur bacteria with internal sulfur globules**

Cells grow by utilizing sulfide (or) sulfur as electron donor. Internal sulfur globules are present, contain bacteriochlorophyll a or b and carotenoids are spirilloxanthin, okenone and rhodopinal.

Ex. Amoebobacter, Chromatium, Thiospirillum.

2. Purple sulfur bacteria with external sulfur globules

Electron donor is sulfide (or) sulfur external sulfur globules occur, contain chlorophyll a or b and carotenoid is spirilloxanthin.

Ex. Ectothiorhodospora

3. Purple nonsulfur bacteria (Rhodospirillaceae)

Grow by photoassimilation of simple organic compounds.

Ex. Rhodobacter, Rhodospirillum, Rhodopseudomonas

4. Green sulfur bacteria (Chlorobiaceae)

Electron donor is sulfide or sulfur. External sulfur globules occur. Contain bacterial chlorophylls c, d or e. Cultures are in green or brown.

Ex. Chlorobium, Anchalochloris.

5. Bacteria with bacteriochlorophyll g

Strictly photo heterotrophic organisms. Contain chlorophylls and carotenoid is neurosporene.

Ex. Heliobacillus, Heliobacterium

6. Filamentous green bacteria (Chloroflexaceae)

Cells have flexible walls

Exhibit gliding movement

Contain various chlorophylls & carotenoids.

Ex. Chloroflexus, Chloronema, Oscillochloris.

7. Aerobic, Chemotrophic bacteria with bacteriochlorophyll a

Cells grow only in presence of molecular oxygen. Metabolism is primarily respiratory, using organic compounds. Bacteriochlorophyll a is present and carotenoids are present.

Ex. Erythrobacter.

II. Oxygenic Phototrophic Bacteria

The oxygenic phototrophic bacteria split water to form oxygen as part of their photosynthetic metabolism. They possess two photosystems (photosystem I & II).

The oxygenic phototrophic bacteria are divided into two groups.

1. Cyanobacteria
2. Prochlorophytes (prochlorales)

Both these occupy intermediary positions between the phototrophic bacteria and the eukaryotic algae, indicating a probable like to these higher photosynthetic organisms.

1. Cyanobacteria

The cyanobacteria (or) blue green algae, are the most diverse and widely distributed group of photosynthetic bacteria. Over 1000 species of cyanobacteria have been reported. Among the cyanobacteria, some genera characteristically are unicellular and others are filamentous. Cell wall structures of cyanobacteria are gram-ve type.

In cyanobacteria the major light-harvesting pigments are chlorophyll a, phycobilli proteins, phycocyamin, allophycocyanin and carotenoids. The light that is absorbed by chlorophyll a molecule is predominantly channeled to the reaction centers in photosystem I, where as light absorbed by phycobili proteins is predominantly channeled to the reaction centers in photosystem II. The combinations of all these pigments causes the cells to be variously coloured-green, blue-green, purple, red brown and black.

The cytoplasm of cyanobacteria is felled with photo synthetic membranes called Thylakoids. The thylakoids are organized into a system of flattened sacs containing primary photosynthetic pigment, chlorophyll a and the electron transport components. The outer surfaces of thylakoids have associated granules known as phycobilisomes, which are composed of the auxiliary photosynthetic pigments, the phycobiliproteins.

Basing on morphological criteria, like cell division and cell arrangement, cyanobacteria are classified into 5 subgroups.

1.Chroococceale

Unicellular rods or coeei.

Reproduce by binary fission (or) budding.

Ex. Chroococcusturgidus.

2. Pleurocapsales

Single cells enclosed in a fibrous layer.

Reproduce by multiple fission, producing baeocytes.

Ex. Pleurocapsa minor

3. Oscillatoriales

Cells form trichomes without heterocysts.

Ex. Spirulina, Oscillatoria, Lyngbya.

4. Nostocales

Cells form trichomes with vegetative cells and heterocysts. Has ability to fix atmospheric N_2.

Ex. Nosboe, anabaena

5. Stigonematales

Filamentous with branching or trichomes and heterocysts.

Ex. Fischerella, Chlorogleopsis.

2. Prochlorophytes

These are similar to the cyanobacteria except that they synthesize chlorophyll b in addition to chlorophyll a.

These bacteria also lack phycobilin pigments.

The only known genera of prochlorophytes are prochloron and prochlorothrix.

These photosynthetic bacteria occur as extra cellular symbionts of marine ascidian invertebrates (tunicates).

These bacteria appear bright green on the surfaces of animals with which they are associated.

The photosynthetic apparatus in the prochlorophytes cotain thylakoid membranes that contain all of the reaction centers, the light harvesting pigments, and the electron transport system.

Ex. Prochloron didemmi – Unicellular and spherieal cells.

Prochlorothrix hollandica – forms unbranched trichomes of various lengths.

1.1.4.10 model questions

1)Explain the generalcharacters of Rickettsias

2)Write detailed account on methanogenic bacteria

1.1.4.10 Reference books

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2. Microbiology → Prescott

L.M. Harley

J.P. Klein, D.A

Lesson 1.2.1

NUTRITIONAL REQUIREMENTS OF BACTERIA

Objective

To study the types of bacteria on their nutritional requirements, the various nutrient elements required by them & the modes of nutritional transport into their from the external environment.

1.2.1.1 Introduction

Bacterial nutrition is a process by which chemical compounds (nutrients) are absorbed from the surrounding environment by an organism and enter into cellular processes like metabolism and growth.

Bacteria, like all other living organisms, require certain nutrients for growth. Nutrients are substances used in biosynthesis, a process known as & energy (Anabolism) production and therefore are required for microbial growth. Anabolism is an energy-requiring process and the cells obtain this energy either directly from light or from the breakdown of organic compounds or inorganic compounds into simpler

substances. This process of breakdown of complex organic or inorganic substances into simple substances is known as catabolism.

The nutrients must also contain those elements that are constituents of the cellular materials & that are necessary for the activity of enzyme and transport systems.

1.2.1.2 Nutritional Elements

Basing on their importance, Nutrients are of 2 types.

1. Macronutrients
2. Micronutrients

1. Macronutrients

Analysis of microbial cell composition shows that over 95% of cell dry weight is made up of a few major elements, such as –

Carbon, Oxygen, Hydrogen, Nitrogen, Sulphur, Phosphorus, Potassium, Calcium, Magnesium and Iron.

These are called Macroelements (or) Macronutrients (or) Major elements, because they are required by microorganisms in relatively large amounts.

The first six (C, O, H, N, S, P) are components of carbohydrates, lipids, proteins & nucleic acids.

The remaining four macroelements (K, Ca, Mg & Fe) exist in the cell as cations & play a variety of roles.

2. Micronutrients

All microorganisms require several TRACE ELEMENTS (also called Microelements (or) Micronutrients) besides macroelements.

The trace elements such as Manganese, Zinc, Cobalt, Molybdenum, Nickel and Copper are needed by most cells.

Cells require such small amounts that contaminants in water, glassware and regular media components often are adequate for growth.

Therefore it is very difficult to demonstrate a trace element requirement. Trace elements are normally a part of enzymes and cofactors, and they aid in the catalysis of reactions and maintenance of protein structure.

3)Requirements for Carbon, Hydrogen and Oxygen

Carbon is required for the skeleton or backbone of all organic molecules and molecules serving as carbon sources usually also contribute both oxygen and hydrogen atoms.

One carbon – source for which this is not true is carbondioxide (CO_2) because it is oxidized and lacks hydrogen.

C, H and O can be utilized by bacteria in the form of organic and inorganic compounds.

Among the inorganic compounds used are CO_2, H_2, H_2S, NH_3, H_2O, O_2, NO_3^- & SO_4^{2-}.

The number of organic compounds utilized by microorganisms in large. Any organic compound utilized by microorganism is degradable.

Microorganisms play an important role in this degradation. Their versatility has led to the formulation of the "doctrine of microbial catabolic in fallibility", meaning that every naturally occurring carbon compound is used by some microbe.

The metabolism of Carbon, Hydrogen and Oxygen-containing compounds is important not only because these elements are the main constituents of the cell. These compounds are important substrates for the energy production in microorganisms.

4)Autotrophs

The organisms which can use CO_2 is a very energy expensive process.

Thus many microorganisms cannot use CO_2 as their sole `carbon-source' but must rely on the presence of more reduced, complex molecules for a supply of carbon.

Organisms that use reduced, preformed organic molecules as carbon sources are heterotrophs (these preformed organic molecules normally come from other organisms).

Most heterotrophs use organic nutrients as a source of both carbon and energy.

For ex, the glycolytic pathway traps energy as ATP & NADH and also produces carbon skeletons for use in biosynthesis.

5)Prototrophs

A microorganism requiring the same nutrients as most naturally occurring members of its species is a prototroph.

A prototrophic microorganism may mutate so that it cannot synthesize a molecule essential for Growth & Reproduction.

It will then require that molecule, or a compound that can be converted to it, as a nutrient.

Auxotroph

A mutated microorganism that lacks the ability to synthesize an essential nutrient & therefore must obtain it or a precursor from the surroundings is an Auxotroph.

The requirement for a specific aminoacid is a common form of auxotrophy.

Many microorganisms can synthesize all the common amino acids needed for growth.

Oceasionally a mutation will block the synthesis of an essential aminoacid and the microorganism becomes auxotrophic for it, that is, the aminoacid must be available for growth to take place.

The production of auxotrophs is useful in the study of microbial genetics.

1.2.1.3 Nutritional types of Microorganisms

All organisms also require sources of energy, hydrogen and electrons for growth to take place.

Basing on the energy sources, these are of 2 types.

1)Phototrophs

These organisms use light as their energy source i.e., light energy trapped during photosynthesis.

2)Chemotrophs

These organisms obtain energy from the oxidation of chemical compounds i.e., the energy derived from oxidizing organic or inorganic molecules.

Basing on the sources of hydrogen atoms or electrons, they are again of 2 types.

3)Lithotrophs

These organisms use reduced inorganic substances as their electron source.

4)Organotrophs

These extract electrons or hydrogen from inorganic compounds.

Despite the great metabolic diversity seen in microorganisms, most of them may be placed in one of the four

nutritional classes based on their primary sources of energy-hydrogen &/or electrons, and carbon.

These are

1. PHOTOLITHORTOPHIC AUTOTROPHS,
2. PHOTOORGANOTROPHIC HETEROTROPHS,
3. CHEMOLITHOTROPHIC AUTOTROPHS, and
4. CHEOORGANOTROPHIC HETEROTROPHS

The large majority of microorganisms thus far studied are either Photolithotrophic Autotrophs (or) Chemoorganotrophic Heterotrophs.

1)Photolithotrophic Autotrophs

- These are often called photoautotrophs
- They use light energy and CO_2 as a carbon source.
- Eukaryotic algae and blue green algae (cyanobacteria) employ water as the electron donor and release oxygen.
- Purple and green sulfur bacteria cannot oxidize water but extract electrons from inorganic donors like hydrogen, hydrogensulfide and elemental sulfur.

Ex: Purple and Green non-sulfur bacteria

2)Chemolithotrophic Autotrophs

- These contribute greatly to the chemical transformations of elements (eg., the conversion of NH_3 to NO_3^- or S to SO_4^{2-}, that continually occur in the ecosystem.
- This group of organisms, oxidizes reduced & inorganic compounds such as iron, nitrogen or sulfur molecules to derive both energy and electrons for biosynthesis.
- CO_2 is the carbon-source.

- A few chemolithotrophs can derive their carbon from organic sources and thus are heterotrophic.

- These include Sulfur-oxidising bacteria (e.g., Thiobacillus thioxydans) Hydrogen bacteria (Hydrogenomonas), Nitrifying bacteria (e.g: Nitrosomonas, Nitrobacter) and Iron bacteria (e.g., Leptothrix, Cladothrix, Ferrobacillus).

3)Chemoorganotrophic Heterotrophs

- These are often called chemoheterotrophs or even heterotrophs.

- They use organic compounds as sources of energy, hydrogen, electrons, and carbon for biosynthesis.

- Frequently, the same organic nutrient will satisfy all these requirements.

- It should be noted that essentially all pathogenic microorganisms are chemoheterotrophs.

- These may include protozoa, fungi, most non-photosynthetic (including most pathogens) bacteria.

5)Mixotrophic

Bacteria relying on inorganic energy sources and organic (or sometimes CO_2) carbon sources may be called Mixotrophic. These have combined autotrophic and heterotrophic metabolic processes.

6)Requirements for Nitrogen, Phosphorus and Sulphur

To grow, a microorganism must be able to incorporate large quantities of Nitrogen, Phosphorus and Sulphur. Although, these elements may be acquired from the same nutrients that supply carbon, microorganisms usually employ inorganic sources as well.

Nitrogen

- Nitrogen is needed for the synthesis of aminoacids, purines, pyrimidines, some carbohydrates and lipids, enzyme cofactors, and other substances.

- Many microorganisms can use the nitrogen in aminoacids, and ammonia often is directly incorporated through the action of such enzymes asglutamate dehydrogenase or glutamine synthetase and Glutamatesynthase.

- Most phototrophs and many nonphotosynthetic microorganisms reduce nitrate to ammonia and incorporate the ammonia in assimilatory nitrate reduction.

- A variety of bacteria (e.g., Many Cyanobacteria and the Symbiotic bacterium, Rhizobium) can reduce and assimilate atmospheric nitrogen using the nitrogenase system.

Phosphorus

- Phosphorus in present in Nucleic acids, Phospholipids, Nucleotides like ATP, several cofactors, some proteins and other cell components.

- Almost all microorganisms use inorganic phosphate as their phosphorus source and incorporate it directly.

- Some microorganisms (e.g., E.Coli) actively acquire phosphate from their surroundings.

- Low phosphate levels actually limit microbial growth in many acquatic environments.

Sulphur

- Sulphur is needed for the synthesis of substances like the aminoacids, Cysteine and Methionine, some Carbohydrates, Biotin & Thiamine.

- Most microorganisms use sulfate as a source of sulfur and reduce it by assimilatory sulfate reduction, a few require a reduced form of sulfur such as Cysteine.

1.2.2.4 Growth Factors

Microorganisms especially many photolithotrophic autotrophs, often grow and reproduce when minerals and sources of energy, carbon, nitrogen, phosphorus and sulfur are supplied.

Many microorganisms, on the other hand, lack one or more essential enzymes. Therefore they cannot manufacture all indispensable constituents but must obtain them or their precursors fro the environment.

Organic compounds required because they are essential cell components or precursors of such compounds and cannot be synthesized by the organism are called Growth Factors.

There are three major classes of growth factors:

1. Amino acids,
2. Purines and pyrimidines, and
3. Vitamins

➔ Although there are many amino acids, only 20 of them are involved in protein synthesis and hence these are called Magic 20.

➔ These 20 amino acids required for protein synthesis include

- ➔ Purines are double-ringed heterocyclic structures. These include Adenine (A) and Guanine (G).
- ➔ Pyrimidines are single – ringed hetero cyclic structures. These include Cytosine ©, Thymine (T) and Uracil (U).
- ➔ Both these purines and Pyrimidives are required for the synthesis of Nucleic acids (DNA & RNA).
- ➔ Vitamins are small organic molecules that usually make up all or part of enzyme and only very small amounts sustains growth.

1.2.1.5 Summary

Knowledge of the specific growth factor requirements of many microorganisms makes possible quantitative – growth – response assays for variety of substances. For ex., species from the bacterial genera Lactobacillus & Streptococcus can be used in microbiological assays of most vitamins and amino acids.

- ➔ The appropriate bacterium is grown in a series of entture vessels – each containing medium with an excess amount of all required components except the growth factor to be assayed.
- ➔ A different amount of growth factor is added to each vessel.

- ➔ The standard curve is prepared by plotting the growth factor quantity or concentration against the total extent of bacterial growth.
- ➔ Ideally the amount of growth resulting is directly proportional to the quantity of growth factor present; if the growth factor concentration doubles, the final extent of bacterial growth doubtes.
- ➔ The quantity of the growth factor in a test sample is determined by comparing the extent of growth caused by the unknown sample with that resulting from the standards.
- ➔ Microbiological assays are specific, sensitive & simple.
- ➔ They still are used in the assay of substances like vit. B_{12} and Biotin, despite advances in chemical assay techniques.

1.2.1.6 Model Questions

1)Write abot different nutritional elements of bacteria?

2)Classify the microorganisms based on their nutrition?

1.2.1.7 Reference books

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Lesson 1.2.2

GROWTH & GROWTH KINETICS OF BACTERIA

Objective

1.2.2.1 Introduction

1.2.2.2 Reproduction of bacterial cell-Binary fission

1.2.2.3 Cell elongation

1.2.2.4 Replication

1.2.2.5 Growth rate

1)Kinetics of bacterial reproduction

2)Effect of growth rate on physiological state

3)Viable nonculturable cells

4)Phases of bacterial growth

1.2.2.6 Growth of bacterial cultures

1)batch cultures

2)Continuous cultures

3)Syncrhonous cultures

1.2.2.7 Effect of nutrient concentration on bacterial growth

1)Specific strategies for coping with periods of low nutrient availability

1.2.2.8 Effect of temperature on bacterial and archael growth rate

1)Enzymatic response to temperature

The present chapter deals with the different steps involved in cellcycle, growth pattern of bacteria and kinetics of bacterial growth

1.2.2.1 Introduction

Growth may be generally defined as a steady increase in all of the chemical components of an organism and usually results in an increase in the size of a cell and frequently results in cell division (except for some filamentous microorganisms). There is an important distinction between the growth of multicellular versus unicellular organisms: growth in multicellular organisms leads to an increase in the size of the organism whereas growth

in unicellular organisms leads to an increase in the number of individuals in the population. Because cell division is usually a tightly related consequence of cell number in a population is often used to assess growth.

The life cycle of a single bacterial cell may be taken as the time of division of a mother cell into two daughter cells, and then when one of the daughter cells divides into two more daughter cells. The cell cycle in eukaryotic cells involves separate phases for cell enlargement, replication of the genome, separation of the replicated genomes by mitosis, and cell division (cytokinesis) that are separated by gaps (Table). The bacterial cell cycle is characterized by continuous macromolecular synthesis and cell elongation occurs while the genome is being replicated. The replicated bacterial chromosomes are not pulled apart by microtubules as in mitosis of eukaryotic cells but appear to be attached to the cytoplasmic membrane. In essence the cytoplasmic membrane of the bacterial cell replaces the mitotic spindle fibers of the eukaryotic cell. Thus the cell cycle in bacteria is relatively simple.

Table Cell Cycle in Eukaryotic Cells

Phase	$G_1 \rightarrow S \rightarrow G_2 \rightarrow M \rightarrow C$ Description
G_1	The primary growth phase of the cell during which cell enlargement occurs; a gap phase separating cell growth from replication of the genome
S	The phase in which replication of the genome occurs
G_2	The phase is which the cell prepares for separation of the replicated genomes; the phase includes synthesis of microtubules and condensation of the chromosomes; a gap phase separating chromosome replication from mitosis
M	The phase called mitosis during which the microtubular apparatus is assembled and

	subsequently used to pull apart the sister chromosomes
C	The phase of cytokinesis during which the cells divides to form two daughter cells

1.2.2.2 Reproduction of bacterial cells-binary fission

Most bacterial cells reproduce asexually by binary fission, a process in which a cell divides to produce two nearly equal-size progeny cells (fig). Binary fission involves three processes: increase in cell size (cell elongation), DNA replication, and cell division. Not all bacteria reproduce by binary fission. Some use other mechanisms such as yeast-like budding for reproduction. Even among the bacteria that reproduce by binary fission. Some use other mechanisms such as yeast-like budding for reproduction. Even among the bacteria that reproduce by binary fission, there is considerable variability in the overall process.

1.2.2.3 Cell elongation

Increase in cell size requires growth of the cell wall. The biosynthesis of new cell surface occurs at specific sites (Fig). Newly synthesized cell wall material in cocci is inserted at specific sites of the pre-existing cell wall. In the coccal bacterium Enterococcus, for example, cell wall synthesis begins at a band that circles the cell perpendicular to a line running from cell pole to cell pole. As additional cell wall material is added, the nascent wall is forced away from the site laterally to form an elongated cell. Incorporation of radioactive cell well precursors and autoradioraphic analyses suggest that rod-shaped bacteria also incorporate new wall at discrete sites. In Gram-negative rod-shaped bacteria, cell wall is added all around the cylindrical region, and outer membrane material is inserted at the specific adhesion sites between the cytoplasmic membrane and outer membrane.

1.2.2.4 DNA replication

DNA replication in Escherichia coli takes 40 minutes to completely copy the bacterial chromosome. However, E.Coli and other bacteria can reproduce every 20 minutes. Thus new DNA synthesis is initiated before a previously initiated round of DNA synthesis is completed. This means that rapidly growing bacteria have multiple initiation forks simultaneously on their bacterial chromosome. When a bacterial cell divided into two cells, each cell receives a complete genome and an additional portion of the genome whose synthesis was initiated part of the way through the life cycle of that cell.

A new round of replication of the bacterial chromosome is initiated every time the cell divides. Thus the initiation of DNA replication is actually coordinated with and controlled by the rate of cell division. It is not clear what regulates the initiation step, although the product of the dna A gene is required for initiation to proceed. Surprisingly, this gene product appears to be self-regulated. When Dna A protein is present in the cell in high concetration, it initiates DNA synthesis more frequently but also binds to the dna A gene to shut off its own synthesis.

Septum formation

At some time during the cell cycle, the cell must partition the DNA and cytoplasmic components by partition the DNA and cytoplasmic components by synthesizing a septum, or crosswall, consisting of cytoplasmic membrane and cell wall peptidoglycan (and outer membrane in Gram-negative bacteria) (Fig). In most bacteria the septum is initiated by invaginations of the cell envelope layers, which leads to formation of a ring-shaped constriction, generally in the center of the cell and perpendicular to the outer surface of the cell. The opening in the ring gradually becomes smaller and smaller as new cell envelope material is added until it completely walls off one compartment of the cell from the other.

In many bacteria, the septum is separated after cell division by autolysis, which leads to two independent daughter cells. In other bacteria, such as streptococci, septum separation is usually incomplete and the cells remain attached to one another to form chains (Fig). In other bacteria, such as Thiopedia, cells divide in one division plane in the first generation, and in the next generation the daughter cells synthesize a septum perpendicular to the first. This leads to the formation of sheets of cells. Yet other bacteria, such as Sarcina, divide in three-dimensional division planes and form cubical arrangements of cells called octads.

It is not known what triggers the initiation of septum formation or ties septum formation to DNA replication. At least 12 proteins in E.coli are responsible for the occurrence of septation. One of these is a cytoplasmic membrane protein, penicillin-binding protein 3 (PBP3), that is believed to be involved in the septation process. The antibiotic cephalexin preferentially binds to and inhibits PBP3, which leads to fiamentous growth of E.coli cells (long cells that lack septa).

Another unknown factor in the bacterial cell cycle is how the DNA is segregated into the two daughter cells before septation is complete. There is no mitotic apparatus (centromere, spindle fibers, or microtubule contraction) in bacterial cells to assure the separation of replicated chromosomes. One explanation may lie in the attachment of the DNA to the cytoplasmic membrane. As the membrane and cell wall grow laterally, the DNA is swept along by virtue of its attachment.

For E.coli, when growth reaches a critical cell mass, bacterial chromosome replication is initiated. Initiation of DNA replication requires a Dna A protein, phosphorylated by ATP, together with replication proteins. The duplicate bacterial chromosomes are partitioned into opposite sides of the cell, perhaps being pulled apart by the protein MukB. Cytokinesis (cell division) starts with the formation of a GTP-binding protein (FtsZ), usually in the interior of the cell. The FtsZ protein aggregates and forms a ring (Z-ring) on the inner surface of the

cytoplasmic membrane. This Z-ring contracts and with the assistance of FtsA protein leads to the inward growth of peptidoglycan and septum formation. Mutations in the ftsZ gene lead to the formation of elongated cells with multiple chromosomes. A specific enzyme (PBP3) and other proteins (FtsQ, FtsL, FtsW) are responsible for the coordinated ingrowth o the peptidoglycan cell wall and cytoplasmic membrane (septum formation) at this location. EnvA protein is required to split the resultant cross-wall to form new cells. A minimum cell length is probably required for this partitioning into two daughter cells.

1.2.2.5 Growth rate

The growth rate of a microorganism is the time that it takes for the cell to reproduce. This characteristic of the cell can be quite variable and depends on several physiological parameters. As a result of microbial growth there is an increase in the number of cells and the biomass.

1)Kinetics of bacterial reproduction

Reproduction by binary fission results in doubling of the number of viable bacterial cells. Therefore during active bacterial growth the bacterial population is continuously doubling. The time required to achieve a doubling of the population size, known as the doubling time, or generation time, is the unit of measure of the bacterial growth rate (Tab).

Table Growth Rates for some Representaive Bacteria under Optimal Conditions

Organism	Temperature (c)	Generation Time (min)
Bacillus stearothermophilus	60	11
Escherichia coli	37	20

Bacillus subtilis	37	27
Bacillus mycoides	37	28
Staphylococcus aureus	37	28
Streptococcus lactis	37	30
Pseudomonas putida	30	45
Lactobacillus acidophilus	37	75
Vibrio marinus	15	80
Mycobacterium tuberculosis	37	360
Bradyrchizobium japonicum	25	400
Nostoc japonicum	25	570
Anabaena cylindrical	25	840
Treponema pollidum	37	1980

Because the bacterial population doubles every generation, if the initial population size is No, after one generation of growth.

$$N1 = 2 \times No$$

After two generations of growth:

$$N1 = 2 \times 2No = 22\ No$$

After three generations of growth:

$$N1 = 2 \times 2\ 2\ No = 23\ No$$

And after n generations of growth:

$$Nn = 2nNo$$

This relationship can be expressed in terms of the generation time. If No is the initial population number, No the population at time t; and n, the number of generations in time, then:

$$N1 = No \times 2n$$

Solving for n (the number of generations):

$$\text{Long } N1 = \log No + n \times \log 2$$

$$N = \frac{\text{Log } N1 - \log No}{\text{Log} 2} \qquad \frac{\log N1 - \log No}{0.301}$$

The growth rate of a bacterial culture can also be expressed as a function of the reciprocal of the doubling time, K:

The growth rate constant represents the number of generations per unit of time and is usually described as generations per hour. A useful calculation, the mean generation time (g), or doubling time, for a population that is actively reproducing, is:

This mathematical formula for the bacterial growth rate is based on the premise that the rate of increase is proportional to the number, or mass, of cells present at any given tie and that the doubling time is constant during a period of growth.

By determining cell numbers during the period of active cell division, the generation time can be estimated. In comparing generation times, one finds that bacteria reproduce more rapidly than higher organisms. A bacterium such as E.coli can have a generation time as short as 20 minutes under optimal conditions, although in nature many bacteria have generation times of several hours. One cell of a bacterium with a 20-minute generation time could multiply to 1,000 cells in 3.3 hours and to 1,000,000 cells in 6.6 hours. The generation times for many archaea is 20 to 30 minutes but some reproduce more slowly and have doubling times of 45-90 minutes. Methanococcus jannashii, for example, has a doubling time of 20 minutes at 85oC.

2)Effect of growth rate on the physiological state

The rate of growth of a cell has various consequences on its physiological state. As the growth rate increases, the mass of the cell also increases. This means that at faster growth rates they become larger. They also contain more cell components – DNA, RNA, and protein. The relative concentration of macromolecules increases as an exponential function (fig).

A significant observation is the relative enrichment of RNA in comparison to other cellular macromolecules in cells with a faster growth rate. This is due to the increase in the number of ribosomes in these cells. Ribosomes are responsible for the biosynthesis of proteins but they polymerize amino acids at a fairly constant rate. When cells need more protein, that is, at faster growth rates, they do not increase the speed of amino acid polymerization but rather increase the number of ribosomes.

Faster growing cells also have increased amounts of DNA. In E.coli it takes 40 minutes for the cell to completely replicate its genome and about 20 minutes thereafter before the cell divides. In cells that divide every 60 minutes, this time frame is adequate to accomplish DNA replication and the onset of cell division. However, in cells that are growing at faster doubling times (between 20 and 60 minutes), they must initiate DNA replication more frequently to have multiple copies of the genome

that will be segregated into daughter cells. As a consequence of more frequent initiations of replication, faster growing cells have more DNA (fig). The initiation of DNA replication is triggered by the size or mass of the cell, called the critical cell mass. This implies that for faster growing cells to have more frequent initiations of DNA replication, they must reach this critical cell mass or size sooner and therefore must be larger. It is not clear what mechanism is involved in triggering the initiation of DNA replication when the cell reaches its critical cell mass.

Bacterial cells have evolved adaptations that permit greater expression of genes whose gene products are required in greater quantities. This involves locating the genes where greater transcription can occur and having longer lived mRNAs so that greater translation can occur. This permits higher rates of gene expression. In E.coli the mRNAs that code for essential structures, such as the outer membrane proteins, have longer half lives than those involved in catabolism, for example, those involved in the metabolism of lactose. Also the genes for outer membrane proteins, which are required in high amounts, are located near the origin of replication. Since rapidly growing bacterial cells carry extra partially completed genomes, there will be multiple copies of genes located near the origin and only single copies of those located near the point of replication termination (fig). Multible copies of genes permit greater transcription and higher rates of gene expression.

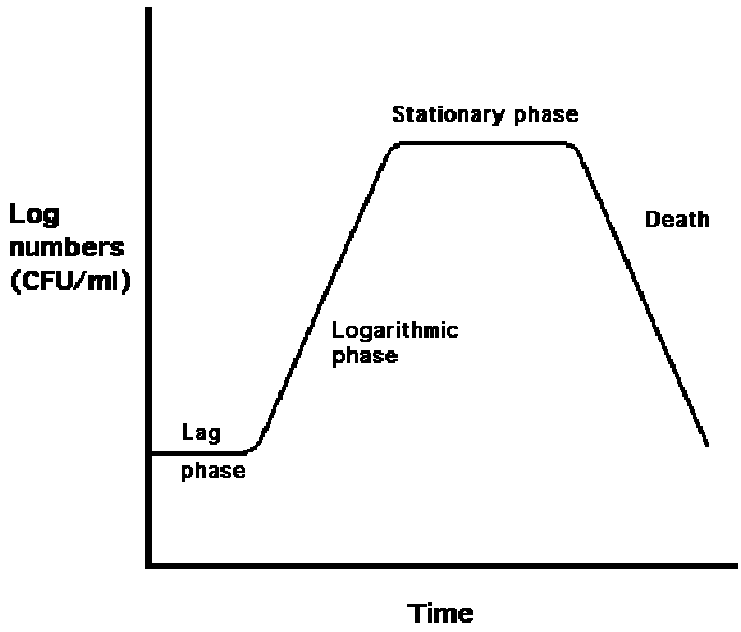
Viable nonculturable cells

Often, cells in the environment are viable but nonculturable. These cells exhibit active metabolism in the form of respiration or fermentation, incorporate radioactive substrates, and have active protein synthesis but cannot be cultured or grown on conventional laboraroty media. They have been detected by observing discrepancies between plate count enumeration of bacterial populations and direct staining and microscopic counts. These cells may be particular problems in the environment if they are pathogens, for example, viable

nonculturable cells of Vibrio cholerae, enteropathogenic E.coli, Legionella pneumophila, and various other bacteria have been shown to regain culturability after they have entered the intestinal tracts of animals.

4)Phases of bacterial growth

If an old culture of bacteria is inoculated or added to a fresh medium and the cell concentration is periodically measured, a curve describing the change in cell number against time can be drawn. This curve, called the growth curve, will be hyperbolic due to the exponential nature of bacterial growth (Fig).



Hypothetical bacterial growth curve.

a)Lag phase

The typical growth curve of a bacterial culture begins with the lag phase. During the lag phase there is little increase in cell

numbers. Rather, during this phase the bacteria are transporting nutrients inside the cell from the new medium, preparing for reproduction, and synthesizing DNA and various inducible enzymes needed for cell division. They increase in size during this process but the number of cells does not increase.

b)Exponential phase

In the log growth phase, also called the exponential growth phase, bacterial cell division begins and proceeds as a geometric progression. One cell divides to form two, each of these cells divides to form four, and so forth in a geometric progression (Fig).

During the log phase of growth, so-named because the logarithm of the bacterial biomass increases linearly with time, bacterial reproduction occurs at a maximal rate for the specific set of growth conditions. This growth phase is better called the exponential growth phase because the number of cells is increasing as an exponential function of time. Growth during much of the exponential growth phase is said to be balanced, that is, the concentrations of all macromolecules of the cell are increasing at the same rate. The average composition of the cells therefore remains constant. During the log phase of the growth curve, the growth rate of a bacterium is proportional to the biomass of bacteria that is present.

The growth rate during the log phase is described by the equation:

$$\frac{\overline{DB}}{Dt} = \alpha B$$

Where B is the bacterial biomass, t is time, and α is the instantaneous growth rate constant. During this period the generation time of the bacterium is determined. If a bacterial culture in the exponential growth phase is inoculated into an identical fresh medium, the lag phase is usually by passed and exponential growth continues. This occurs because bacteria are already actively carrying out the metabolism necessary for continued growth. If, however, the chemical composition of the new medium differs significantly from that of the original growth medium, the bacteria go through a lag phase wherein they synthesize the enzymes needed for growth in the new medium before entering the log-arithmic growth phase.

c)Stationary phase

A growing bacterial culture eventually reaches a phase during which there is no further net increase in bacterial cell numbers. This is called the stationary growth phase. The transition between the exponential and stationary phases involves a period of unbalanced growth during which the various cellular components are synthesized at unequal rates. Consequently, cells in the stationary phase have a different chemical composition from cells in the exponential phase. During the stationary phase, the growth rate is exactly equal to the death rate. A bacterial population may reach stationary growth when a required nutrient is exhausted, when inhibitory end products accumulate, or when physical conditions change. In all cases, there are feedback mechanisms that regulate the bacterial enzymes involved in key metabolic steps. The duration of the stationary phase varies; some bacteria exhibit a very long stationary phase.

When ells enter the stationary phase their overall metabolic rate decreases. They become more resistant to environmental stresses, such as elevated temperatures, osmotic pressure, and hydrogen peroxide. This physiological adaptation is due in part to the synthesis of protein KatF, which plays a regulatory role in trascription. The katF gene is required for induction by carbon starvation. KatF may be a sigma factor that regulates transcription of several genes, including a catalse, exonuclease,

and acid phosphatase, that are synthesized at the beginning of the stationary phase.

The physiological changes between the exponential and stationary phases often are very significant so that cells in stationary phase have distinct characteristics. When Arthrobacter, for example, reaches stationary phase there is a change from rod-shaped cells to coccoid cells. These coccoid cells are called arthrospores or cystitis. The formation of arthrospores represents the beginning of a regular life cycle that is characteristic of eukaryotic microorganisms but is rare among prokaryotes. The sequence of morphological changes in the growth cycle distinguishes Arthrobacter from other genera.

d)Death phase

Eventually the number of viable bacterial cells begins to decline, signaling the onset of the death phase. The kinetics of bacterial death, like those of growth, are exponential because the death phase really represents the result of the inability of the bacteria to carry out further reproduction. The rate of the death phase need not be equal to the rate of growth during the exponential phase, however. The rate of death phase need not be equal to the rate of growth during the exponential phase, however. The rate of death is proportional to the number of survivors. Modifying environmental conditions can alter the death rate of a bacterium.

1.2.2,6 Growth of bacterial cultures

In laboratory and natural situations, some environmental parameter or interaction of environmental parameters controls a given bacterial species' rate of growth. In nature, where conditions cannot be controlled and many species co-exist, fluctuating environmental conditions favor population shifts because of the varying growth rates of individual bacterial populations within the community at a given location. In the laboratory, it is possible to adjust conditions to achieve optimal growth rates for a given bacterial species. Similarly, inindustrial fermentors, conditions can be adjusted to optimize bacterial growth rates, thereby maximizing the accumulation of desired

metabolic products. Many laboratory and industrial applications use pure cultures of bacteria, facilitating the adjustment of the growth conditions so that they favor optimal growth of the particular bacterial species.

1)Batch culture

The normal bacterial growth curve is characteristic of bacteria in batch culture, that is, under conditions in which a fresh medium is simply inoculated with a bacterium. A flask containing a liquid nutrient medium or broth inoculated with a bacterium such as E.coli is an example of a batch culture. In batch culture growth, nutrients are expended, and metabolic products accumulate in the closed environment. The batch culture models situations such as occur when a canned food product is contaminated with a bacterium.

During exponential growth in a batch culture the instantaneous growth rate constant (μ) is related to the generation time (g) by the equation:

$$\mu = \frac{0.693}{g}$$

This equation is derived from the fact that during exponential growth the rate of change of a population of cells from a given cell number (N) is described by the equation:

$$\frac{DN}{Dt} = \mu N$$

The generation time represents the average time that it takes a population to double in size, whereas the instantaneous growth rate constant more closely resembles the growth (reproduction) of individual cells.

As a batch culture approaches stationary phase it is necessary to modify the equation describing growth because there is a maximal obtainable population:

$$\frac{DN}{Dt} = \mu N - \frac{\mu}{Nmax} N2$$

According to this logistics equation, as the population size (N) approaches its maximal obtainable limit (Nmax) the change in population size (dN/dt) approaches zero, which is what occurs during the stationary growth phase.

2)Continuous culture

In continuous culture systems, fresh medium replaces some of the spent medium, thus permitting continuous growth of a culture. In a turbidostat the system includes an optical sensing device that measures the turbidity of the culture in the growth vessel and generates an electrical signal that is used to regulate the flow of fresh medium into the vessel and the flow of spent medium and cells out of it. Thus, in a turbidostat, the number of cells in the culture controls the flow rate, and the rate of growth of the culture adjusts to this flow rate.

In a chemostat the flow rate from a reservoir of a growth medium is set at a particular value and the rate of growth of the culture adjusts to this flow rate (fig). Because end products do not accumulate and nutrients are not completely expended, the bacteria never reach stationary phase in a chemostat. Bacteria grown in a chemostat, in which nutrients are supplied and end products continuously removed, are maintained in the exponential growth phase. Continuous growth of bacteria is accomplished in this device by continuously feeding a liquid medium into the bacterial culture. The liquid medium contains some nutrient in growth-limiting concentration, and the concentration of the limiting nutrient in the growth medium determines the rate of bacterial growth. During steady-state operation of a continuous culture device, the concentration of

the limiting nutrient remains constant because the rate of addition of the nutrient equals the rate at which it is used by the culture, plus that lost through overflow.

Even though bacteria are continuously reproducing, a number of bacterial cells are continuously being washed out and removed from the culture vessel. Thus a constant number of bacterial cells are maintained in the chemostat culture vessel.

The instantaneous growth rate of the bacterial population in the chemostat is:

$$\frac{DN}{Dt} = \mu N$$

The rate at which cells are lost as a result of being washed out of the chemostat is:

$$\frac{DN}{Dt} = \mu N - DN = (\mu - D)N$$

Where N is the size of the steady state population and D is the rate of dilution.

Because the rate of cell washout is equal to the growth rate, the dilution rate is equal to the growth rate of a bacterium growing in a cheostat.

The relationship between the culture generation time and the concentration of the limiting substrate is:

$$\mu = \mu max \frac{S}{(ks + s)}$$

where μ is the culture generation time, μmax is the maximal growth reate at saturating concentrations of substrate, s is the

substrate concentration, and ks is the saturation constant defined as the substrate concentration at ½ μmax. Cell numbers and the concentration of the limiting nutrient change little at low dilution rates. As the dilution rate approaches ks, the cell concentration drops rapidly to zero, and the concentration of the limiting nutrient approaches its concentration in the reservoir. A chemostat is a good model for bacterial growth in open systems such as rivers and oceans, and by using chemostats and the papropriate mathematical calculations, the growth rates of bacteria in nature can be estimated.

3)Synchronous culture

Synchronous growth of bacteria occurs when all cells divide at the same time. Adjusting environmental conditions, for example, by repeatedly changing the temperature or by adding fresh nutrients to cultures as soon as they enter the stationary phase, can induce synchronous growth. A synchrophase, can induce synchronous growth. A synchronous population of bacterial cells can be obtained also by physical separation procedures. For example, an unsynchronized culture of bacteria can be filtered through a membrane filter. The loosely associated bacteria are washed from the filter, leaving some cells tightly adsorbed to it. The filter is inverted and fresh medium allowed to flow through it. New bacterial cells that also through cell division are not tightly bound to the membrane and are washed into the effluent. All of the cells in the effluent are newly formed and are therefore at the same stage of the cell cycle. Such synchronous growth, however, can be maintained for only a few generations.

1.2.2.7 Effects of nutrient concentrations on bacterial growth

To grow, bacteria must utilize various substances called nutrients, which they obtain from their environment and use for the production of energy and for the biosynthesis of cellular macromolecules. Water comprises a large part of the cell by weight, about 80% to 90%, and is therefore an essential nutrient. The remaining solids of the cell are largely composed

of hydrogen, oxygen, carbon, nitrogen, phosphorus, and sulfur. Also vital for proper cell functioning, although in substantially smaller amounts, are metal cations of potassium, magnesium, calcium, iron, manganese, cobalt, copper, molybdenum, and zinc; as well as anions such as chloride; and, for some microorganisms, growth factors such as vitamins. Each nutrient plays an important role in the overall growth of the cell (Table).

Table Principal Elements of the Cell and their Physiological Functions

Element	Percentage of Cell Dry Weight	Physiological Functions
Carbon	50	Constituent of all organic cell components
Oxygen	20	Constituent of cellular water and most organic cell components; molecular oxygen serves as an electron acceptor in aerobic respiration
Nitrogen	14	Constituent of proteins, nucleic acids, coenzymes
Hydrogen	8	Constituent of cellular water and organic cell components
Phosphorus	3	Constituent of nucleic acid, phospholipids, coenzymes
Sulfur	1	Constituent of some amino acids in proteins and some coenzymes
Potassium	1	Important inorganic cation and cofactor for some enzymatic reactions
Sodium	1	One of the principal inorganic cations in eukaryotic cells and important in membrane transport

Calcium	0.5	Important inorganic cation and cofactor for some enzymatic reactions
Magnesium	0.5	Important inorganic cation and cofactor for many enzymatic reactions
Chlorine	0.5	Important inorganic anion
Iron	0.2	Constituent of cytochromes and some proteins
All others	0.3	Various functions

Table Regulatory Systems that Respond to Starvation (Nutrient Depletion) in Bacteria

Starvation Factor	System	Microorganism	Genetic Control	Description
Low concen-trations of amino acids	Stringent response	Enterbacte-rianceae and other bacteria	relA (Stringent factor) and spot (ppGpp degradation)	General response to poor growth conditions triggered by amino acid depletion in which cells decrease rates of ribosomal RNA and transfer RNA synthesis
Low concen-trations of ammonia	Ntr system	Enterobacte-riaceae	glnB, glnD, glnG and glnL (gluta-mine)	General response to nitrogen stavation triggered by growth limiting concentrations of ammonia in which cells synthesize proteins aimed at scavenging very low concentrations of ammonia and

				obtaining nitrogen from alternate source
Low concen-trations of ammonia	Nif system	Klebsiella aerogenes and other bacteria	More than 12 nitro-genase regulator y genes	Response of some bacteria to limiting concentrations of ammonia that results in activation of nitrogen fixation enzyme system
Low concen-trations of glucose	Catabo-lite repre-ssion	Enterobacte-riaceae	cya (aden-ylate cyclase) and crp (catabolit e repressor protein)	General response to limiting concentrations of readily utilizable organic matter triggered by low concentrations of glucose by which the cell is able to synthesize enzymes for the utilization of various other organic carbon sources
Low concen-trations of molecula r oxygen	Arc System	Escherichia coli	arcA, arcB (aerobic respira-tion regulator y genes)	General system of facultative anaerobes that responds to conditions of anoxia (lack of molecular oxygen) and activates metabolic pathways that permit the utilization of alternate terminal electron acceptors for respiratory metabolism so that a shift can occur from aerobic to anaerobic metabolism
Low concen-	Pho system	Enterobacte-riaceae	PhoB, phoR,	General response system to phosphate

trations of Phos-phate			phoU, and phoA (phos-phate utilizatio n genes)	starvation triggered by low concentrations of inorganic phosphate in which cells turn on genes for utilization of organic phosphate compounds and for the scavenging of trace amounts of inorganic phosphate

1)Specific strategies for coping with periods of low nutrient availability

Bacteria preferentially growing at low nutrient concentrations are called oligotrophs, or low nutrient bacteria. Most oligotrophs have slow growth rates. In contrast to oligotrophs, bacteria that grow at high nutrient concentrations – called copiotrophs, such as those found in most culture media, exhibit high rates of reproduction.

1.2.2.8 Effects of temperature on bacterial and archaeal growth rates

Temperature is one of the most important factors that influence growth of cells. Cells grow within a well defined temperature growth range (fig). This growth range is defined by a minimum temperature below which cells are metabolically inactive and a maximum temperature above which cells do not grow. Within this range of extremes is an optimal growth temperature at which cells exhibit their highest rates of growth and reproduction.

1)Enzymatic response to temperature

Temperature influences the rate of chemical reactions and the three-dimensional configuration of proteins, thereby affecting

the rates of enzymatic activities. As long as the enzyme is not denatured, that is, as long as its three-dimensional structure is not disrupted, a rise of 10oC generally results in the approximate doubling of the rate of its reaction. The Q10 of a reaction describes the change in reaction rate that occurs when the temperature is increased by 10oC. Enzymatic reactions typically exhibit Q10 values of 2 to 3.

2)Heat shock response

The heat shock response is a rapid change in gene expression that occurs when there is a temperature shit to an elevated temperature. This is an evolutionarily conserved response and it occurs in bacterial, archaeal, and eukaryotic cells. The heat shock response results in the production of a set of heat shock proteins (Hsps). One family of heat shock proteins. Hsp70, exhibits 50% homology between the bacterium E. coli and the yeast Saccharomyces cerevisiae.

3)Growth range and optimal growth temperatures

Bacteria and archaea grow over a wide range of temperatures. Within the growth range for a particular microorganism there is an optimal growth temperature at which the highest rate of reproduction occurs. The optimal growth temperature is defined by the maximal growth rate, not the maximal cell yield. Sometimes greater cell or product yields are achieved at lower or higher temperatures. Because the generation time is the reciprocal of the instantaneous growth rate, the shortest generation time occurs at the optimal temperature.

Microorganisms that grows best at low temperatures (<20oC) are called psychrophiles; those that reproduce fastest at moderate temperatures (20o to 40oC) are called mesophiles; those with fastest growth rates at high temperatures (>40oC) are called thermophiles; and those that grow best at very high temperatures (>80oC) are called extreme thermophiles (also called hyperthermophiles).

The classification of an organism as a psychrophile, mesophile, or thermophile refers to the organism's optimal growth temperature and the temperature range at which it can grow. Many Bacillus and Clostridium species, for example, are mesophiles, not thermophiles, even though their ability to produce endospores permits them to survive in a dormant state at very high temperature.

Some bacteria, known as stenothermal bacteria, grow only at temperatures near their optimal growth temperature, whereas eurythermal bacterial grow over a wider range of temperatures. Laboratory incubators, which are simply controlled – temperature chambers, are normally used to establish conditions that permit the growth of a bacterial culture at temperatures favoring optimal grown rates.

1.2.2.9 Effects of oxygen concentration – Reduction potential

The presence or absence of oxygen in the environment is important in the growth of microorganisms. Oxygen has limited solubility in water; in nonturbulent aqueous environments, availability of oxygen may be a limiting factor in the growth of microorganisms. Many cells may utilize O2 for their metabolism, and since O2 cannot easily diffuse back into the solution, the environment becomes oxygen-depleted.

1)Oxygen relationships of microorganisms

Microorganisms can be grouped into categories based on their requirement or intolerance of O2. Aerobes grow in the presence of air that contains molecular oxygen. Obligate aerobes require O2 for growth and carry out aerobic respiration.

Other microorganisms, called microaerophiles, grow only at reduced concentrations of molecular oxygen. Such organisms require O2 for growth and carry out aerobic respiration.

Other microorganisms, called microaerophiles, grow only at reduced concentrations of molecular oxygen. Such organisms require O2 for growth but only at concentrations (-5%) reduced

from that of atmospheric levels (20%). Generally, microaerophilic organisms will not grow in air. However, some microaerophiles grow at elevated CO2 concentrations (5% to 10%) and are called capnophiles.

Facultative anaerobes can grow in the presence or absence of air. Many facultative anaerobes such as E.coli switch between aerobic respiration and fermentation, depending on the availability of molecular oxygen. They usually carry out fermentative metabolism in the absence of O2 and aerobic respiration in the presence of O2. This group of facultative anaerobes also includes strictly fermentative bacteria, such as streptococci, that are insensitive to oxygen (oxyguric) and hence can grow in the presence of O2.

Other bacteria are anaerobes and grow only in the absence of air. Obligate anaerobes carry out fermentative metabolism. Various bacteria (for example, the sulfate-reducer Desulfovibrio), archaea (for example, methanogenic archaea), and protozoa are obligate anaerobes. Strict anaerobes are sensitive to oxygen and even a brief exposure to O2 will kill such organisms. Clostridium species can be classified as obligate, strict anaerobes.

1.2.2.10 Effects of water activity

All bacteria require water for growth and reproduction. Water is an essential solvent and is needed for all biochemical reactions in living systems. The availability of water has a marked influence on bacterial growth rates. Pure distilled water has a water activity (Aw) of 1.0. Water activity is an index of the amount of water that is free to react . It is equivalent to the atmospheric measure of water availability known as relative humidity. Adsorption and solution factors, however, can reduce that availability of water and thus lower the water activity. Water, for example, may be bound by a solute and hence unavailable to bacteria. A saturated solution of NaCl has an Aw

of 0.8. Seawater, however, which has a salt concentration of only about 3%, has an Aw of 0.98.

In the atmosphere, the availability of water is expressed as relative humidity (RH). The determination of the relative humidity can be found using the equation.

RH = 100 Aw

Thus a relative humidity of 90% corresponds to an Aw of 0.90. The relatively low availability of water in the atmosphere accounts for the inability of bacteria to grow in the air. Bacteria, likewise, are unable to grow on dry surfaces except when the relative humidity is high. Bacterial growth on surfaces is a problem in tropical zones, where the available water in the atmosphere can support bacteria growth, permitting bacteria to grow on clothing, tents, and numerous other surfaces there this normally does not occur in temperate regions.

Water activity can be used as an index of the water that is actually available for utilization by bacteria. Most bacteria require an Aw above 0.9 for active metabolism (Table). Some bacteria, known as xerotolerant organisms, can grow at much lower water activities. Some yeasts grow on concentrated sugar solutions with an Aw of 0.60. As a rule fungi can grow at lower water activities than bacteria. Fungi, therefore, grow on many surfaces where the available water will not support bacterial growth.

Table Approximate Limiting Water Activities for Microbial Growth

Water Activity (A_w)	Bacteria/Archaea	Fungi	Algae
1.00	Caulobacter Spirillum		
0.90	Lactobacillus Bacillus	Fusarium Mucor	
0.85	Staphylococcs	Debaromyces	
0.80		Penicillium	

0.75	Halobacterium Halococcus	Aspergillus Chryosporum	Denaliella
0.60		Saccharamyces rouxii Xeromyces Bisporus	

1.2.2.11 Effects of pressure

The growth of all cells is affected by the external and internal pressures. These forces include osmotic pressure and hydrostatic pressure. Hydrostatic pressure results from the weight o a column of water on cells such as those found in the deepest parts of the ocean. Osmotic pressure results from water diffusing across the cell membrane in response to solute concentrations. Solute concentration affects the availability of water and also the osmotic pressure. This is associated often with the salt concentration (salinity) surrounding the cell.

1)Osmotic pressure and salinity

The cell wall structures of bacteria make them relatively reistant to changes in osmotic pressure. Extreme osmotic pressures can result in the death of bacteria. In hypertonic solutions, bacteria may shrink and become desiccated. In hypotonic solutions the cell may burst. Organisms that can grow in solutions with high solute concentrations are called osmotolerant. These organisms can withstand high osmotic pressures and also grow if low water activities. Some microorganisms are osmophiles, requiring a high solute concentration for growth. For example, the fungus Xeromyces is an osmophile, with an optimum Aw of approximately 0.9. Additionally, solutions with high sugar concentrations are used in laboratory procedures to product protoplasts (cells with their cell walls completely removed) and spheroplasts (cells with their cell walls partially removed) against rupture due to osmotic pressure.

Fig.

2)Hydrostatic pressure

Hydrostatic pressure, the pressure exerted by a water column as a result of the weight of the column, can influence bacterial growth rates. Each 10 m of water depth is equivalent to approximately 1 atm. Most bacteria are relatively tolerant of the hydrostatic pressures in most natural systems but cannot withstand extremely high hydrostatic pressures. Hydrostatic pressures of more than 200 atm generally inactivate enzymes and disrupt membrane transport processes. However, some bacteria – referred to as barotolerant – can grow at high hydrostatic pressures. There even appear to be some bacteria-referred to as barophiles – that grow best at such pressures.

1.2.2.12 Effects of acidity and pH

The pH of a solution describes the hydrogen ion concentration (H+) (Fig). The pH is equal to –log H+ or 1/log H+. A neutral solution has a pH of 7.0 acidic solutions have pH values below 7, and alkaline or basic solutions have pH values greater than 7. Bacterial growth rates are greatly influenced by pH values and are based largely on the nature of proteins.

In culture media and industrial fermentors, pH values are controlled to achieve optimal growth rates. This is normally accomplished by buffering the solution. Buffers are salts of weak acids or bases that keep the hydrogen ion concentration constant by maintaining an equilibrium with the hydrogen ions of the solution. Buffers are used to maintain the pH value within a certain range, thus dampening changes in pH and permitting continued bacterial growth. At neutral pH values, a

phosphate buffer may be used. At alkaline pH values, borate buffers are often employed. Citrate buffers often are used for maintaining acidic conditions.

Bacteria vary in their pH tolerance ranges (Table) and most grow well within a range of 6 to 9 pH. Most bacteria can therefore be considered neutralophiles because they tend to thrive under neutral, conditions. Although most bacteria are unable to grow at low pH values, there are exceptions, and certain bacteria tolerate pH values as low as 0.8. Fungi generally exhibit a wider pH range, growing within a range of 5 to 9 pH. Some eukaryotic microorganisms, including protozoa and algae, can grow at low pH values, with the lower limit for growth of some protozoa at approximately 2, and, for some algae, at approximately 1. Some fungi can grow well at lower pH values – as low as 0.

Fig.

1)Acidophiles

Some bacteria, called acidophiles, are restricted to growth at low pH values. The cytoplasmic membrane of an acidophilic bacterium breaks down and cannot function at neutral pH values. Thiobacillus species, for example, are acidophilic and grow only at pH values near 2. Sulfolobus and Thiobacillus species possess physiological adaptations that permit enzymatic and membrane transport activities.

An important factor that regulates bacterial growth under any condition is the protonmotive force. The essential energy source that is derived from the separation of protons (H+) across the cytoplasmic membrane. The protonmotive force is actually composed of two interchangeable components: a ΔpH due to the H+ ion gradient and a $\Delta\Psi$ (membrane potential) due to the charge separation across the membrane. Bacteria can use either of these components to drive protons back across the membrane

and perform work (ATP synthesis, flagellar rotation, and active transport).

Due to low pH environment, acidophiles have a very large ΔpH across the membrane. The outside of the membrane is negatively charged while the inside is positively charged. These two features in acidophiles (a large naturally occurring ΔpH and reversed ΔΨ) produce a protonmotive force that supports cell function.

2)Alkaliphiles

A small group of bacteria prefer growth under very alkaline conditions. Environments with high sodium concentrations such as some salt lakes or soils high in sodium carbonate can have pH values in the range of 9 to 11. Bacteria that live at these pH extremes, alkaliphiles, have developed mechanisms for keeping sodium ions outside the cell.

The internal pH is about 7 to 9 and the external pH is between 9 to 11. To accommodate this feature, alkaliphiles use a Na+/H+ antiporter K+/H+ antiporter to maintain a sufficiently high ΔΨ to drive the protonmotive force. The Na+/H+ antiporter in alkalophilic bacteria is largely responsible for returning protons to the cytoplasm during growth at alkaline pH.

1.2.2.13 Effects of light

Photosynthetic bacteriautilize specific light wavelengths for generation of ATP Certain photosynthetic bacteria move through their environment in response to light, called phototaxis. Some of these phototactic bacteria have mechanisms that regulate flagellar rotation in response to changing intensities of light. Bacteria may also respond to specific wavelenthts of light.

Visible light, ultraviolet light, cause structural damage to proteins and DNA. Many bacterial cells that are exposed to bright light in their environment protect themselves from harmful radiation damage by synthesizing carotenoids and other pigments. These pigments absorb light of certain wavelengths before the light can cause damage and kill the cell (Fig).

Many phototatic bacteria that live in aquatic environments can adjust their vertical position in the water column by synthesizing gas vesicles (Fig). Gas vesicles are intracytoplasmic vacuoles that contain gas. The amount of gas in the vesicles determines the buoyancy of the cell. When light intensities are low, the bacteria form gas vesicles so they can float closer to the surface of the lake or pond, and hence brighter light. When light intensities are high, the bacteria collapse their gas vesicles and sink to a lower level.

1.2.2.14 Model questions

1)Explain different stages involved in growth curve of bacteria.

2)Explain in detail about the kinetics of bacterial growth.

3)Explain the factors that effect growth kinetics of bacteria

1.2.2.15 Reference books

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Lesson 1.2.3

METHODS OF STERILIZATION

Objective

Objective

The present lesson deals with the definition, different procees of sterilizations,different physical and chemical agents used as antimicrobial agents, their properties and mechanismof action

1.2.3.1 Introduction

Sterilization is the process of removing or killing all micro-organisms and viruses on or in a material. In contrast, disinfection only reduces the number of infections agents to a point where they no longer present a hazard. Time, temperature, growth stage of the organism, nature of the suspending medium, and the numbers of organisms present must all be considered when determining which sterilization or disinfection technique

heat, filtration, chemicals, or radiation. Preservation techniques slow or halt the growth of organisms to delay spoilage.

1.2.3.2 Definitions

1)Sterilization

The process of removing or killing all microorganisms and viruses on or in a product. A sterile object is free of living microorganisms. The terms sterile, sterilize, and sterilization therefore refer to the complete absence or destruction of all microorganisms and should not be used in a relative sense. An object or sustance is sterile or nonsterile; it can never be semisterile or almost sterile.

2)Disinfection

The process that reduces the number of potential disease-causing bacteria and viruses on a material until they no longer represent a hazard. It does not kills the resistant spore forms of disease-producing microorganisms. The term is commonly applied to substances used on inanimate objects. Disinfection is the process of destroying infections agents.

A disinfectant is a chemical used to disinfect inanimate objects.

3)Antiseptic

It is a disinfectant that is non toxic enough to be used on human tissues. Antiseptic is a substance that opposes sepsis, ie., prevents the growth or action of microorganisms either by destroying microorganisms or by inhibiting their growth and metabolism. Usually associated with substances applied to the body.

4)Sanitizer

An agent that reduces the microbial population to safe levels as judged by public health requirements. Usually it is a chemical agent that kills 99.9 percent of the growing bacteria. Sanitizers are commonly applied to inanimate objects and are

generally employed in the daily care of equipment and ntensils in diaries and food plants and for glasses, dishes, and ntensile in restawants. The process of disinfection would produce sanitization; however, in the strict sense, sanitization implies a sanitary condition which disinfection does not necessarily imply.

The term decontamination is often used interchangeably with disinfection, but it implies a broader role, including inactivation or removal of both microbial toxins and the living microbial pathogens themselves.

5)Germicide

(-cide cones from – cida meaning "to kill") → An agent that kills the growing forms (microorganisms and viruses) but not necessarily the resistant spore forms of germs. It is a disinfectant. But germicides are commonly used for all kinds of germs (microbes) for any application.

6)Bactericide → An agent that kills bacteria

7)Fungicide → An agent that kills fungi.

8)Virucide → An agent that kills viruses

9)Sporicide → An agent that kills spores

1.2.3.3 Pasteurization & Preservation

1)Pasteurization

Involves a brief heat treatment that is used to reduce the number of spoilage organisms in products such as wine or to kill disease-causing organisms in milk and other foods.

2)Preservation

Preservation is the process that delays spoilage of food or other perishable products by inhibiting the growth of microorganisms common methods of preservation include storage at low temperature, lowering the pH, adding chemicals that inhibit microbial growth, and reducing the available water in the product being preserved.

3)Bacteriostasis

(Static comes from a Greek word Statikas meaning "causing to stand" – to cease activity)→ A condition in which the growth of bacteria is prevented. The word bacteriostatic indicates that an antimierobial agent is primarily inhibitory in its action, preventing growth without substantial killing.

4)Fungistatic

An agent that stops the growth of fungi.

5)Microbistatic

Agents that have in common the ability to inhibit growth of microorganisms.

6)Antimicrobial agent

One that interferes with the growth and metabolism of microbes. This term denotes inhibition of growth, and with reference to specific groups of organisms, such terms as antibacterial, or antifungal are frequently employed. Some antimicrobial agents are used to treat infections, and they are called chenotherapeutic agents.

1.2.3.4 Principles of Sterilization and Disinfection

Agents called disinfectants are typically applied to inanimate objects, and agents called antiseptics are applied to living tissue. A few agents are suitable as both disinfectants and antiseptics, although most disinfectants are too harsh for use on delicate skin tissue. Antibiotics are also applied to skin.

Sterilization is the killing or removal of all microorganisms in a material or on an object. There are no degrees of sterility – sterility means that there are no living organisms in or on a material. When properly carried out, sterilization procedures ensure that even highly resistant bacterial endospores and fungal spores are killed. Much of the controversy regarding spoataneous generation in the nineteenth century resulted from the failure to kill resistant cells in materials that were thought to

be sterile. In contrast with sterilization, disinfection means reducing the number of pathogenic organisms on objects or in materials so that they pose no threat of disease.

1.2.3.5 The control of microbial growth

Both the growth and death of microorganisms occur at logarithmic rates. Here we are concerned with the death rate and the effects of antimicrobial agents obey the same laws regarding death rates as those declining in numbers from natural cause. For example when heat is applied to a material, the death rate of the organisms in or on it remains logarithmic but is greatly accelerated. Heat acts as an antimicrobial agent. If 20 percent of the organisms die in the first minute, 20 percent of those remaining alive will die in the second minute, and so on. If, at a different temperature, 30 percent die in the first minute, 30 percent of the remaining ones will die in the second minute, and so on. From these observation we can derive the principle that a definite proportion of the organisms die in a given time interval.

Table Terms related to sterilization and disinfection

TERM	DEFINITION
Sterilization	The killing or removal of all microorganisms in a material or on an object
Disinfection	The reduction of the number of pathogenic microorganisms to the point where they pose no danger of disease
Antiseptic	A chemical agent that can safely be used externally on living tissue to destroy microorganisms or to inhibit their growth
Disinfectant	A chemical agent used on inanimate objects to destroy microorganisms
Decontamination	Most disinjectants do not kill spores
Sanitizer	A chemical agent typically used on food

	handling equipment and eating utensils to reduce bacterial numbers so as to meet public health standards. Sanitization may simply refer to through washing with only soap or detergent
Bacteriostatic agent	An agent that inhibits the growth of bacteria
Germicide	An agent capable of killing microbes rapidly; some such agents effectively kill certain microorganism but only inhibit the growth of others
Bactericide	An agent that kills bacteria. Most such agents do not kill spores
Viricide	An agent that inactivates viruses
Fungicide	An agent that kills fungi
Sporocide	An agent that kills bacterial endospores or fungal spores

1.2.3.6 Chemical antimicrobial agents

1)The potency of chemical agents

The potency, or effectiveness, of a chemical antimicrobial agent is affected by time, temperature, pH and concentration. The death rate of organisms is affected by the length of time the organisms are exposed to the antimicrobial agent. Thus, adequate time should always be allowed for an agent to kill the maximum number of organisms. The death rate of organisms subjected to a chemical agent is accelerated by increasing the temperature. Increasing temperature by 10^{o}C roughly doubles the rate of chemical reactions and thereby increases the potency of the chemical agent. Acidic or alkaline pH can increase or decrease the agent's potency. A pH that increases the degree of ionization of a chemical agent often increases its ability to

penetrate a cell. Such a pH also can alter the contents of the cell itself. Finally, increasing concentration may increase the effects of most antimicrobial chemical agents. High concentrations may be bacterial (killing), whereas lower concentrations may be bacteriostatic (growth-inhibiting).

Both ethyl and isopropyl alcohol are exceptions to the rule regarding increasing concentrations. They have long been believed to be more potent at 70 percent than at higher concentrations, although they are also effective up to 99 percent concentration. Some water must be present for alcohols to disinfect because they act by coagulating (permanently denaturing) proteins, and water is needed for the coagulation reactions. Also, a 70 percent alcohol – water mixture penetrates more deeply than pure alcohol into most materials to be disinfected.

2)Evaluating the effectiveness of chemicals agents

Many factors affect the potency of chemical antimicrobial agents, so evaluation of effectiveness is difficult. No entirely satisfactory method is available. However, we need some way to compare the effectiveness of disinfecting agents, especially as new ones come on the market.

a)The Phenol coefficient

Since Lister introduced phenol (carbolic acid) as a disinfectant in 1867, it has been the standard disinfectant to which other disinfectants are compared under the same conditions. The result of this comparison is called the phenol coefficient. Two organisms, Salmonella typhi, a pathogen of the digestive system, and Staphylococcus aureus, a common wound pathogen, are typically used to determine phenol coefficients. A disinfectant with a phenol coefficient of 1.0 has the same effectiveness as phenol. A coefficient less than 1.0 means that the disinfectant is less effective than phenol: a coefficient of greater than 1.0 means that it is more effective. Phenol coefficients are reported separately for the different test organisms. Lysol, for instance, has a coefficient of 5.0 against

Staphylococcus aureus but only 3.2 when used on Salmonella typhi, whereas ethyl alcohol has a value of 6.3 against both.

The phenol coefficient can be determined by the following steps. Prepare several dilutions of a chemical agent, and place the same volume of each in different test tubes. Prepare an identical set of test tubes, using phenol dilutions. Put both sets of tubes in a 20^{o}C water bath for at least 5 minutes to ensure that the contents of all tubes are at the same temperature. Transfer 0.5ml of a culture of a standard test organism to each tube. After 5, 10 and 15 minutes, use a sterile loop to transfer a specific volume of liquid from each tube into a separate tube of nutrient broth, and incubate the tubes. After 48 hours, check cultures for cloudiness, and find the smallest concentration (highest dilution) of the agent that killed all organisms in 10 minutes but not in 5 minutes. Find the ratio of this dilution to the dilution of phenol that has the same effect. For example, if a 1:1000 dilution of a chemical agent has the same effect as a 1:100 dilution of phenol, the phenol coefficient of that agent is 10 (1000/100). If you performed this test on a new disinfectant and obtained these results, you would have found a very good disinfectant ! The phenol, the phenol coefficient provides an acceptable means of evaluating the effectiveness of chemical agents derived from phenol, but it is less acceptable for other agents. Another problem is that the materials on or in which organisms are found may affect the usefulness of a chemical agent by complexing with it or inactivating it. These effects are not reflected in the phenol conefficient number.

b)The filter paper method

The filter paper method of evaluating a chemical agent is simpler than determining a phenol coefficient. It uses small filter paper disks, each soaked with a different chemical agent. The disks are placed on the surface of an agar plate that has been inoculated with a test organism. A different plate is used for each test organism. After incubation, a chemical agent that inhibits growth of a test organism is identified by a clear area around the disk where the bacteria have been killed. Note: What is effective against one organism may have little or no effect on the others.

c)The use-dilution test

A third way of evaluating chemical agents, the use-dilution test, uses standard preparations of certain test bacteria. A broth culture of one of these bacteria is coated onto small stainless steel cylinders and allowed to dry. Each cylinder is then dipped into one of several dilutions of the chemical agent for 10 minutes, removed, rinsed with water, and placed into a tube of broth. The tubes are incubated and then observed for the presence or absence of growth. Agents that prevent growth at the greatest dilutions are considered the most effective. Many microbiologists feel that this measurement is more meaningful than the phenol coefficient.

3)Disinfectant selection

An ideal disinfectant should

1. Be fast acting even in the presence of organic substances, such as those in body fluids:
2. Be effective against all types of infectious agents without destroying tissues or acting as a poison if ingested:
3. Easily penetrate material to be disinfected without damaging or discoloring the material:
4. Be easy to prepare and stable even when exposed to light, heat, or other environmental factors:
5. Be inexpensive and easy to obtain and use:
6. Not have an unpleasant odor.

No disinfectant is likely to satisfy all these criteria, so the agent that meets the greatest number of criteria for the task at hand is chosen.

In practice, many agents are tested in a wide range of situations and are recommended for use where they are most effective. Thus, some agents are selected for sanitizing kitchen equipment and eating utensils, whereas other agents are chosen for rendering pathogenic cultures harmless. Furthermore,

certain agents can be used in dilute concentration on the skin and in stronger concentration on inanimate objects.

4)Mechanisms of action of chemical agents

Chemical antimicrobial agents kill microorganisms by participating in one or more chemical reactions that damage cell components. Although the kinds of reactions are almost as numerous as the agents, agents can be grouped by whether they affect proteins, membranes, or other cell components.

a)Reactions that affect proteins

Much of a cell is made of protein, and all its enzymes are proteins. Alteration of protein structure is called denaturation. Denaturation, hydrogen and disulfide bonds are disrupted, and the functional shape of the protein molecule is destroyed. Any agent that denatures proteins prevents them from carrying out their normal functions. When treated with mild heat or with some dilute acids, alkalis, or other agents for a short time, proteins are temporarily denatured. After the agent is removed, some proteins can regain their normal structure. However, most antimicrobial agents are used in a strong enough concentration over a sufficient length of time to denature proteins permanently. Permanent denaturation of a microorganism's proteins kills the organism. Denaturation is bactericidal if it permanently alters the protein such that the protein's normal state cannot be restored. Denaturation is bacteriostatic if it temporarly alters the protein, and the normal structure can be recovered.

Fig. 12.2

Reactions that denature proteins include hydrolysis, oxidation, and the attachment of atoms or chemical groups. (Recall that hydrolysis is the breaking down of a molecule by the addition of water and that oxidation is the addition of oxygen to, or the removal of hydrogen from, a molecule). Acids, such as boric acid, and strong alkalis destroy protein by hydrolyzing it.

Oxidizing agents (electron acceptors), such as hydrogen peroxide and potassium permanganate, oxidize disulfide linkages (-S-S-) or sulfhydryl groups (-SH). Agents that contain halogens the elements chlorine, fluorine, bromine, and iodine – also sometimes act as oxidizing agents. Heavy metals, such as mercury and silver, attach to sulfhydryl groups. Alkylating agents, which contain methyl (-CH_3) or similar groups, donate these groups to proteins. Formaldehyde and some dyes are alkylating agents. Halogens can be substituted for hydrogen in carboxyl (- COOH), sulfhydryl, amino (- NH_2), and alcohol (- OH) groups. All these reactions can kill microorganisms.

b)Reactions that affect membranes

Membranes contain proteins and so can be altered by all the preceding reactions. Membranes also contain lipids and thus can be disrupted by substances that dissolve lipids. Surfactants (surfak tantz) are soluble compounds that reduce surface tension, just as soaps and detergents break up grease particles in dishwater (Fig). Surfactants include alcohols, detergents, and quaternary ammonium compounds, such as benzalkonium chloride, which dissolve lipids. Phenols, which are alcohols, dissolve lipids and also denature proteins. Detergent solutions, also called wetting agents, are often used with other chemical agents to help the agent penetrate fatty substances. Although detergent solutions themselves usually do not kill microorganisms, they do help get rid of lipids and other organic materials so that antimicrobial agents can reach the organisms.

Fig. 12.2

Fig. 12.3

c)Reactions that affect other cell components

Other cell components affected by chemical agents include nucleic acids and energy-producing systems. Alkylating agents can replace hydrogen on amino or alcohol groups in nucleic acids. Certain dyes, such as crystal violet, interfere with cell wall formation. Some substances, such as lactic acid and propionic acid (end products of fermentation), inhibit fermentation and thus prevent energy production in certain bacteria, molds, and some other organisms.

d)Reactions that affect viruses

Like many cellular microorganisms, viruses can cause infections and must be controlled. Control of viruses requires that they be inactivated – that is, rendered permanently incapable of infeeting or replicating in cells. Inactivation can be effected by destroying either the viruses' nucleic acid or their proteins.

Alkylating agents, such as ethylene oxide, nitrous acid, and hydroxylamine act as chemical mutagens – they alter DNA or RNA. If the alteration prevents the DNA or RNA from directing the synthesis of new viral particles, the alkylating agents are effective inactivators. Detergents, alcohols, and other agents that denature proteins act on bacteria and viruses in the same way. Certain dyes, such as acridine orange and methylene blue, render viruses susceptible to inactivation when exposed to visible light. This process disrupts the structure of the viral nucleic acid.

Viruses sometimes remain infective even after their proteins are denatured, so methods used to rid materials of bacteria may not be as successful with infectious viruses. Also, use of an agent that does not inactivate viruses can lead to laboratory-acquired infections.

5)Specific chemical antimicrobial agents

The structural formulas of some of the most important compounds are (Figure).

Fig.

a)Soaps and detergents

Soaps and detergents remove microbes, oily substances, and dirt. Mechanical scrubbing greatly enhances their action, vigorous hand washing is one of the easiest and cheapest means of preventing the spread of disease among patients in hospitals, in medical and dental offices, among employees and patrons in food establishments, and among family members. Unlike surgical scrubs, germicidal soaps usually are not significantly better disinfectants than ordinary soaps.

Soaps contain alkali and sodium and will kill many species of Streptococcus. Micrococcus, and Neisseria and will destroy influenza viruses. Many pathogens that survive washing with soap can be killed by a disinfectant applied after washing. A common practice after washing and rinsing hands and inanimate objects is to apply a 70 percent alcohol solution. Disposable gloves are used where there is a risk that health care workers may become infected or may transmit pathogens to other patients.

Detergents, when used in weak concentrations in wash water, allow the water to penetrate into all crevices and cause dirt and microorganisms to be lifted out and washed away. Detergents are said to be cationic if they are positively charged and anionic if they are negatively charged. Cationic detergents are used to sanitize food utensils. Although not effective in killing endospores, they do inactivate some viruses. Anionic detergents are used for laundering clothes and as household cleaning agents. They are less effective sanitizing agents than cationic detergents, probably because the negative charges on bacterial cell walls repel them.

Many cationic detergents are quaternary ammonium compounds, or quats, which have four organic groups attached to a nitrogen atom. A variety of quats are available as

disinfecting agents; their chemical structures vary according to their organic groups. One problem with quats is that their effectiveness is decreased in the presence of soap, calcium or magnesium ions, or porous substances such as gauze. An even more serious problem with these agents is that they support the growth of some bacteria of the genus Pseudomonas rather than killing them. Zephiran (benzalkonium chloride) was once widely used as a skin antiseptic. It is no longer recommended because it is less effective than originally thought and is subject to the same problems as other quats. Quats are now often mixed with another agent to overcome some of these problems and to increase their effectiveness.

b)Acid and Alkal Soap is a mild alkali, and its alkaline properties help destroy microbes. A number of organic acids lower the pH of materials sufficiently to inhibit fermentation. Several are used as food preservatives. Lactic and propionic acids retard mold growth in breads and other products. Benzoic acid and several of its derivatives are used to prevent fungal growth inn soft drinks, catsup, and margarine. Sorbic acid and sorbates are used to prevent fungal growth in cheeses and a variety of other foods. Boric acid, formerly used as an eyewash, is no longer recommended because of its toxicity.

c)Heavy metals

Heavy metals used in chemical agents include selenium, mercury, copper, and silver. Even tiny quantities of such metals can be very effective in inhibiting bacterial growth. Silver nitrate was once widely used to prevent gonococcal infection in newborn infants. A few drops of silver nitrate solution were placed in the baby's eyes at the time of delivery to protect against infection by gonococci entering the eyes during passage through the birth canal. For a time, many hospitals replaced silver nitrate with antibiotics such as erythromycin. However, the development of antibiotic-resistant strains of gonococci has led some localities to require the use of silver nitrate, to which gonococci do not develop resistance.

Organic mercury compounds, such as merthiolate and mercurochrome, are used to disinfect surface skin wounds. Such agents kill most bacteria in the vegetative state but do not

kill spores. They are not effective against Mycobacterium. Merthiolate is generally prepared as a tincture (tingk'chur), that is, dissolved in alcohol. The alcohol in a tincture may have a greater germicidal action than the heavy metal compound can be used to disinfect skin and instruments and as a preservative for vaccines. Phenylmercuric nitrate and mercuric naphthenate inibit both bacteria and fungi and are used as laboratory disinfectants.

Selenium sulfide kills fungi, including spores. Preparations containing selenium are commonly used to treat fungal skin infections. Shampoos that contain selenium are effective in controlling dandruff. Dandruff, a crusting and flaking of the scalp, is often, though not always, caused by fungi.

Copper sulfate is used to control algal growth. Although algal growth usually is not a direct medical problem, it is a problem in maintaining water quality in heating and air-conditioning systems and outdoor swimming pools. (The Environmental Protection Agency, however, is evaluating copper sulfate as an environmental hazard).

d)Halogens

Hypochlorous acid, formed by the addition of chlorine to water, effectively controls microorganisms in drinking water and swimming pools. It is the active ingredient in household bleach and is used to disinfect food utensils and dairy equipment. It is effective in killing bacteria and inactivating many viruses. However, chlorine itself is easily inactivated by the presence of organic materials. That is why a substance such as copper sulfate is used to control algal growth in water to be purified with chlorine.

Iodine also is an effective antimicrobial agent. Tincture of iodine was one of the first skin antiseptics to come into use. Now iodophors, slow-release compounds in which the iodine is combined with roganic molecules, are more commonly used, in such preparations, the organic molecules act as surfactants. Betadine and Isodine are used for surgical scrubs and on skin where an incision will be made. These compounds take several

minutes to act and do not sterilize the skin. Betadine in concentrations of 3 to 5 percent destroys fungi, amoebas, and viruses as well as most bacteria, but it does not destroy bacterial endospores. Contamination of Betadine with Pseudomonas cepacia has been reported.

Bromine is sometimes used in the form of gaseous methyl bromide to fumigate soil that will be used in the propagation of bedding plants. It is also used in some pools and indoor hot tubes because it does not give off the strong odor that chloriinc does.

e)Alcohols

When mixed with water, alcohols denature protein. They are also lipid solvents and dissolve membranes. Ethyl and isopropyl alcohols can be used as skin antiseptics. Isopropyl alcohol is more often used due to legal regulation of ethyl alcohol. It disinfects skin where injections will be made or blood drawn. Alcohol disinfects but does not sterilize skin because it evaporates quickly and stays in contact with microbes for only a few seconds. It also does not penetrate deeply enough into pores in the skin. It kills vegetative microorganisms on the skin surface but does not kill endospores, resistant cells, or cells deep in skin pores.

f)Phenols

Phenol and phenol derivatives called phenolics disrupt cell membranes, denature proteins, and inactivate enzymes. They are used to disinfect surfaces and to destroy discarded cultures because their action is not impaired by organic materials. Amphyl, which contains amylphenol, destroys vegetative forms of bacteria and fungi and inactivates viruses. It can be used on skin, instruments, dishes, and furniture. When used on surfaces, it retains its antimicrobial action for several days. The orthophenylphenol in Lysol gives it similar properties. A mixture of phenol derivatives called cresols is found in creosote, a substance used to prevent the rotting of wooden posts, fences, railroad ties, and such, However, because creosote is irritating to skin and a carcinogen, its use is limited. The addition of

halogens to phenolic molecules usually increases their effectiveness. Hexachlorophene and dichlorophene, which are halogenated phenols, inhibit staphylococci and fungi, respectively, on the skin and elsewhere. Chlorhexidine gluconate (Hibiclens), which is chlorinated and similar in structure to hexachlorophene, is effective against a wide variety of microbes even in the presence of organic material. It is a good agent for surgical scrubs.

g)Oxidizing agents

Oxidizing agents disrupt disulfide bonds in proteins and thus disrupt the structure of membranes and proteins. Hydrogen peroxide (H_2O_2), which forms highly reactive superoxide (O_2), is used to clean puncture wounds. When hydrogen peroxide breaks down into oxygen and water, the oxygen kills obligate anaerobes present in the wounds. Hydrogen peroxide is quickly inactivated by enzymes from injured tissues. A recently developed method of sterilization that uses vaporized hydrogen peroxide can now be used for small rooms or areas, such as glove boxes and transfer hoods. Another oxidizing agent, potassium permanganate, is used to disinfect instruments and, in low concentrations, to clean skin.

h)Alkylating agents

Alkylating agents disrupt the structure of both proteins and nucleic acids. Because they can disrupt nucleic acids, these agents may cause cancer and should not be used in situations where they might affect human cells. Formaldehyde, glutaraldehyde, and β-propiolactone are used in aqueous solutions. Ethylene oxide is used in gaseous from.

Fromaldehyde inactivates viruses and toxins without destroying their antigenic properties. Glutaraldehyde kills all kinds of microorganisms, including spores, and sterilizes equipment exposed to it for 10 hours. Beta-propiolactone destroys hepatitis viruses, as well as most other microbes, but penetrates materials poorly. It is, however, used to inactivate viruses in vaccines.

Gaseous ethylene oxide has extraordinary penetrating power. Used at a concentration of 500 mg /1 at 50°C for 4 hours, it sterilizes rubber goods, mattresses, plastics, and other materials destroyed by higher temperatures. Special equipment used during ethylene oxide sterilization. As will be explained when we discuss autoclaving, an ampule (a sealed glass container) of endospores should be processed with ethylene oxide sterilization to check the effectiveness of sterilization.

All articles sterilized with ethylene oxide must be well ventilated with sterile air to remove all traces of this toxic gas, which can cause burns if it reaches living tissues and is also highly explosive. After exposure to ethylene oxide, articles such as catheters, intravenous lines, in-line valves, and rubber tubing must be thoroughly flushed with sterile air. Both the toxicity and flammability of ethylene oxide can be reduced by using it in gas containing 90 percent carbon dioxide. It is exceedingly important that workers be protected from ethylene oxide vapors, which are toxic to skin, eyes, and mucous membranes and may also cause cancer.

i)Dyes

The dye acridine, which interferes with cell replication by causing mutations in DNA can be used to clean wounds. Methylene blue inhibits growth of some bacteria in cultures. Crystal violet (gentian violet) blocks cell wall synthesis, possibly by the same reaction that causes this dye to bind to cell wall material in gram staining. It effectively inhibits growth of Gram-positive bacteria in cultures and in skin infections. It can be used to treat protozoan (Trichomonas) and yeast (Candida albicans) infections.

j)Other agents

Certain plant oils have special antimicrobial used. Thymol, derived from the herb thyme, is used as a preservative, and eugenol, derived from oil of cloves, is used in dentistry to disinfect cavities. A variety of other agents are used primarily as food preservatives. They include sulfites and sulfur dioxide, used to preserve dried fruits and molasses; sodium diacetate,

used to retard mold in bread; and sodium nitrite, used to preserve cured meats and some cold cuts. Foods containing nitrites should be eaten in moderation because the nitrites are converted during digestion to substances that may cause cancer.

1.2.3.7 Physical antimicrobial agents

Physical antimicrobial agents have been used to preserve food. Ancient Egyptians dried perishable foods to preserve them. Scandinavians made holes in the centers of pieces of dry, flat, crisp bread in order to hang them in the air of their homes during the winter; likewise they kept seed grains in a dry place. Otherwise, both flour and grains would have molded during the long and very moist winters. Europeans used heat in the food-canning process 50 years before Pasteur's work explained why heating prevented food from spoiling. Today, physical agents that destroy microorganisms are still used in food preservation and preparation. Such agents remain a crucial weapon in the prevention of infectious disease. Physical antimicrobial agents include various forms of heat, refrigeration, desiccation (drying), irradiation, and filtration.

Table Properties of Chemical Antimicrobial Agents

AGENT	ACTIONS	USES
Soaps and detergents	Lower surface tension, make microbes accessible to other agents	Hand washing, laundering, sanitizing kitchen and dairy equipment
Surfactants	Dissolve lipids, disrupt membranes, denature proteins, and inactivate enzymes in high concentrations; act as wetting agents in low concentrations	Cationic detergents are used to sanitize utensils; anionic detergents to launder cloths and clean household objects; quaternary ammonium compounds are sometimes used as antiseptics on skin
Acids	Lower pH and	Food preservation

	denature proteins	
Alkalis	Raise pH and denature proteins	Found in soaps
Heavy metals	Denature proteins	Silver nitrate is used to prevent gonococcal infections, mercury compounds to disinject skin and inanimate objects, copper to inhibit algal growth, and selenium to inhibit fungal growth
Halogens	Oxidize cell components in absence of organic matter	Chlorine is used to kill pathogens in water and to disinject utensils iodine compounds are used as skin antiseptics
Alcohols	Denature proteins when mixed with water	Isopropyl alcohol is used to disinfect skin; ethylene glycol and propylene glycol can be used in aerosols
Phenols	Disrupt membranes, denature proteins and inactivate enzymes; not impaired by organic matter	Phenol is used to disinject surfaces and destroy discarded cultures amylphenol destroys vegetative organisms and inactivates virus on skin and inanimate objects; chlorhexidine gluconate is especially effective as a surgical scrub
Oxidizing agents	Disrupt disulfide bonds	Hydrogen peroxide is used to clean puncture wounds, potassium permanganate to disinject instruments
Alkylating	Disrupt structure of	Formaldehyde is used to

agents	proteins and nucleic acid	inactivate viruses without destroying antigenic properties, glutaraldehyde to sterilize equipment betapropiolactone to destroy hepatitis viruses and ethylene oxygen to sterilize inanimate objects that would be harmed by high temperatures
Dyes	May interfere with replication or block cell wall synthesis	Acridine is used to clean wounds, crystal violet to treat some protozoan and fungal infections

1)Principles and applications of heat killing

Heat is a preferred agent of sterilization for all materials not damaged by it. It rapidly penetrates thick materials not easily penetrated by chemical agents. Several measurements have been defined to quanify the killing power of heat. The thermal death point is the temperature that kills all the bacteria in a 24-hour-old broth culture at neutral pH in 10 minutes. The thermal death time is the time required to kill all the bacteria in a particular culture at a specified temperature. The decimal reduction time, also known as the DRT or D value, is the length of time needed to kill 90 percent of the organisms in a given population at a specified temperature. (The temperature is indicated by a subscript: D_{80^oc}, for example).

These measurements have practical significance in industry as well as in the laboratory.

2)Dry heat, moist heat, and pasteurization

Dry heat probably does most of its damage by oxidizing molecules, Moist heat destroys microorganisms mainly by denaturing proteins; the presence of water molecules helps disrupt the hydrogen bonds and other weak interactions that

hold proteins in their three-dimensional shapes. Moist heat may disrupt membrane lipids as well. Heat also inactivates many viruses, but those that can infect even after their protein coats are denatured require extreme heat treatment, such as steam under pressure, that will disrupt nucleic acids.

a)Dry Heat

Dry (oven) heat penetrates substances more slowly than moist (steam) heat. It is usually used to sterilize metal objects and glassware and is the only suitable means of sterilizing oils and powders. Objects are sterilized by dry heat when subjected to 171^{o}C for 1 hour, 160^{o}C for 2 hours or longer, or 121^{o}C for 16 hours or longer, depending on the volume.

An open flame is a form of dry heat used to sterilize inoculating loops and the mouths of culture tubes by incineration and to dry the inside of pipettes. When flaming objects in the laboratory, avoid the formation of floating ashes and aerosols (droplets released into the air) which are a means of spreading infectious agents if the organisms in them are not killed by incineration as intended. For this reason, specially designed loop incinerators with deep throats are often used for sterilizing inoculating loops.

b)Moist heat

Moist heat, because of its penetrating properties, is a widely used physical agent. Boiling water destroys vegetative cells of most bacteria and fungi and inactivates some viruses, but it is not effective in killing all kinds of spores. The effectiveness of boiling can be increased by adding 2 percent sodium bicarbonate to the water. However, if water is heated under pressure, its boiling point is elevated, so temperatures above 100^{o}C can be reached. This is normally accomplished by using an autoclave (aw to-klav), as shown in figure 12.9, in which a pressure of 15lb/in^{2} above atmospheric pressure is maintained for 15 to 20 minutes, depending on the volume of the load. At this pressure, the temperature reaches 121^{o}C, which is high enough to kill spores as well as vegetative organisms and to disrupt the structure of nucleic acids in viruses. In this

procedure it is the increased temperature, and not the increased pressure that kills microorganisms.

Sterilization by autoclaving is invariably successful if properly done and if two common-sense rules are followed: First, articles should be placed in the autoclave so that steam can easily penetrate them: second, air should be evacuated so that the chamber fills with steam. Wrapping objects in aluminum foil is not recommended because it may interfere with steam penetration. Steam circulates through an autoclave from a steam outlet to an air evacuation port. In preparing items for autoclaving, containers should be unsealed and articles should be wrapped in materials that allow steam penetration. Large packages of dressings and large flasks of media require extra time for heat to penetrate them. Likewise, packing many articles close together in an autoclave lengthens the processing time to as much as 60 minutes to ensure sterility. It is more efficient and safer to run two separate, uncrowded loads than one crowded one.

Tapes or other sterilization indicators should be placed inside and near the center of large packages to determine whether heat penetrated them. This precaution is necessary because when an object is exposed to heat, its surface becomes hot much more quickly than its center.

The Centers for Disease Control and Prevention recommends weekly autoclaving of a culture containing heat-resistant endospores, such as those of Bacillus stearothermophilus, to check autoclave performance. Endospore strips are commercially available to make this task easy. The spore strip and an ampule of medium are enclosed in a soft plastic vial. The vial is placed in the center of the material to be sterilized and is autoclaved. Then the inner ampule is broken, releasing the medium, and the whole container is incubated. If no growth appears in the autoclaved culture, sterilization is deemed effective.

c)Pasteurization

Pasteurization, a process invented by Pasteur to destroy organisms that caused wine to sour, does not achieve sterility. It does kill pathogens, especially Salmonella and Mycobacterium, that might be present in milk, other dairy products, and beer, Mycobacterium used to cause many cases of tuberculosis among children who drank raw milk. Milk is pasteurized by heating it to 71.6^{o}C for at least 15 seconds in he flash method or by heating it to 62.9^{o}C for 30 minutes in the holding method. Some years ago certain strains of bacteria of the genus Listeria were found in pasteurized milk and cheeses. This pathogen causes diarrhea and encephalitis and can lead to death in pregnant women.

Fig

Although most milk for sale in the United States is pasteurized fresh milk, sterile milk also is available. All evaporated or condensed canned milk is sterile, and some milk packaged in cardboard containers also is sterile. The canned milk is subjected to steam under pressure and has a “cooked” flavor. Sterilized milk in cardboard containers is widely available in Europe and can be found in some stores in the United States. It is subjected to a process that is similar to pasteurization but uses higher temperatures. It too has a “cooked” flavor but can be kept unrefrigerated as long as the container remains sealed. Such milk is often flavored with vanilla, strawberry, or chocolate. Ultrahigh temperature (UHT) processing raises the temperature from 74^{o}C in less than 5 seconds. A complex cooling process that prevents the milk from ever touching a surface hotter than itself prevents development of a “cooked” flavor, some, but not all, small containers of coffee creamer are treated by this method.

3)Refrigeration, freezing, drying, and freeze-drying

Cold temperature retards the growth of microorganisms by slowing the rate of enzyme-controlled reactions but does not kill many microbes. Heat is much more effective than cold at killing microorganisms. Refrigeration is used to prevent food spoilage. Freezing, drying, and freeze-drying are used to preserve both foods and microorganisms, but these methods do not achieve sterilization.

a)Refrigeration

Many fresh foods can be prevented from spoiling by keeping them at 5^{o}C (ordinary refrigerator temperature). However, storage should be limited to a few days because some bacteria and molds continue to grow at this temperature. To convince yourself of this, recall some of the strange things you have found growing on leftovers in the back of your refrigerator. In rare instances strains of Clostridium botulium have been found growing and producing lethal toxins in a refrigerator when the organisms were deep within a container of food, where anaerobic conditions exist.

b)Freezing

Freezing – at 20^{o}C is used to preserve foods in homes and in the food industry. Although freezing does not sterilize foods, it does significantly slow the rate of chemical reactions so that microorganisms do not cause food to spoil. Frozen foods should not be thawed and refrozen. Repeated freezing and thawing of foods causes large ice crystals to form in the foods during slow freezing. Cell membranes in the foods are ruptured, and nutrients leak out. The texture of foods is thus altered, and they become less palatable. It also allows bacteria to multiply while food is thawed, making the food more susceptible to bacterial degradation.

Freezing can be used to preserve microorganisms, but this requires a much lower temperature than that used for food preservation. Microorganisms are usually suspended in glycerol or protein to prevent the formation of large ice crystals (which could puncture cells), cooled with solid carbon dioxide (dry ice)

to a temperature of –78°C, and then held there. Alternatively, they can be placed in liquid nitrogen and cooled to –180°C.

c)Drying

Drying can be used to preserve foods because the absence of water inhibits the action of enzymes. Many foods, including peas, beans, raisins, and other fruits, are often preserved by drying. Yeast used in baking also can be preserved by drying. Endospores present on such foods can survive drying, but they do not produce toxins. Dried pepperoni sausage and smoked fish retain enough moisture for microorganisms to grow. Because smoked fish is not cooked, eating it poses a risk of infection. Sealing such fish in plastic bags creates conditions that allow anaerobes such as Clostridium botulinum to grow.

Drying also naturally minimizes the spread of infectious agents. Some bacteria, such as Treponema pallidum, which causes syphilis, are extremely sensitive to drying and die almost immediately on a dry surface; thus they can be prevented from spreading by keeping toilet seats and other bathroom fixtures dry. Drying of laundry in dryers or in the sunshine also destroys pathogens.

d)Freeze-drying

Freeze-drying, or lyophlization (li-of"I-li-za'shun), is the drying of a material from the frozen state. This process is used in the manufacture of some brands of instant coffee, freeze-dried instant coffee has a more natural flavor than other kinds. Microbiologists use lyophilization for long-term preservation rather than for destruction of cultures of microorganisms. Organisms are rapidly frozen in alcohol and dry ice or in liquid nitrogen and are then subjected to a high vacuum to remove all the water while in the frozen state. Rapid freezing allows only very tiny ice crystals to form in cells, so the organisms survive this process. Organisms so treated can be kept alive for years, stored under vacuum in the freeze-dried state.

4)Radiation

Four general types of radiation-ultraviolet light, ionizing radiation, microwave radiation, and strong visible light (under certain circumstances) – can be used to control microorganisms and to preserve foods. Fig. 3.4 pg 51.

a)Ultraviolet light

Ultraviolet (UV) light consists of light of wavelengths between 40 and 390 nm, but wavelengths in the 200-nm range are most effective in killing microorganisms. UV light is absorbed by the purine and pyrimidine bases of nucleic acids. Such absorption can permanently destroy these important molecules. Ultraviolet light is especially effective in inactivating viruses. However, it kills far fewer bacteria than one might expect because of DNA repair mechanisms. Once DNA is repaired, new molecules of RNA are protein can be synthesized to replace the damaged molecules.

Ultraviolet light is of limited use because it does not penetrate glass, cloth, paper, or most other materials, and it does not go around corners or under lab benches. It does penetrate air, effectively reducing the number of airborne microorganisms and killing them on surfaces in operating rooms and rooms that will contain caged animals. UV lights lose effectiveness over time and should be monitored often. To help sanitize the air without irradiating humans, these lights can be turned on when the rooms are not in use. Exposure to ultraviolet light can cause burns, also damage the eyes; years of exposure of skin can lead to skin cancer.

b)Ionizing radiation

X-rays, which have wavelengths of 0.1 to 40 nm, and gamma rays, which have even shorter wavelengths, are forms of ionizing radiation, so named because it can dislodge electrons from atoms, creating ions. (Longer wavelengths comprise nonionizing radiation). These forms of radiation also kill microorganisms and viruses. Many bacteria are killed by absorbing 0.3 to 0.4 millirads of radiation; polioviruses are inactivated by absorbing 3.8 millirads. A rad is a unit of

radiation energy absorbed per gram of tissue; a millirad is one-thousandth of a rad. Humans usually do not become ill from radiation unless they are subjected to doses greater than 50 rad.

Ionizing radiation damages DNA and produces peroxides, which act as powerful oxidizing agents in cells. This radiation can also kill or cause mutations in human cells if it reaches them. It is used to sterilize plastic laboratory and medical equipment and pharmaceutical products. It can be used to prevent spoilage in seafoods by doses of 100 to 250 kilorads, in meats and poultry by doses of 50 to 100 kilorads, and in fruits by doses of 200 to 300 kilorads. (one kilorad equals 1000 rads). Many consumers in the United States reject irradiated foods for fear of receiving radiation, but such foods are quite safe – free of both pathogens and radiation. In Europe, milk and other foods are often irradiated to achieve sterility.

c)Microwave radiation

Microwave radiation, in contrast with gamma, X-ray, and ultraviolet radiation, falls at the longwavelength end of the electromagnetic spectrum. It has wavelengths of approximately 1 mm to 1m, a range that includes television and police radar wavelengths. Microwave oven frequencies are tuned to match energy levels in water molecules, In the liquid state, water molecules quickly absorb the microwave energy and then release it to surrounding materials as heat. Thus, materials that do not contain water, such as plates made of paper, china, or plastic, remain cool while the moist food on them becomes heated. For this reason the home microwave cannot be used to sterilize items such as bandages and glassware. Conduction of energy in metals leads to problems such as sparking, which makes most metallic items also unsuitable for microwave sterilization. Moreover, bacterial endospores, which contain almost no water, are not destroyed by microwaves. However, a specialized microwave oven has recently become available that can be used to sterilize media in just 10 minutes. It has 12 pressure vessels, each of which holds 100 ml of medium. Microwave energy increases the pressure of the medium inside the vessels until sterilizing temperatures are reached.

Caution should be observed in cooking foods in the hone microwave oven. Geometry and differences in density of the food being cooked can cause certain regions to become hotter than others, sometimes leaving very cold spots. Consequently, to cook foods thoroughly in a microwave oven, it is necessary to rotate the items either mechanically or by hand. For example, pork roasts must be turned frequently and cooked thoroughly to kill any cysts of the pork roundworm Trichinella. Filure to kill such cysts could lead to the disease trichinosis, in which cysts of the worm become embedded in human muscles and other tissues. All experimentally infected pork roasts, when microwaved without rotation, showed live worms remaining in some portion at the end of standard cooking time.

d)Strong visible light

Sunlight has been known for years to have a bactericidal effect, but the effect is due primarily to ultraviolet rays in the sunlight. Strong visible light, which contains light of wavelengths from 400 to 700 nm (violet to red light), can have direct bactericidal effects by oxidizing light-sensitive molecules such as riboflavin and porphyrins (components of oxidative enzymes) in bacteria. For that reason, bacterial cultures should not be exposed to strong light during laboratory manipulations. The fluorescent dyes eosin and methylene blue can denature proteins in the presence of strong light because they absorb energy and cause oxidation of proteins and nucleic acids. The combination of a dye and strong light can be used to rid materials of both bacteria and viruses.

e)Sonic and ultrasonic waves

Sonic, or sound, waves in the audible range can destroy bacteria if the waves are of sufficient intensity: Ultrasonic waves, or waves with frequencies above 15,000 cycles per second, can cause bacteria to cavitate. Cavitation (kav “I-ta’shun) is the formation of a partial vacuum in a liquid – in this case, the fluid cytoplasm in the bacterial cell. Bacteria so treated disintegrate, and their proteins are denatured. Enzymes used in detergents are obtained by cavitating the bacterium Bacillus subtilis. The disruption of cells by sound waves is called sonication (son”I-

ka'shun). Neither sonic nor ultrasonic waves are a practical means of sterilization. We mention them here because they are useful in fragmenting cells to study membranes, ribosomes, enzymes, and other components.

5)Filtration

Filtration is the passage of a material through a filter, or straining device. Sterilization by filtration requires filters with exceedingly small pores. Filtration has been used since Pasteur's time to separate bacteria from media and to sterilize materials that would be destroyed by heat. Over the years, filters have been made of porcelain, asbestos, diatomaceous earth, and sintered glass (glass that has been heated without melting). Membrane filters, thin disks with pores that prevent the passage of anything larger than the pore size, are widely used today. They are usually made of nitrocellulose and have the great advantage that they can be manufactured with specific pore sizes from 25 μm. Particles filtered by various pore sizes are summarized in table 12.4.

Membrane filters have certain advantages and disadvantages. Except for those with the smallest pore sizes, membrane filters are relatively inexpensive, do not clog easily, and can filter large volumes of fluid reasonably rapidly. They can be autoclaved or purchased already sterilized. A disadvantage of membrane filters is that many of them allow viruses and some mycoplasmas to pass through. Other disadvantages are that they may absorb relatively large amounts of the filtrate and may introduce metallic ions into the filtrate.

Table Pore sizes of membrane filters and particles that pass through them.

PORE SIZE (in μm)	PARTICLES THAT PASS THROUGH THEM
10	Erythrocytes, yeast cells, bacteria, viruses, molecules
5	Yeast cells, bacteria, viruses, molecules

3	Some yeast cells, bacteria, viruses, molecules
1.2	Most bacteria, viruses, molecules
0.45	A few bacteria, viruses, molecules
0.22	Viruses, molecules
0.10	Medium-sized to small viruses molecules
0.05	Small viruses, molecules
0.025	Only the very smallest viruses, molecules
Ultrafilter	Small molecules

Membrane filters are used to sterilize materials likely to be damaged by heat sterilization. These materials include media, special nutrients that might be added to media, and pharmaceutical products such as drugs, sera, and vitamins. Some filters can be attached to syringes so that materials can be forced through them relatively quickly. Filtration can also be used instead of pasteurization in the manufacture of beer. When using filters to sterilize materials, it is important to select a filter pore size that will prevent any infectious agent from passing into the product.

In the manufacture of vaccines that require the presence of live viruses, it is important to select a filter pore size that will allow viruses to pass but prevent bacteria from doing so. By selecting a filter with a proper pore size, scientists can separate polioviruses from the fluid and debris in tissue cultures in which they were grown. This procedure simplifies the manufacture of polio vaccine. Cellulose acetate filters with extremely tiny pores are now available and are capable of removing many viruses (although not the very smallest) from liquids. However, these filters are expensive and clog easily.

Membrane filters used to trap bacteria from air and water samples can be transferred directly to agar plates, and the

quantity of bacteria in the sample can be determined. Alternatively, the filters can be transferred from one medium to another, so organisms with different nutrient requirements can be transferred from one medium to another, so organisms with different nutrient requirements can be detected. Filtration is also used to remove microorganisms and other small particles from public water supplies and in sewage treatment facilities. This technique, however, cannot sterilize; it merely reduces contamination.

High-efficiency particulate air (HEPA) filters are used in the ventilation systems of areas where microbial control is especially important, such as in operating rooms, burn units, and laminar flow transfer hoods in laboratories. HEPA filters also capture organisms released in rooms occupied by patients with tuberculosis or in laboratories where especially dangerous microbes are studied, such as the maximum containment units. These filters remove almost all organisms larger than 0.3μm in diameter. Used filters are soaked in formalin before they are disposed of.

Osmotic pressure

High concentrations of salt, sugar, or other substances create a hyperosmotic medium, which draws water from microorganisms by osmosis. Plasmolysis (plaz-mol'I-sis), or loss of water, severely interferes with cell function and eventually leads to cell death. The use of sugar in jellies, jams, and syrups or salt solutions in curing meat and making pickles plasmolyzes most organisms present and prevents growth of new organisms. A few halophilic organisms, however, thrive in these conditions and cause spoilage, especially of pickles, and some fungi can live on the surface of jams.

Table Properties of physical antimicrobial agents.

AGENT	ACTION	USE
Dry heat	Denatures proteins	Oven heat used to sterilize glassware and metal objects; open flame used to incinerate microorganisms
Moist heat	Denatures	Autoclaving sterilizes media,

	proteins	bandages, and many kinds of hospital and laboratory equipment and damaged by heat and moisture; pressure cooking sterilizes canned foods
Pasteurization	Denatures proteins	Kills pathogens in milk, dairy products, and beer
Refrigeration	Slows the rate of enzyme controlled reactions	Used to keep fresh foods for a few days; does not kill most microorganisms
Freezing	Greatly slows the rate of most enzyme controlled reactions	Used to keep fresh foods for several months; does not kill microorganisms; used with glycerol to preserve microorganisms
Drying	Inhibits enzymes	Used to preserve some fruits and vegetables; sometimes used with smoke to preserve sausages and fish
Freeze-drying	Dehydration inhibits enzymes	Used to manufacture some instant coffees; used to preserve microorganisms for years
Ultraviolet light	Denatures proteins and nucleic acids	Used to reduce the number of microorganisms in air in operating rooms, animal rooms, and where cultures are transferred
Ionizing radiation	Denatures proteins and nucleic acids	Used to sterlize plastics and pharmaceutical products and to preserve foods
Microwave radiation	Absorbs water molecules, then releases microwave energy to surroundings as heat	Cannot be used reliably to destroy microbes except in special media-sterilizing equipment
Strong visible light	Oxidation of light-sensitive	Can be used with dyes to destroy bacteria and viruses;

	materials	may help sanitize clothing
Sonic and ultrasonic waves	Cause cavitation	Not a practical means of killing microorganism but useful in fractionating and studying cell components
Filtration microbes	Mechanically removes microbes	Used to sterilize media, pharmaceutical products, and vitamins, in manufacturing vaccines, and in sampling microbes in air and water
Osmotic pressure	Removes water from microbes	Used to prevent spoilage of foods such as pickles and jellies

1.2.3.8 Model Questions

1.What is sterilization?Explain different sterilization processes?

2.Write a detail note on different anti microbial agents ?

1.2.3.9 Reference books

1. Microbiology → Michael J. Pelczar, JR.

E.C.S. Chan

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2. Microbiology → Prescott

L.M. Harley

J.P. Klein, D.A

Lesson 1.2.4

MODES OF GENE TRANSFER

Objetive:

This chaptr explains different gene transfer mechanisms that take place in different microorgansms

1.2.4.1 Introduction

Sexuality and recombination in bacteria will involve transfer of genetic material from one bacterial cell to another. Following three methods for such a transfer are known and they are

a) Transformation

In this case, genetic material is released from one strain and a part may be acquired by another strain, which thus gets transformed without coming in contact with the other strain.

b) Transduction

In this phenomenon, a virus when released from a host bacterial strain may carry with it a part of genetic material of the host strain and may transfer it to another strain later infected by this virus.

c) Conjugation

In this case, genetic material is transferred through a conjugation tube, when two strains come in contact.

These three parasexual processes of transformation, conjugation and transduction are distinguished by two simple criteria

- Sensitivity to deoxyribonuclease (Dnase); &
- Dependence on cell-cell contact

These two criteria are easily tested.

The first criterion is tested by simply adding Dnase to the medium containing the bacterial strains involved in recombination.

If recombination occurs in its absence but not in the presence of D Nase, transformation must be occurring because, the DNA, which is free in the medium, is Vulnerable to the enzyme's activity.

In conjugation and transduction, DNA is not free in the medium, so it is not subject to degradation by Dnase. Conjugation, which requires cell-cell contact, can be identified by the U-tube experiment in which bacteria of two different

genotypes are placed in opposite arms of a U-shaped culture tube.

The arms of the U-tube, and hence the two bacterial cultures, are separated by a sintered glass filter that allows DNA molecules and bacterial viruses but not bacterial cells to pass through. Conjugation cannot occur under these conditions because the filter presents cell-cell contact. If D Nase is present in the culture medium of the U-tube and if recombination occurs, it is likely taking place by the process of transduction.

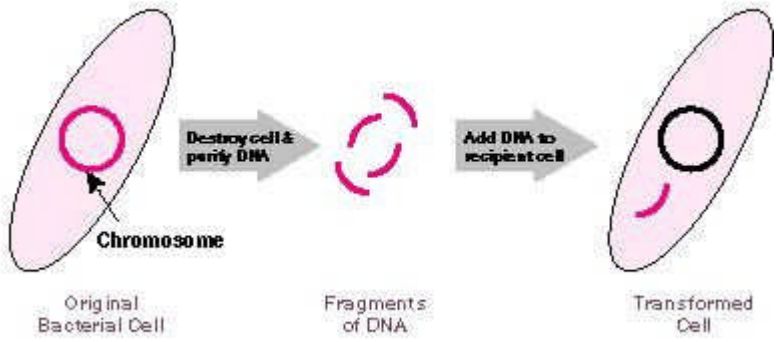
Key points

To determine which of the three parasexual processes may be operating in a particular bacterial species, it is necessary to determine whether cell-cell contact is required and whether the process can be disrupted by the presence of D Nase in the medium.

1.2.4.2 . **TRANSFORMATION**

Transformation was the earliest of the three mechanisms of genetic exchange in bacteria to be described. It was first discovered in 1928 in pathogenic strains of Streptococcus pneumoniae (also known as Diplococcus pneumoniae) by Frederick Griffith, although he knows nothing of the actual nature of the process. Griffith's recorded observations were crucial to the eventual identification of DNA as the genetic material. In 1944, Avery, Mac Leod, and Mc Carty demonstrated convincingly that the "transforming principle" described by Griffith was DNA. Their discovery that DNA was the genetic material was confirmed by the experimental work of A.D. Hershey and M.Chase in 1952.

8.2 PRINCIPLE OF TRANSFORMATION



a)Process of Transformation

The movement of donor DNA molecules across the membrane and into the cytoplasm of recipient bacteria is an active, energy-requiring process. It does not involve the passive diffusion of DNA molecules through permeable cell walls and membranes. Transformation is not a naturally occurring process in all species of bacteria; rather, it takes place only in those species possessing the protein and enzymatic machinery required to bend free DNA molecules in the medium and transport them to the cytoplasm. Most transformation studies have used three species of bacteria ---- Streptococcus pneumonial, Bacillus subtilis and Haemophilus influenzae.

Even in these species all cells in a given population are not capable of being transformed. Only competent cells, which secrete a competence factor, a small protein that induces the synthesis of 8 to 10 new proteins required for transformation, are capable of serving as recipients in transformation. The proportion of bacteria in a culture that are physiologically

competent to be transformed depends on the growth conditions. In most bacterial species, cells that are likely to be transformed are dividing at their maximal rate. These populations of cells are growing exponentially and are fast approaching the plateau phase where nutrients in the medium become a limimiting factor in the continued growth of the population.

In Haemophilus cells, the process of transformation is different. The bacterial species does not produce a competence factor. While Streptococcus can take up DNA from a variety of source, Haemophilus can take up DNA from only closely related species. Double stranded DNA is not degraded but rather is complexed with proteins and then taken into the cell by membrane vesicles. The specificity of Haemophilus transformation is due to a special 11 base pair sequence (5'-A GTG GGG TCA-3') that is repeated about 600 times in the Haemophilus genome. For DNA to be transported successfully across the membrane, it must have this nucleotide sequence.

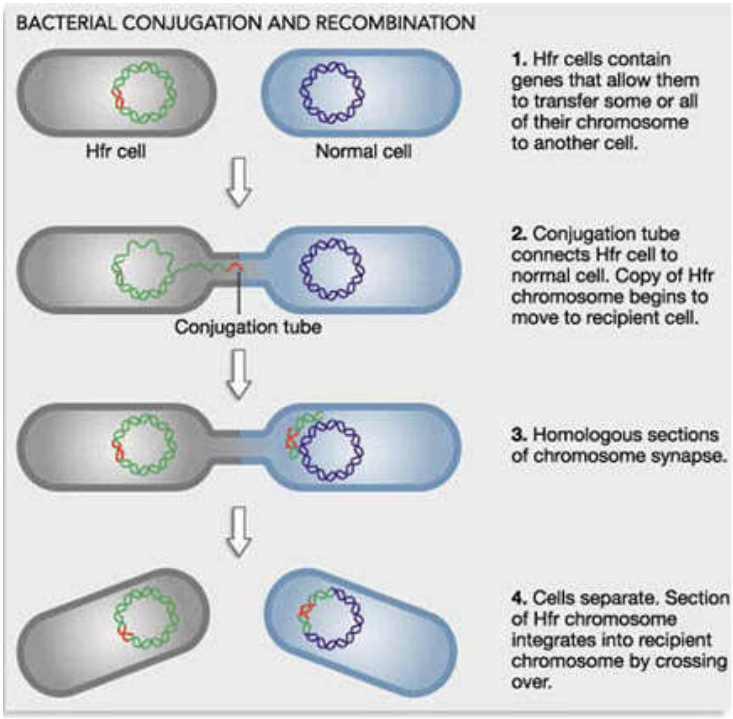
1.2.4.3 CONJUGATION

Escherichia coli is the bacterial species most widely used in genetic research, but it does not normally undergo transformation. Nevertheless, other processes have evolved in this species to generate recombinant genotypes. In 1946, Josha Lederberg and Edward L.Tatum established for the first time that E.Coli engages in a form of sexual reproduction involving a physical mating between sexually differentiated strains of bacteria. This mating process, called conjugation, accomplishes the transfer of genetic material from one strain to another indirectly, enabling bacteria of one strain to exchange genetic information with bacteria of another strain. During conjugation, chromosomal DNA may or may not be transferred to a recipient cell. Because genetic transfer is unidirectional and does not involve gamete formation or gamete fusion, conjugation is Para sexual process. Conjugation creates recombinant genotypes that have provided investigators the opportunity to analyse the E. Coli genome with some rigor.

a)The Discovery of Conjugation

The discovery of a sexual process in bacteria by Lederberg and Tatum grew out of an effort to study the genetic control of metabolic processes. They analyzed various nutritional mutants in bacteria and in 1946 published a paper in the British Journal Nature that began, "Analysis of mixed cultures of nutritional mutants have revealed the presence of new types which strongly suggest the occurrence of a sexual process in the bacterium Escherichia coli". They had mixed together two auxotrophic strains of E.Coli, requiring two different nutritional supplements in the medium. One of the auxotrophic strains (a) required methionine and biotin for growth (met^- bio^- thr^+ leu^+); a second strain (B) required threonine and leucine for growth (met^+ bio^+ thr^- leu^-). After plating the mixture of cells on a minimal medium that would not support the growth of either strain, they discovered prototrophic colonies that could grow. These results suggested that genetic recombination had occurred, but what was the mechanism ?

When Lederberg and Tatum mixed strain A (met^- bio^- thr^+ leu^+) cells with strain B cells (met^+ bio^+ thr^- leu^-) on a nutrient medium for several hours and then placed cells on a minimal medium, genetically stable, met^+ bio^+ thr^+ leu^+, prototrophs appeared.



Strains a and B alone did not yield prototrophic colonies. Considering the relatively high frequency of prototrophic colonies from the mixed strains, they concluded that the prototrophs could not have arisen by reverse mutation (- → +) in the auxotrophic strains because two mutant strains were some how mating and exchanging genetic material.

Lederberg and Tatum eliminated transformation as a possibility by showing the DNA extracted from either auxotrophic strain could not transform the other strain. Transformation does not normally occur in E.Coli. They did not prove that physical contact was required. Proof the cell-cell contact was required for gene exchange came in 1950 when Bernard Davis

developed the U-tube. When strains A & B were separated by a sintered glass filter, gene transfer could not take place

b)F^+ F^- Mating

Two years after Davis proved that cell-cell contact was required for conjugation. William Hayes discovered that gene transfer was unidirectional from a donor (F^+) strain to a recipient (F^-) strain. Hayes further discovered that in F^+ x F^- matings, genes on the main bacterial chromosome were rarely transferred but that F^- cells usually become F^+.

Donar F^+ cells carry a plasmid called the fertility or F factor, a circular extra chromosomal molecule containing about 94,000 bp's. Genes on the F factor are responsible for facilitating cell-cell contact & F-factor transfer from donor to recipient cell. Many F-factor genes direct the synthesis of sex pili (singular, pilus), filamentous appendages on the bacterial surface that attach the F^+ cell to an F^- cell.

The F factor and the main bacterial chromosome have insertion sequences (IS) that enable the F factor to insert into the chromosome by homologous recombination. Insertion sequences are short, transposable DNA sequences that contain a gene coding for an enzyme called transposase which facilitates the movement of the insertion sequence around the genome. Because the F factor can exist independently of the bacterial chromosome or be integrated into it, it is a special type of plasmid called an episome.

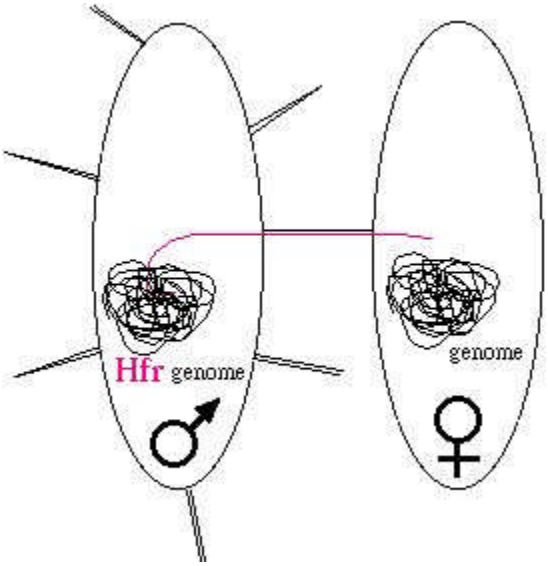
During F^+ x F^- conjugation, the F factor replicates by the rolling circle mechanism, and a single-stranded copy of it moves into the F^- cells. It is not clear whether the DNA passes through the hollow sex pilus or through another conjugation channel. The stugle-stranded DNA entering the recipient becomes a

double stranded molecule. Because genes on the main bacterial chromosome are rarely transferred into the recipient with the independent F factor, the recombination frequency is low. It can only occur if the F factor integrates into the chromosome.

c)Hfr Conjugation

Some F^+ strains transfer bacterial genes from the main chromosome into recipient cells with a high frequently, but they do not usually transfer the F factor. This is quite different from the F^+ cell we have just considered. In these strains, the F factor does not exist independently of the main bacterial chromosome; rathee, it is integrated into it. The F factor can integrate into the bacterial chromosome at several location by recombination between homologous insertion sequences present on both are F factor and the chromosome. The integrated plasmid directs the synthesis of sex pili, undergoes, rolling circle replication, and transfers genetic material of an F^- cell. This type of donor cell is called Hfr cell (high frequency recombination) because of the high frequency of chromosomal gene transfer into F^-cells.

DNA transfer is initiated at a point on the integrated F-factor. Rolling circle replication moves the donor strand from the main chromosome into the recipient cell where it becomes double-stranded. Because only a small part of the F-factor is transferred to the recipient,



The recipient remains F^- unless the entire bacterial chromosome is transferred. It takes about 100 minutes for the entire bacterial chromosomes to be transferred, but the connection-between conjugating cells is usually severed before the process is finished. Thus the recipient usually remains F^-.

Hfr strains vary in the chromosomal locations of F factor integration and in the orientation of the F factor, which determines whether the direction of gene transfer is clockwise or counterclock wise around the circular chromosome. When the donor DNA enter the F^- cell, it may be degraded or it may recombine with the recipient chromosome. Hfr conjugation is the most efficient natural mechanism of gene transfer in bacteria.

d)F Conjugation (or) Sexduction

The integrated F factor in an Hfr cell may exit the bacterial chromosome by reversing the steps that resulted in its integration. Sometimes there is an error in the excision of the F

factor from the chromosome. This error results in the formation of a F^1 factor – one containing a segment of the chromosome. The mating of an F^+ times F^- mating, with one important difference; bacterial genes incorporated in the F plasmid are transferred with very high frequency to recipient cells. These genes do not have to be integrated into the recipient cells chromosome to be expressed. The recipient acquires the F factor and is partially diploid for the genes that it carries. The transfer of bacterial genes by F factors is called sexduction. Sexduction results in the rapid spread of bacterial genes from the main chromosome through a population.

Sexduction is a valuable tool for nierobial geneticists. Because of the cell's partially alipolid condition, it allows investigators to answer questions about dominance and reeessiveness for alleles carried by the F factor. Furthermore, if two genes are incorporated together into an F factor, investigators conclude that the two genes are close together on the bacterial chromosome.

1.2.4.4 TRANSDUCTION

Transduction, the third mechanism of gene transfer in bacteria, differs significantly from transformation and conjugation in that it is mediated by bacterial viruses. Bacterial genes are incorporated into a phage capsid because of errors that occur during the virus life cycle. The virus then injects these genes into another bacterium, transferring genetic material from one bacterium, transferring genetic material from one bacterium to another. Two different types of different transduction have been identified.

a) Generalized transduction

In which the phage can transfer any sequent of the bacterial genome to another bacterium, &

b) Specialized transduction

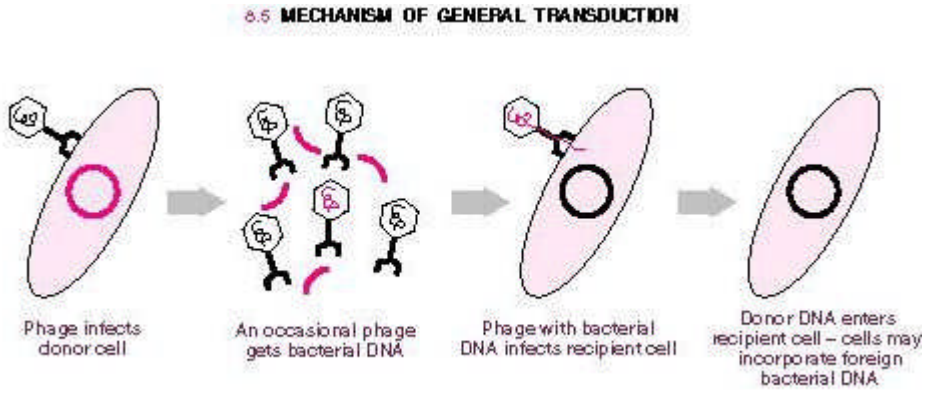
In which the only restricted segments of the bacterial genome are transferred. Transduction is a common mechanism for gene exchange and recombination in bacteria and, like transformation and conjugation, has been extremely important in bacterial gene mapping.

c)The Discovery of Transduction

Generalized transduction was discovered in 1952 by Joshna Lederberg and his student, Norton Linder. They were attempting to show that conjugation, which Lederberg and Tatum discovered in E.Coli in 1946, could occur in other bacterial species. While studying Salmonella typhimurium, they found that when two multiple auxotrophic strains were penentrated together (Phe Trp Tyr and Met His) on an amino acid free minimal medium, protrotrophs appear with a frequency of about 1 per 100,000 cells. Because this frequency was too high to be accounted for by reverse mutation, they concluded that bacteria were undergoing a process of genetic exchange to test for transformation and conjugation, they carried out a U-tube experiment. The two strains were placed in the two arms of the U-tube, separated by a sintered glass filter, in a medium containing DNase prototrophs still appeared but in only one of U-tube arms. These results could not be explained by transformation because DNase destroys free DNA. Conjugation could not explain the results because the filter presented cell-cell contact. Lederberg and Lindee had discovered a new mechanism of bacterial gene transfer.

Further analysis of this gene exchange process in Salmonella revealed that one of the bacterial strains harboured a virus called P_{22}. When cells carrying these P_{22} viruses lysed, transduring particles were released. These particles passed through the filter and infected cells in the other arm of the U-

tube, carrying with them genes from the virus infected strain. These genes were subsequently incorporated into the genomes of the newly infected cells.



Conjugation

Not possible because cell to cell contact is not there due to the presence of filter.

Transduction

Due to the presence of a virus infected strain, after lysis of cells, the phage particle (transducing partice) released into the medium, can pass through the filter and infect / transfer the genane of the host bacterium into the other bacterium.

Transformation

Not possible because there is not possibility for the naked DNA to be in the medium and if it is present, it will be degraded by the D Nase enzyme.

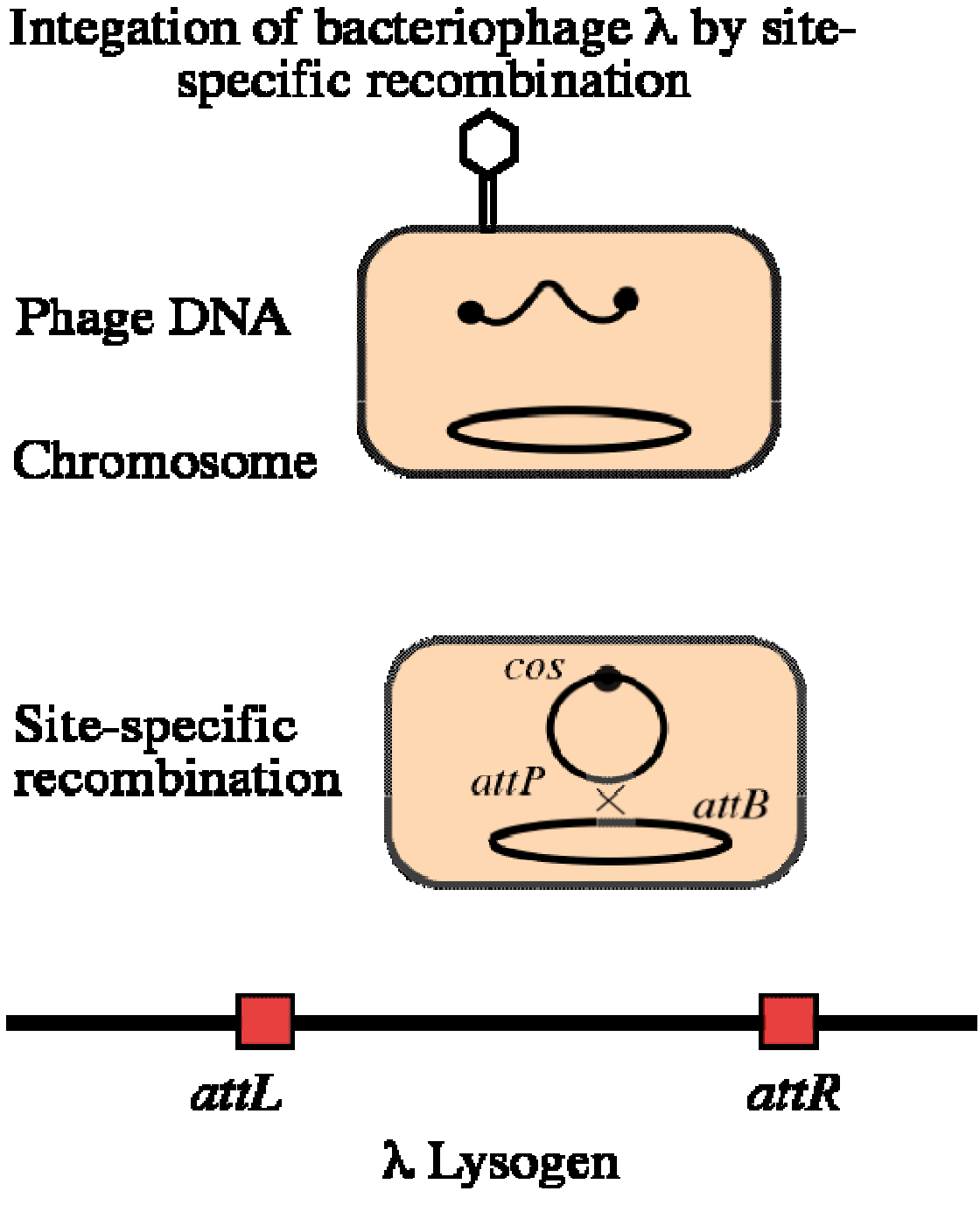
So, there is a possibility of transduction, but not the other 2 processes.

Transduction is of 2 types.

1. Generalized
2. Specialized / Restricted

a) Generalized Transduction

In generalized transduction, a phage may carry any bacterial gene from cell to another. Towards the end of the phage lytic cycle, when viral DNA is being packaged inside viral protein veoats, fragments of the bacterial chromosome are mistakenly packaged into the phage head. This packing error produces a phage particle that carries bacterial DNA and no phage DNA. The size of the bacterial DNA fragment packaged into the phage varies, but it is not unusual for it to consist of 1 or 2 percent of the bacterial genome. Thus, for a bacterium with 3000 genes, 30 to 60 genes may be packaged into a tansducting virus particle.



A phage particle that carries donor bacterial DNA is released upon lysis of the cell. The phage protein coat functions as a vehicle for the transfer of bacterial DNA from one cell to another. The transducing particle attaches to a recipient cell and injects donor bacterial DNA into that cell. This transducing

particle, which carries no phage DNA, is incapable of going through a life cycle. Once the DNA is injected, it must be incorporated into the host genome in order to be preserved.

The donor DNA is not always incorporated into the recipient's genome. Occasionally, the fragment of donor DNA survives and is expressed in the recipient's cytoplasm. This cell is partially diploid for the introduced genes. The donor DNA is not replicated because it does not have the proper recognition site for DNA polymerase. Thus, when the bacterium divides, only one of the two daughter cells receives the donor fragment and expresses the donor genotype. The other cell, having lost the donor fragment, reverts back to its original phenotype. This phenomenon is called abortive transduction and is the result of partially diploid bacteria containing non integrated, transduced DNA.

b) Specialized transduction

In specialized transduction, the transducting particle carries only specific portions of the bacterial genome. In order to understand the nature of specialized transduction, it is first necessary to understand the life cycle of one of the most extensively studied and fascinating specialized transducing viruses, lambda, Lambda (λ) , unlike the T-phages, is a temperate phage because it has two options when it infects a cell ., it can proceed through the lytic cycle, like the T phage, and destroy the host cell; or it can enter into a lysogenic relationship with the host, a relationship in which the chromosome circularizes and hen integrates into the host chromosome by recombination at a specific site. In the integrated state, the λ genome is genetically in active, except for a single gene, encoding repressor, that maintains all other λ genes in a transcriptionally inactive state. However, if the repression breaks down, the λ chromosome can excise from the bacterial chromosome and commence a lytic infection. Thus, an integrated λ is still able to cause cell lysis at some time in the future. λ DNA is replicated along with the host chromosome during the cell's replication cycle.

The integrated λ genome, called a prophage, can be inducted by various means to exist the host chromosome. Excision of the λ prophage occurs when the λ DNA forms a loop and a site-specific recombination event mediated by λ-encoded enzymes releases a circular phage chromosome from the larger bacterial chromosome. Sometimes this recombination even occurs aberrantly, releasing a phage genume that has had some of its own genetic material replaced by bacterial genetic material. This process is similar to the formation of an F factor during conjugation. When it excises improperly, it can pick up only those bacterial genes that bracket its insertion site in the bacterial chromosome. A λ chromosome is packaged into a phage coat, it forms a specialized transducing particle. Unlike the generalized transducing particle discussed earlier (which carries only bacterial DNA), a specialized transducing particle carries a hybrid chromosome containing both bacterial and viral genetic material. Following lysis from host cell, the specialized transducing particle injects its chromosome into another bacterium, thus transmitting bacterial genes from one cell to another. However, the defective chromosome of this particle is unable to direct the synthesis of progeny viruses. Instead, the bacterial genes carried by the transducing particle recombine with the genes of the newly infected cell and become incorporated into the recipient cell's genome.

1.2.4.5 RECOMBINATION

The rearrangement of genetic information in and among DNA molecule, results in reshuffling of genes. This process can produce numerous new combinations of genetic recombination. A variety of process occur in making recombinants. These processes are called recombination.

- Recombination of genetic information from two different cells produces progeny that contain genetic information derived from two potentially different genomes.

- Recombination results in an exchange of allelic forms of genes that can produce new combinations of alleles.

- In eukaryotic cell, genetic exchange during sexual reproduction affords a mechanism for gene re assortment within the -- & maintenance of genetic heterogenicity.

- In bacteria, various genetic exchange processes are involved that lead to the recombination of genetic information.

Genetic recombination events fall into 3 general classes,

1. Homologous genetic recombination – Involves genetic exchange between any two DNA molecules, (or) segments of the same molecule, that share an extended region with homologous sequence. DNA's have same (or) nearly same – sequences.

2. Site-specific recombination – The genetic exchanges occur only at defined DNA sequences.

3. DNA transposition – It is a distinct process, usually involves a short segment of DNA with the remarkable capacity to move from one location in a chromosome to another.

a) **Homologous Recombination**

In diploid eukaryotes, his occurs commonly during the meiosis when the duplicated chromosomes line up in parallel in pachytene of prophase I of Meiosis I. Bacteria also perform this recombination such as in conjugation.

This process of recombination starts with the alignment of homologous DNA segments by an unknown mechanism. The Rec BED enzyme binds to one linear DNA & use ATP as energy to travel along the DNA duplex, unwinding the DNA ahead & rewinding it behind. Rewinding is slower than unwinding so that a bubble are cut when the enzyme encounters a sequence called "chi" (GCTGG TGG). The single strand DNA carrying 5'

end of the nicks becomes coated with Rec A protein to form RecA-SSDNA filaments. This filament is aligned with a second duplex DNA & search for the corresponding sequences (invasion), after which the nicks on both the strands are sealed & four branched holiday structure is formed. This structure is dynamic & cross over point can move a considerable distance in either direction (branch migration). The holiday intermediate can be resolved into 2 ways. If two invading structures are cut, the resulting recombinants are similar to the original ones. If the non invading structures are cut, products have one half from parental duplex & the other half from the 2^{nd} duplex.

b) Site Specific Recombination

This involves non homologous but specific pieces of DNA & is mediated by proteins that recognize specific DNA sequences. It does not require Rec A & SSDNA. Bacteriaphage "λ" has the ability to insert its genome into a specific site on E.Coli chromosome. The attachment site for λDNA in E. Coli DNA, called act B is located between "gal" & "bio" genes. The base sequence of att B is symbolized by B-O-B' (B-Bacteria). The specific attachment site on λ phage, called att p is located next to the genes int 8 x is. The base sequence of att p is symbolized by P-O-P' (p-for phage). "O" – which is common, in both P-O-P' & B-O-B', represents the core sequence (15 bp identical sequence) through which they recombine. Integration of λDNA into E. Coli is mediated integrase (λ-phage coded) & integration host factor (IHF) froming

a prophage. The prophage is stable in the absence of excisionase, an enzyme coded by λ DNA will be helpful in the excision of λDNA.

c) Recombination by Transposition

Transpositions are segments of DNA, found in virtually all cells, that move or hop from one place on a chromosome to another. No homology is usually required for the movement, called transposition. There are 2 general pathways for transposition in bacteria.

In direct or simple transposition, cuts are made on either side of the transposon to excise it, & the transposon moves to a new site leaving the double stranded break in the DNA from which it came. At the target site, a staggered cut is made & some replication is needed to duplicate the target site sequence.

In the replicative transposition, the transposon is replicated so that a copy is left behind in its original donor location. An intermediate with 2 complete transposons (cointegrate), in which the donor region is covabntly linked to DNA at the target site. The intermediate will be resolved by the help of an enzyme called resolvase.

1.2.4.6 Model questions

1) Discuss in detail the process of conjugation in bacteria

2) Give Griffith experiment to understand bacterial transformation

1.2.4.7 Reference books

Shlegel, Microbiology, Cambridge University press

Atlas, Principles of Microbiology, McGraw-Hill Science Publishers

Jogdand, S.N. (2003) Gene Biotechnology, Himalayan Publishing House, Mumbai

Lessons 1.3.1

PURE CULTURE TECHNIQUES

Objective

1.3.1.1Introduction

1.3.1.2 Establishment of pure cultures

1.3.1.3 Maintainence and preservation of pure cultures

1.3.1.4 Culture collections

1.3.1.5 Culture characterstics

1.3.1.6 Colony characterstics

1.3.1.7 Optical features

1.3.1.8 Characterstics broth cultures

1.3.1.9 Enumeration of Bacteria

1.3.1.10 modelQuestions

1.3.1.11 Reference books

Objective

Isolation of pure cultures from a mixed culture is very difficult.So that different techniques have been evolved for getting pure culture which were described in this chapter

1.3.1.1 Introduction

The ability to examine and study the characteristics of micro-organisms including obtaining organisms for micro-scopic crisualization depends on growing micro-organisms in the laboratory. Pure cultures contain only one kind of organisms and free from all other types. Isolation of pure cultures involves separating samples of micro-organisms into individual cells that are than allowed to reproduce and form clones of single micro-organisms. Each clone represents a pure culture.

Isolation is achieved by the physical seperation of the micro-organism, but the success of an isolation method also depends on the ability to maintain the viability and growth of a pure culture of the micro-organism. Eare (f) must be taken to ensure that the micro-organisms are not killed during the isolation procedure, which can easily occur by exposing the micro-organisms to conditions they cannot tolerate, such as air in the case of obligatly anaerobic micro-organisms that are sensitive to oxygen. The success of an isolation depends on the ability to grow the micro-organisms that is to define the growth medium and to establish the appropriate incubation conditions that permit its growth. Several different methods are used for the establishment pure cultures of micro organisms.

1.3.1.2 Establishing a pure culture

1)Sterilization

There are carious ways of eliminating micro-organisms in order to establish a pure microbial culture from the liquids containers and instruments used in pure culture procedures. These include exposure to elwated temperatures, toxic chemicals or radiation to kill micro-organism and filtration to remove micro-organism from liquid.

Removal of microorganisms by filtration is achieved by passage of solution through a filter with 0.21 to 0.45 um diameter pores. Most bacteria are traped on the filter but irruses and some very small bacteria may pass through it.

Heat sterilization at a temperature that all microorganisms including their endospores is also used to eliminate unwanted microorganisms. Dry heat sterilization requires high temperature and long exposure periods to kill all of the micro-organisms in a sample. Exposure in an over for two hours 170^{o}C (320^{o}F) is generally used for the dry heat sterilization of glassware and other laboratory items.

An autoclave is used for sterilization of culture media by steam under pressure. It is an instrument that exposses substances to steam at elevated temperatures. Steam has a high pevetrating power and a much higher heat capacity than dry heat. Thus it is very effective at killing micro-organisms. It is operated for 15 minutes at 121^{o}C using a pressure of 15lb/in^{2}, inorder to sterilize microbiological culture media.

2)Aseptic technique

Aseptic technique involves avoiding ay contact of the pure culture sterile medium and sterile surfaces of the growth vessel with contaminating micro-organisms. The steps for transferring a culture from one vessel to another by aseptic technique involves

i. Flame the inoculating or transfer loop.

ii. Open and flame the mouths of the culture tubes.

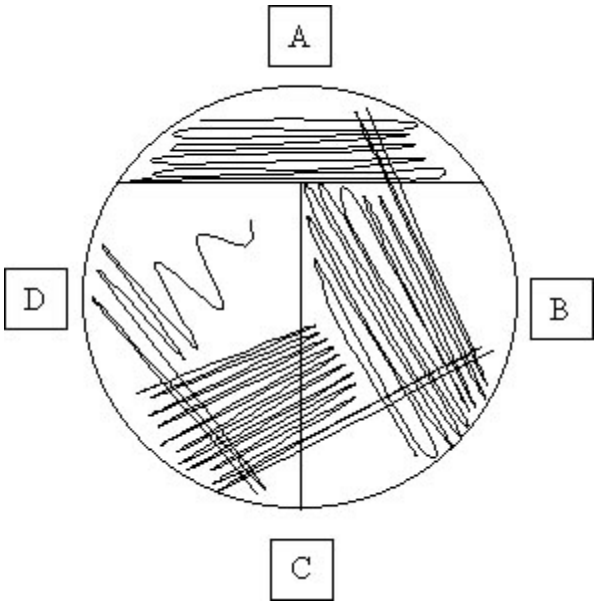
iii. Pick up some of the culture growth and transfer it to fresh medium.

iv. Flame the mouths of the culture vessels and reseal them and

v. Relame the inoculating loop.

3)Streak plate

In this technique for isolating pure cultures of bacteria, a loopful of bacterial cells is streaked across the surface of a sterile, solidified, agar nutrient medium. Contained in a petri plate.

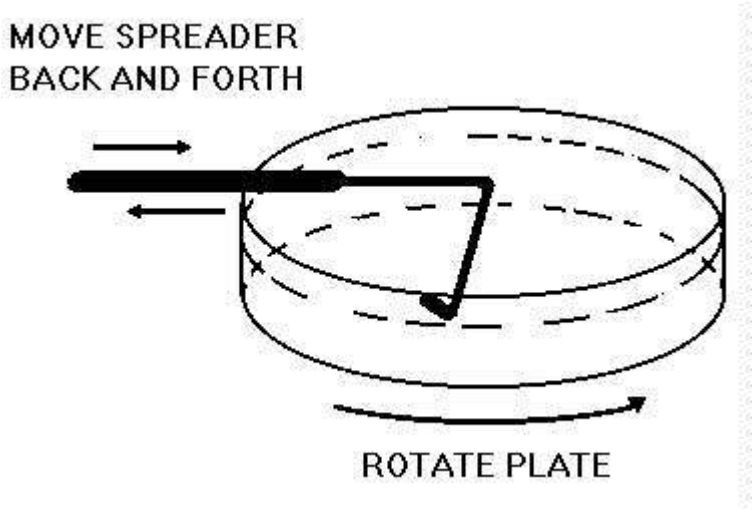


Many different streaking patterns can be used to separate individual bacterial cells on the agar surface. The plates are then incubated under favourable conditions to permit the growth of the bacteria. The key principle of this method is that by streaking, a dilution gradient (numbers of cells decreased as they move across the agar and away from the point inoculation) is established across the face of the plate as bacterial cells are deposited on the agar surface. Because of this dilution gradient, confluent growth (from the latia for flow together) occurs on part of the plate were the bacterial cells are not sufficiently separated in other regions of the plate were few enough bacteria are deposited to permit space between individual cells, separate macroscopic colonies develop that can easily be seen with naked eye.

Each well-isolated colony is assumed to arise from a single bacterium and therefore to represent a clone of a pure culture. If this premise is not sustained, for example because two bacterial cells are deposited at the same location on the plate, the methods fails to produce a pure culture. Assuming that each colony comes from a single cell samples of the isolated colonies can be picked up using a sterile inoculating loop and restreaked on to a fresh medium to ensure a purity. A new sample colony is then picked up and transferred to an agar slant or other suitable medium for maintenance of the pure culture.

4)Spread plate

In this method a drop of liquid containing a suspension of micro-organisms is placed on center of an agar plate and spread over the surface of the agar, using a sterile hocky stick shaped glass rod.



The glass rod is normally sterilized by being dipped in alcohol and ignited to burn off the alcohol. When the suspension is

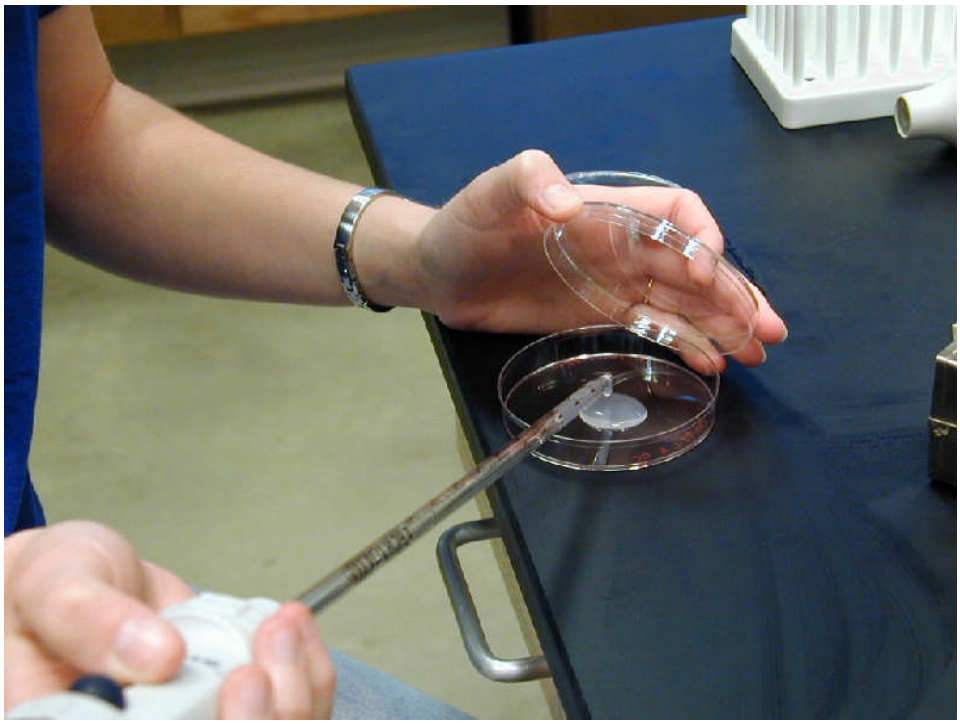
spread over the plate individual micro-organisms are separated from others in the suspension and are deposited at discrete locations. To accomplish this seperation, it is often necessary to dilute the suspension before application to the agar plate to prevent over crowiding and the formation fo confluent rather than the descried development of isolated colonies. After incubation isolated colonies are picked up and streaked on to a fresh medium to ensure purity.

5)Pour plate

In this technique suspensions of micro-organisms are added to tubes containing melted agar cooled to approximately 42-45^{o}C.

The bacteria and agar medium are mixed well and the suspensions are poured into sterile petridishes using aseptic techniques. The agar is allowed to solidified, trapping the bacteria at separate discrete positions with in the medium. While the medium holds bacteria in place, it is still soft enough to permit the growth of the formation of discrete isolated colonies with in the gel and on the surface of the agar.

As with the other isolation methods, individual colonies are them pecked up and streaked on to another plate for purification. In addition to its use in isolating pure cultures the pour plate technique is used for quantification of numbers of viable bacteria. The facts that agar solidifieds below 42^{o}C and that many bacteria survive at these temperatures ensure the success of this isolation technique. Because in some cases such as in marine samples significant members of bacteria are killed under these condition this method cannot always be used.



1.3.1.3 Maintenance & Preservation of Pure Cultures

Most microbiology laboratories maintain a large collection of strains, frequently referred to as a stock-culture collection. These organisms are needed for laboratory classes and research work as test for particular procedures, or as reference strains for foxonomic studies. Most major biological companies maintain large culture collections. The strains are used for screening of new, potentially effective chemotherapeutic agents; as assay tools for vitamins and amino acids, as agents for the production of vaccines; antisera; antitumor agent, enzymes, and organic chemicals, and as reference cultures that are cited in company patents. For these to other purposes it is extremely important to have properly identified and cataloged strains of bacteria available. Consequently a considerable amount of research has been performed to develop methods whereby bacterial strains can be preserved & stored until they are needed several different methods have been developed, since not all bacteria respond in a similar manner to a specific meathod. Moreover, there are various practical considerations such as the amount of labour

involved and the amount of storage space required. However, all the methods which we will now describe have the same objective; t maintain strains alive & uncontaminated & to prevent any change in their characteristics.

1)Methods of maintenance and preservation

Strains can be maintained by periodically preparing a fresh stock culture from the previous stock culture. The culture medium, the storage temperature and the time interval at which the transfers are made vary which the species & must be ascertained before hand.

2)Periodic transfer to fresh media

The temperature and the type of medium chosen should support a slow rather than a rapid rate of growth so that the time interval between transfers can be as long as possible. Many of the more common heterophs remain viable for several weeks or months on a medium like nutrient agar. The transfer method has the disadvantage of failing to prevent changes in the characteristics of a strain due to the development of variants and mutants.

3)Presentation by overlaying cultures with mineral oil

Many bacteria can be successfully preserved by covering the growth on an agar slant with sterile mineral oil. The oil must cover the slant completely; to ensure this, the oil should be about ½ in above the tip of the slanted surface. Maintenance of viability under this treatment varies with the species (1 month to 2 years). This method of maintenance has the unique advantage that you can remove some of the growth under the oil with a transfer needle, inoculate a fresh medium, and still preserve the original culture. The simplicity of the method makes it attractive, but changes in the characteristics of a strain can still occur.

4)Preservation by lyophilization (Freeze-drying)

Most bacteria die if cultures are allowed to become dry, although spare-and cyst-formers can remain viable for many years. However, freeze-drying can satisfactorily preserve many kinds of bacteria that would be killed by ordinary drying. In this process a dense cue suspension is placed in small vials and frozed at –60 to – 78^{o}C. The vials are then connected to a high-vacuum line.

The ice present in the frozen suspension sublimes under the vacuum, i.e., evaporates with out first going through a liquid water phase. This results in dehydration of the bacteria with a minimum of damage to delicate cul structures. The vails are then sealed off under a vaccum & stored in a refrigerator one arrangement of equipment employed to lyophilize cultures is shown in Fig. May species of bacteria preserved by this method have remained viable and inchanged in their characteristics for more than 30 years. Only minimal storage space is required; hundreds of lyophilized cultures can be stored in a small area. Furthermore, the small vials can be sent conveniently through the mail to other microbiology laboratories when packaged in special sealed mailing containers. Lyophilized cultures are revived by opening the vials, adding liquid medium, and transferring the rehydrated culture to a suitable growth medium.

5)Storage at low temperatures

The ready availability of liquid nitrogen has provided the microbiologist with another very useful means for long-term preservation of cultures. In this procedure cells are prepared as a dense suspension in a medium containing a cryoprotective agent such as glycerol or dimethyl sulfoxide (DMSO), which prevents cell damage due to ice crystal formation during the subsequent steps. The cell suspension is sealed in to small ampoules or vials and then frozen at a controlled rate to –150^{o}C. The ampoules or vials are then stored in a liquid nitrogen refrigerator either by immersion in the liquid nitrogen (-196^{o}C) or by storage in the gas phase above the liquid nitrogen (-150^{o}C). The liquid nitrogen meathod has been successful with many species that cannot be preserved by lyophilization, and most species can remain viable under these conditions for 10-30 years

as more with out undergoing change in their characteristics. However, the method is relatively expensive, since the liquid nitrogen in the refrigerators method is relatively expensive, since the liquid nitrogen in the refrigerators must be replenished at regular intervals to replace the loss due to evaporation.

1.3.1.4 Culture collections

When microbiologists first began to isolate pure cultures each microbiologist kept a personal collection of those strains having special interest, subcultures of some strains were often sent to other microbiologists; other subculturies were of wome strains were received and added to the scientist's own collection. Certain strains had taxanomic importance because they farmed the basis far descriptions of species and genera others had special properties useful for various purposes. However, many important strains became last or were inadequately maintained. They, it became imperative to establish large central collections whose main purpose would be the acquisition, preservation, and distribution of authentic cultures of living microorganisms.

Many countries have at least one central collection. As example, in France a collection of bacteria is maintained at the institute Pasteur in paris, in England the National collection of type cultures is in London, the Federal Republic of Germany maintains the Deutsche sammlung Von Midroorganismen in Darmstadt, and Japan maintains large collection at the institute for fermentation is Osaka. Many other such collections exist.

In the United States the major collection is the American Type Culture Collection (ATCC), located in Rockville, Maryland. In 1980 the collection included the following numbers of strains: bacteria, 11,500; bacterophages , 300; fungi and fungal viruses, 13,700, protozoa, 720; algae 130; animal-cell cultures 500; animal viruses rickutsiae, and chlamydiae, 1,135; and plant viruses, 220. More than 1 million ampoules of lyopnilized or frozen living strains are inventoried and stored at the ATCC. Other large collections in the United States are more specialized in scope. For example, the Northern Utilization Research and Development Division. USDA, at Peoria, Illinois – maintains a

collection of yeastes, molds, and bacteria especially for use in fermentations. The quarter-master Research and Development Center, U.A Army, Natick, Massachuseits, maintains a collection of microbial strains that are associated with dterioration processes. A number of smaller collections of a specialized nature also exist, such as the collection of anaerobic bacteria maintained by the department of Anaerobic Microbiology, Virginia Polytechnic Institute and State University, Blacksburg.

One of the major functions of a large national collection is the preservation of type strains. As discussed in chap. 3, the type strain of a species has great taxonomic importance because it is the `name-bearer' strain, or permanent example of the species. Microbiologists who propose a new species are expected to deposit the type strain with one or more national collections so that it can be preserved and so that sub-cultures can be distributed to other workers for study and comparison with other microorganisms.

1.3.1.5 Cultural characteristics

One of the major features of a bacterial strain is it's apperance following growth on various media. Such commonplace characteristics as the abundance of the growth, the size of the colonies and the color (or chromogenesis) of the colonies provide useful clues for identification.

To determine the growth characteristics of a bacterial strain, it is customary to observe the features of colonies and broth cultures. Inoculation of agar plates to obtain isolated colonies has already been described. Tubes of broth can be inoculated with transfer needle or the loop, generally the loop is used when the inoculum is liquid.

After inoculation of the medium and subsequent incubation, the cultural characteristics can be determined. The main features can be summarized as fallows.

1.3.1.6 Colony characteristics

1)Size

Colonies range in size from extremely small (pinpoint) measuring only a fraction of a millimeter in diameter, to large colonies measuring 5 to 10 nm in diameater. Although the colonies of a given species have a characteristic diameter. One must be aware of certain factors affecting colony diameter. For instance, only well-separated colonies should be measured, since such colonies tend to have a larger diameter than those which are crowded together (for example, sec) This is because widely separated colonies are subject to less competition for nutrients and less inhibition by toxic products of metabolism. Moreover, young colonies are smaller than older colonies, therefore the time at which mea swrements are mode must be stated. There is generally an upper limit to the final size of the colonies of a given species; i.e., a point is reached where further incubation no longer results in a corresponding increase in size. However: some bacteria (e.g. certain species of proteins and bacillus) can spread across the entire agar surface: and the colony size is limited only by the dimensions of the petridish.

2)Margin of edge

The periphery of bacterial colonies may take one of several different patterns, depending on the species. It may be evenly circular like the edge of a droplet or it may show irregularities such as rounded projections, notches and fnreadllike or rootlike projections.

Surface texture

Depending on the species, the colony surface may be smooth, rough, cor, mucoid certain species have colonies possessing a highly wrinkled surface.

For a pure culture, all the colonies on the plate should have a similar type of surface; however; you should bear in mind that same pure cultures may exhibit surface variation. One of the commonest variations is known as the S→ R variations. This

is due to (the commonest variations is known as the) presence of mutant cells that give rise to some rough ® colonies in a population that otherwise produces smooth (S) colonies. Some R mutants produce rough colonies because they lack the ability to make capsules; or if the species is Gram – negative; they no-longer be able to form a antigens.

For several species of pathogenic bacteria, the surface texture of colonies may bear a relation to virulence. For instance, S colonies of S. pneumomiae or of salmonella species are usually virulent, wheras R colonies are not on the other hand for strains of mycobacterium tuberculosis a rough surface showing serpentine cords is usually a good indicator of virulence.

3)Elevation

Depending on the species, colonies be thin to thick, and the surface may be flat or it may exhibit varying degrees of convexity.

4)Consistency

This can be determined by touching a transfer needle to the colony. Some bacterial species form colonies having a butyrous or butterlike consistency. Others may form colonies that are viscous, stringy, or rubbery, in the latter type the whale colony, rather than `sust a portion of it, may come off the agar surface with the transfer needle. Still other species may form dry, brittle, or powdery colonies that break up when touched with the needle.

Optical features

Colonies may be opaque, translucent, or oparlescent.

1)Chromogenesis or pigmentation

Some bacterial species produce and retain water-insoluble pigments intracellularly, thus causing the colonies to become colored some species which from pigmented colonies are:

1. Flectobacillus major - Pink
2. Serratia marcescens - Red
3. chromobacterium violaceum – Violet
4. Staphylococcus awrecs – Gold
5. Micrococcus luteus – Yellow
6. Oerxia gummosa – Brown
7. Bacteroides mechainogeniccy – Black

Some colonies produce pigments that are water-soluble, these diffuse in to the surrounding agar and stain it. For instance, pseudomonas aerouginosa forms a blue water-soluble pigment called pyocyanin, some pigments are only sparingly water soluble (pigment called P) and may precipitate in the medium. For example, psendomonas chlororaphis forms a pigment called chlorophin which accumulates in the form of green crystals around the colonies.

Certain water-soluble pigments are fluorescent, i.e., that agar medium around the colonies glows white or blue-green when exposed to ultraviolet light. For example, P.aeruginosa produce not only the non fluorescent pigment pyocyanin but also a fluorescent pigment, pyoverdin.

For a bacterial stain to exhibit its characterstic pigmentation, special media, incubation temperatures, or other conditions may be required. For instance, mycobacterium kansasii forms a characteristic yellow pigment (β-carotence) only when the colonies are exposed to light.

Several types of bacterial colonies are shown to

1.3.1.8 Characteristics of broth cultures

1. Amount of growth, scanty, moderate, or abundant.

2. Distribution and type of growth. The growth may be uniformly distributed throughout the medium. Alternatively , it may be confined to the surface of the broth as a scum or film or it may accumurate as a sediment which may be granular or viscons.

The scheme for interpreting the apperance of bacterial growth has been described in some detail to emphasize the fact that may differences in cultural charactergtics do occur among bacteria. With experience, familiarity with such characteristics becomes very helpful as a guide for the recognition of major groups of bacteria. Too often students pay little attenction to these features of bacterial growth and thus deprive themselves of much useful information in the laboratory study of cultures.

To further emphasize the importance of cultural characteristics, suppose that we have prepared plate and broth cultures of an unidentified strain designated as strain 24. The colonies are irregular and raised and appear dry with a roughened, granular surface. When we touch a colony with a trasfer needle it proves to be brittle and when a protion of the colony is removed it will not emulsify easily when spread in a drop of water. Growth in broth cultures occurs mainly in the from of a heavy surface pellicle, and the (cultural) medium below the pellicle is only slightly turbid. Familiarity with the cultural appearance of bacteria would suggest that strain 24 migh be an acid-fact bacterium (Mycrobacterium). Additional tests must be performed to varity this possibility, but the cultural characteristics have provided a clue to the type of organism we are working with.

1.3.1.9 Enumeration of bacteria

To assess the rate of microbial reproduction, it is necessary to determine the numbers of microorganisms present. There are various methods that can be employed for counting bacteria. Some of these methods count only live bacteria that are capable of reproducing in laboratory culture media. Others count all microorganisms, alive and dead.

1)Viable count procedures

a)Viable plate count

The viable plate count method is a common procedure for the enumeration of living bacteria (Fig. 2.34). Srial dilutions of a suspension of bacteria are plated onto a suitable solid growth medium. In streak or spread plate techniques, serial dilutions of the suspension are spread over the surfaces of solid agar plates, hence their general name of "spread surface techniques". In the pour plate technique, the serial dilutions are mixed with melted agar in separate tubes and then poured into culture plates where the agar solidifies. The plates are then incubated so that the bacteria can reproduce.

Bacterial reproduction on a solid medium results in the formation of a macroscopic colony visible to the naked eye. The formation of visible colonies generally takes 16 to 24 hours. It is assumed that each colony arises from an individual bacterial cell. Therefore by counting the number of colonies that develop, colony-forming units (CFUs), and by taking into account the dilution factors, the concentration of bacteria (number of bacteria /ml) in the original sample can be determined. Countable plates are those having between 30 and 300 colonies. Fewer than 30 colonies are not acceptable for statistical reasons, and more than 300 colonies on a plate are likely to produce colonies too close to each other to be distinguished as individual CFUs.

A limitation of the viable plate count procedure in enumerating bacteria from natural environments is its selectivity. There is no set of incubation conditions and medium composition that permits the growth of all bacterial types. The nature of the growth medium and the incubation conditions determine which bacteria can grow and thus be counted. Viable counting measures only cells that are capable of growth on the given plating medium under the set of incubation conditions that

are used. Sometimes bacterial cells are viable but non-culturable in the medium and incubation conditioning chosen by the experimenter.

b)Most probable number (MPN) procedures

Another approach to viable bacterial enumeration, determination of the most probable number (MPN), is a statistical method based on probability theory. In an MPN enumeration procedure, multiple serial dilutions are performed to reach a point of extinction, that is, a dilution level at which not even a single cell is deposited into one or more of the multiple tubes at that dilution level. A criterion, such as the development of cloudiness or turbidity in a liquid growth medium is established for indicating whether a particular dilution tube contains bacteria. The pattern of positive and negative test results is then used to estimate the concentration of bacteria in the orginal sample, that is, the MPN of bacteria, by comparing the observed pattern of results with a table of statistical probabilities for obtaining those results.

2)Direct count procedures

Bacteria can be enumerated by direct counting procedures, that is, counting without the need to first grow the cells in culture. These procedures generally count all bacterial cells whether they are viable or not.

In one type of direct counting procedure, dilutions of samples are observed under a microscope and the number of bacterial cells in a given volume of sample is counted and used to calculate the concentration of bacteria in the original sample. Special counting chambers such as a hemocytometer or Petroff-Hausser chamber are sometimes used (Fig.2.36). These chambers are ruled with squares of known area and are constructed so that a film of liquid of known depth can be introduced between the slide and the cover slip. Consequently, the volume of liquid overlying each square is known. To help visualize bacterial cells, it is often desirable to stain the cells.

Alternatively, a known volume of a sample containing a suspension of bacteria is passed through a filter, for example, a bacteriological filter with a 0.2 um pore size. The bacteria are stained on this filter and counted under a microscope. Fluorescent dyes and frequently used to stain bacteria in direct counting procedures. However, such dyes stain all of the cells, making it impossible to differentiate living bacteria from dead bacteria. The difficulty in establishing the metabolic status o f the observed bacteria, that is, whether the cells are living or dead, is a major limitation of this procedure.

Some procedures can be used with direct microscopic observations to distinguish viable from non-viable cells. Cells can be incubated with nalidixic acid before direct microscopic observation. Nalidixic acid blocks cell division and so in its presence viable cells become elongated. The observation of elongated cells is objective evidence of cell viability, and cells that are not elongated show no sign of the growth that characterizes viable bacterial cells. Alternately, cells can be incubated with chemicals that change color when acted on by specific enzymes that are active in viable cells. The chemical INT, for example, forms pink-red deposits in cells when acted on by dehydrogenases. The observation of cells with pink-red inclusions after treatment with INT indicates that those cells were metabolically active (viable) and that cells lacking such inclusions were metabolically inactive (non-viable).

Another approach to direct counting is to use an electronic particle counter such as a Coulter counter. This instrument can register the magnitude and duration of the changes in conductivity of a suspension of bacterial cells as they pass through a small orifice and thus can register and record both the number and distribution of the size of a cellular population. Such instruments permit the discrimination of particles based on size so that particles the size of bacteria can be counted automatically. If there are no nonliving interfering particles in the same size range of bacteria, this is a rapid counting method.

3)Turbidimetric procedures

When a beam of light passes through a suspension of particles the size of bacteria, the light is scattered. In effect, the turbidity of the solution reduces the amount of light that can pass through. Measuring the amount of light that passes through a suspension of microorganisms with a spectrophotometer (Fig. 2-37) or other optical measuring device can be used for estimating cell mass, since the amount of light absorbed or scattered by the microorganisms is proportional to the concentration of cells.

Spectrophotometers measure absorbency units (A), which follow the log of (I_o/I) where I_o is the intensity of light striking a supension and I is the intensity of light transmitted by the suspension. The absorbency or optical density (OD) of a solution is related to the percentage of light transmitted (%T) through the solution according to the formula:

$$OD = \log 100 - \text{long } \% T$$

When calibrated against bacterial suspensions of known concentration, a requirement for estimating cell concentrations, spectrophotometers provide an accurate and rapid way to estimate the dry weight (mass) of bacteria per unit volume of culture. An increase in cell mass, which can be equated with increases in the numbr of bacterial cells, is useful for establishing a growth curve for a bacterium.

Because bacteria are in suspension, not in solution, a measure of the absorbency of a bacterial suspension is not a direct measure of the bacterial cell concentration. In fact, because light scattering also contributes so significantly to the determination, the measured value of A depends on the precise geometry of the instrument used. The value of A of a bacterial suspension measured on one instrument will not be the same as that measured on a different instrument.

The instrument must be calibrated for the particular bacterium and medium being studied by directly determining the number of bacteria in a dense suspension and by measuring its absorbance A, as well as the absorbance of known dilutions of the suspension. At low densities A is roughly proportional to the cell number, but at higher densities there is a significant deviation from linearity. Such deviation is a consequence of double scattering, where at high culture densities the probability of a scattered ray of light being scattered back so that it strikes the photodetection system is increased.

1.3.1.10 Model questions

1.Explain different methods for establishment of pure cultures

2)Explain the different procedures for enumerating bacteria

1.3.1.11 References Books

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Lesson 1.3.2

HETEROTROPHIC METABOLISM OF BACTERIA

&

PHOTOTROPHIC METABOLISM

Objective:

Based on the type of nutrition bacteria are divided in to two types they are phototrophic and heterotrophic bacteria.Photo trophic bacteria are able to synthesize he their food material on their own by photosynthesis .In this chapter we have discussed the entire reactions involved in the metabolism of both phototrophic and heterotrophic bacteria.

1.3.2 Introduction

Energy flows through living cells, it becomes transformed and redistributed in ways that allow cells to grow, multiply, carry out other cellular functions, and perform cellular work. The chemical reactions accompanying this flow of energy collectively form the process known as cellular metabolism. The word metabolism is derived from the Greek metabole, meaning transition or change. Cellular metabolism consists of a complex network of chemical reactions that capture energy and raw materials from the environment and allows them to be changed into forms that are used to sustain cells. The formation and utilization of ATP and the protonmotive force (PmE) are keys to cellular energy transformations and the central focus of cellular metabolism.

1.3.2.2 Metabolic strategies for generating cellular energy

There are various metabolisms that sources for generating cellular energy: Autotrophy and heterotrophy have evolved among diverse microorganism. These include (1) chemoorganotrophic (heterotrophic) metabolism using organic compounds as sources for generating cellular energy and as the source of carbon for incorporation into cellular structures, (2) chemoautotrophic metabolism using inorganic compounds as energy sources and for biosyntehsis of the organic compounds that make up cellular structures, and (3) photoautotrophic metabolism using light as the energy source and carbon dioxide as a source of carbon.

Heterotrophic metabolism (chemoorganotrophic metabolism), in contrast, requires a supply of preformed organic matter for production of cellular biomass and as a source of the chemical energy used to form ATP. It involves the conversion of the organic substrate molecule to end products via a metabolic pathway that releases sufficient energy for it to be coupled to the formation of ATP. There are two basic types of heterotrophic metabolism in which cells generate ATP. Respiration and fermentation. The distinction between these two types is in the nature of the final electron acceptor of the pathways.

Respiration requires an external terminal electron acceptor that is not derived from the organic substrate, whereas fermentation uses a terminal electron acceptor that is derived from the organic substrate.

Table Types of Heterotrophic Microbial Metabolism used to Generate ATP.

Type of Metabolism	Description
Respiration	Uses complete oxidation of organic compounds, requiring an external electron acceptor to balance oxidation reduction reactions used to generate ATP; much of ATP is formed as a result of chemiosmosis based on establishment of a proton gradient across a membrane
Aerobic respiration	Uses oxygen as the terminal electron acceptor in the membrane-bound pathway that establishes the proton gradient for chemiosmotic ATP generation
Anaerobic respiration	Uses compounds other than oxygen e.g., nitrate or sulfate, as the terminal electron acceptor in the membrane-bound pathway that establishes the proton gradient for chemiosmotic ATP generation
Fermentation	Does not require an external electron acceptor, achieving a balance of oxidation reduction reactions using metabolic intermediates of the organic substrate molecule; various fermentation pathways produce different end products.

1)Metabolic pathways

Each type of metabolism involves a specific series of chemical reactions, called metabolic pathways, in which energy is transformed to generate a protonmotive force and ATP. A metabolic pathway has discrete steps between a starting substance (substrate molecule) and the products of the chemical

reactions (end products). Several central metabolic pathways play key rotes in the metabolism of microbial cells.

2)Generation of protonmotive force

As a consequence of their metabolic activities, cells are capable of channeling energy into the synthesis of a protonmotive force (PMF). The protonmotive force is an electrochemical gradient across a membrane that can be used by the cell to do work. PMF is based on the establishment of a proton gradient (differential concentration of protons, H^+) across the cytoplasmic membranes of bacterial and archael cells and the mitochondrial and chloroplast membranes of eukaryotic cells. The PMF develops when cells, as part of their metabolic activities, move protons (H^+) across their membranes by specific proton translocating systems.

The gradient of protons across a membrane consists of electrical and chemical energy. The electrical component is due to positively charged protons being translocated across a membrane, which results in one side of the membrane being more positively charged and one side being more negatively charged. This electrical component is called $\Delta\psi$ (Delta Psi). In addition, protons form a hydrogen ion concentration, more commonly known as pH, and this chemical component of the protonmotive force is called ΔpH. The contributions of the electrical component and the chemical component are referred to as electrochemical energy of $\Delta\mu_{H+}$. For one mole of protons,

$$\Delta\mu_{H+} = F\Delta\psi + RT \text{ in } \frac{(H^+)_{inside}}{(H^+)_{outside}}$$

Where F is the Faraday constant, R is the universal gas constant, and T is the absolute temperature in “Kelvin. This equation can be rewritten as

$$(\Delta\mu_{H+} / F) = \Delta\psi - 60\Delta\text{pH at } 30^oC$$

Where ($\Delta\mu_{H+}$ / F) is referred to as the proton potential or protonmotive force, Δp.

The electrical component, $\Delta\psi$, and the chemical component, ΔpH, of the protonmotive force may be used of bacteria and the mitochondria and chloroplasts of eukaryotic cells growing at neutral pH develop a protonmotive force, Δp, with contributions from both $\Delta\psi$ and ΔpH. For acidophilic bacteria and archaea that live at pH 1-2, the protonmotive force is derived mainly from the very large ΔpH that is a consequence of their environment:

$$\Delta pH = (pH_{inside} - pH_{outside})$$

In acidophilic microorganisms, the $\Delta\Psi$ is reversed from that seen in other microorganisms, that is, the outside of the membrane is negatively charged and the inside is positively charged. In alkaliphilic bacteria, the Δp is generated almost entirely from the $\Delta\Psi$ with little or no contribution from the ΔpH.

With the establishment of a PMF, the protons can move back through the membrane via appropriate carriers only and the nergy of the PMF is harnessed to do work. The protonmotive force may be used to provide energy for the generation of ATP. In bacterial and archaeal cells the protonmotive force is also used independently of ATP to perform various cellular work, including solute transport and flagellar rotation.

3)Generation of ATP

Energy is transformed into chemical energy and is stored within molecules of ATP. All living organisms use ATP as the "central currency of energy". A growing bacterial cell of Escherichia coli, for example, synthesizes approximately 2.5 million molecules of ATP per second to support its energy needs.

ATP contains bonds that are called high energy phosphate bonds (Fig. 4-2). When a high energy phosphate bond is broken, a large amount of free energy is leased. Though energy is released when the high energy phosphate bond of ATP is broken,

the actual bond breaking process-like all bond breaking processes-requires an input of nergy. Bond breaking requires energy. Bond making releases energy. In the case of breaking the high energy phosphate bond of ATP, however, the immediate formation of new bonds from products of the reaction (ADP + P_i) releases considerably more energy than was taken in to break the original bond. When ATP is converted to ADP, the electrostatic repulsion between the negatively charged phosphate groups is reduced, and this accounts for the relatively large release of free energy associated with this reaction. Thus the conversion of ATP to ADP and P_i releases energy overall, and this energy is referred to as the energy given out when the high energy phosphate bond "breaks". Breaking the terminal phosphate bond in ATP releases – 7.3 kcal/mole of free energy.

Fig.

Many of the metabolic pathways of a cell are involved with coupling energy-yielding reactions (exorganic reactions) with the energy-requiring (endergonic) conversion of ADP and inorganic phosphate (p_i) to ATP, where the ATP can then serve as a common "energy currency" within the cell. Many metabolic pathways require inputs of ATP to drive forward endergonic reactions, splitting the ATP into ADP and phosphate ions as they do so. The cycling of ADP and ATP within the cell is fundamental to cellular energetics, and a living cell continuously forms and consumes ATP.

Organisms have two different processes for generating ATP: substrate level phosphorylation hat directly couples a chemical reaction with the generation of ATP and oxidative phosphorylation that uses the protonmotive force to generate ATP.

Substrate level phosphorylation occurs by coupling an energy-yielding reaction in a catabolic pathway with the energy-requiring formation of ATP. ATP is formed by combining inorganic phosphate (P_i) or phosphate from an organic molecule with ADP. An example of substrate level phosphorylation is the coupling of the exergonic conversion of phosphoenolpyruvate to pyruvate with the endergonic conversion of ADP to ATP.

Phosphoenolpyruvate + ADP → Pyruvate + ATP

In contrast to substrate level phosphorylation, in oxidative phosphorylation, the protonmotive force (not a coupled chemical reaction) supplies the energy for generating ATP. During oxidative phosphorylation, protons are translocated outside of a membrane as a consequence of a flow of electrons through membrane carriers, establishing a protonmotive force. The formation of the protonmotive force is then coupled to ATP synthesis. The two processes, formation of the protonmotive force and its utilization to generate ATP, can be separated (uncoupled) from each other by chemicals such as dinitrophenol.

The process that forms ATP is catalyzed by a proton-conducting membrane-bound enzyme called adenosine triphosphatase (ATPase). (ATPase is sometimes referred to by other names, including F_oF_1 ATPase, proton translocating ATPase, ATP synthetase, and ATP synthase. As these names imply, this enzyme can catalyse thehydrolysis of ATP or its synthesis). When protons move through ATPase, energy is captured and transferred to form ATP from ADP and P_i. This process is called chemiosmosis. The generation of ATP by chemiosmosis depends on the fact that protons cannot simply diffuse back across the membrane but can recross it only via a specific proton channel, such as the one established by a membrane-bound ATPase.

ATPase is multicomponent enzyme system containing two major polypeptide complexes called F_o and F_1 (Fig. 4.3). The F_oF_1 complex couples the synthesis of ATP with proton diffusion. The F_1 polypeptides sit on the inner surface of the membrane, whereas the F_o polypeptides are embedded in the membrane. F_o

forms a channel across the membrane through which protons flow to F_1. Then F_1 catalyzes the synthesis of ATP from ADP+P_1. The energy within ATP can then serve to drive forward the various energy-requiring reactions essential to all cells.

Fig.

4)Respiration

Respiratory metabolism of glucose and other substrates can be divided into three distinct phases:

1. A catabolic pathway, during which organic molecule is broken down into smaller molecules, usually with the generation of some ATP and reduced coenzymes. In the case of carbohydrates, a substrate molecule such as glucose is initially broken down to pyruvate via a glycolytic pathway.

2. The tricarboxylic acid cycle (TCA), during which the small organic molecules produced in the first phase are oxidized to inorganic carbon dioxide and water, accompanied by the production of more ATP and reduced coenzymes.

3. A process known as oxidative phosphorylation, during which the reduced coenzymes are reoxidized. The electrons and protons they release are transported through a series of membrane-bound carries to establish a proton gradient (protonmotive force) across a membrane. A terminal acceptor, such as oxygen, is reduced and ATP is synthesized by chemiosmosis as a result of electron transport and the protonmotive force.

In respiration and external molecule (not derived from the initial organic substrate) is needed to act as the final electron acceptor whose reduction balances the oxidation of the initial

substrate. Therefore in respiration, the initial organic substrate molecule (electron donor) undergoes a net oxidation, while the external electron acceptor is reduced to form a balanced oxidation-reduction process.

Table Electron Acceptors used in Respiratory Metabolism

Type of Metabolism	Electron Acceptor	Products Formed	Microorganism (Exampls)
Aerobic respiration	O_2	H_2O	Escherichia coli, Pseudomnonas aeruginosa, and numerous other bacteria, fungi, algae and protozoa
Anaerobic respiration	NO_3^-	NO_2^-	E.coli and other enteric bacteria
Anaerobic respiration (denitrification)	NO_3^-	NO_2^-, N_2O or N_2	Paracoccus denitrificans
Anaerobic respiration (sulfate reduction)	SO_4^{2-}	H_2S	Desulfovibrio desulfuricons
Anaerobic respiration (methanogenesis)	CO_2	CH_4	Methanobacterium autotrophicum
Anaerobic respiration	S^O	H_2S	Desulfuromonas acetoxidans
Anaerobic respiration	Fe^{3+}	Fe^{2+}	Bacillus licheniformis

The most common external electron acceptor in respiration pathways is molecular oxygen, but some bacteria use alternate terminal electron acceptors (Table 4-4). When molecular oxygen serves as the terminal electron acceptor of respiration, the process is known as aerobic respiration (meaning it requires the presence of air). The pathway of aerobic respiration begins

with an organic substrate molecule and typically combines it with oxygen in an oxidation-reduction process that ends with the formation of carbon dioxide and water. In the process, a substantial amount of ATP is also formed. The classic equation that described this pathway for aerobic respiration of glucose is:

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6\ H_2O$$

The oxidation of glucose by this pathway can generate up to 38 molecules of ATP for each molecule of glucose converted to carbon dioxide and water.

When another inorganic chemical, such as nitrate or sulfate, serves as the terminal electron acceptor the process is known as anaerobic respiration (meaning it does not require the presence of air). Some microorganisms can use oxygen or some other inorganic chemical as the terminal electron acceptor and therefore can carry out both aerobic and anaerobic respiration. The bacterium paracoccus denitrificans, for example, can use oxygen or nitrate as the terminal electron acceptor. Therefore while many other bacterial species are restricted to one or the other form of respiration, others can perform both aerobic and anaerobic respiration.

Fig.

5)Glycolysis

The process of breaking down a sugar is called glycolysis (from the Greek glyco, sweet or sugar, and lysis, breaking down) and the catabolic pathways of sugar metabolism and aro called glycolytic pathways.

In the Embden-Meyerhof pathway, glucose is first converted in a series of reactions to form fructose 1.6-bisphosphate. The 1.6-bisphosphate, in turn, is cleaved to form

two interconvertable 3-carbon sugars that enter a common set of catabolic reactions to form two pyruvates. The breakdown of one molecule of glucose to two molecules of pyruvate by this pathway releases sufficient free energy to permit a net synthesis of two ATP molecules (Table-). The conversion of glucose to form pyruvate is also accompanied by the formation of two reduced coenzyme (NADH) molecules).

Cells initiate the Embden-Meyerhof pathway by coupling the conversion of glucose to glucose 6-phosphate with the conversion of ATP to ADP.

Glucose 6-phosphate is then isomerized to fructose 6-phosphate, which is converted to fructose 1.6-bisphosphate in a reaction that requires input of energy from ATP. The conversion of fructose 6-phosphate to fructose 1.6-bisphosphate, is catalyzed by phosphofructokinase, which is a key enzyme in regulating the rate of glycolysis.

Fructse 1,6-bisphosphate, which contains six carbon atoms, is split into two 3-carbon molecules – glyceraldehydes – 3-phosphate and dihydroxyacetone phosphate – by the action of the enzyme aldolase. There is no loss of energy this splitting of fructose 1,6-bisphosphate into two 3-carbon units is called the aldolytic reaction. Dihydroxyacetone phosphate, which is not in the direct glycolytic pathway, can be converted to glyceraldehydes-3-phosphate. The equilibrium between dihydroxyacetone phosphate and glyceraldehydes-3-phosphate. However, the constant removal of glyceraldehydes-3-phosphate, which si in the direct glycolytic pathway, shifts the balance of reactants and products so that the dihydroxyacetone is convertedto glyceraldehydes-3-phosphate. Thus for each 6-carbon glucose substrate molecule, two molecules of glyceraldehydes-3-phosphate are formed.

Each of the steps subsequent to the formation of glyceraldehydes-3-phosphate occurs twice for each 6-carbon glucose molecule that is metabolized.

Each glyceraldehydes-3phosphate molecule is converted to 1,3-bisphosphoglycerate by the incorporation of inorganic

phosphate (P_i) into the molecule during an exergonic reaction. The oxidative conversion of glyceraldehydes-3-phosphate to form 1,3-bishosphoglycerate is coupled with the conversion of oxidized NAD^+ to the reduced coenzyme NADH. Because there are two molecules of 1,3-bisphosphoglycerate generated from each glucose molecule, there is a net production of two NADH molecules per molecule of glucose.

The 1,3-bisphosphoglycerate is converted to 3-phosphoglycerate, an exergonic reaction that can be coupled with the synthesis of ATP. The formation of ATP in this coupled reaction is a substrate level phosphorylation reaction. It is so designated because ATP is formed from ADP by the direct transfer of a high energy phosphate group from the 1,3-bisphosphoglycerate, an intermediate substrate in the pathway. Because this reaction occurs for each of the two 3-carbon molecules generated per glucose molecule. Therefore the synthesis and utilization of ATP are balanced at this point in the metabolic pathway and the net procution of ATP is 0.

The 3-phosphoglycerate is then further converted to phosphoenolpyruvate and finally to pyruvate. The conversion of phos- phoenolpyruvate to pyruvate is coupled with the synthesis of additional ATP. Thus this glycolytic pathway results in the conversion of the 6-carbon molecule glucose to two molecules of the 3-carbon molecule pyruvate, with the net production of two molecules of reduced coenzyme, NADH, and the net synthesis of two ATP molecules.

The overall equation for glycolysis by the Embden-Meyerhof pathway can be written as follows:

$$\text{Glucose} + 2ADP + 2P_i + 2NAD^+ \rightarrow 2 \text{ pyruvate} + 2NADH + 2ATP$$

6)Enter-doudoroff pathway

Some bacteria and archaea use an alternate pathway of glycolysis called the Enter-Doudoroff pathway (Fig). This pathway is used by many aerobic bacteria, such as pseudomonas species. The net equation for the Enter-Doudoroff pathway of glycoysis is:

Glucose + $NADP^+$ + NAD^+ + ADP + P_i ⟶

2 pyruvate + NADPH + NADH + ATP

Bacteria and archaea that carry out the Enter-Doudoroff pathway lack a key enzyme, 6-phosphofructokinase of the Embden_Meyerhof pathway. In the Enter-Doudoroff pathway, glucose 6-phosphate is oxidized to 6-phosphogluconate and then converted to 2-keto-3-deoxy-6-phosphogluconate (KDPG). KDPG is then cleaved to yield glyceraldehydes 3-phosphate and pyruvate directly. Since pyruvate is formed directly, some of the ATP-generating steps are lost. The catabolism of glucose via the Enter-Doudroff pathway results in the net production of only one ATP molecule per molecule of glucose, in contrast to the two ATP molecules produced in the Embden-Meyerhof pathway. Thus the Enter-Doudoroff pathway is only 50% as efficient in ATP production as the Embden-Meyerhof pathway.

Fig.

Another difference between the Enter-Doudoroff pathway and the Embden-Meyerhof pathway is that in the Entner-Doudoroff pathway the reduced coenzyme NADPH is generated from $NADP^+$, rather than NADH from NAD^+. NADPH is a phosphorylated form of NADH. Generally, NAD^+ and its reduced form, NADH, are used in metabolic reactions that generate ATP, whereas $NADP^+$ and NADPH are used in biosynthetic reactions that build up molecules needed by the cell from simpler substrates. The Enter-Doudoroff pathway provides an important mechanism for producing NADPH and the 3-carbon building blocks that are used in biosynthetic reactions, where the need for them is greatr than the need for ATP.

7)Pentose phosphate pathway

The pentose phosphate pathway generates ATP and reduced coenzymes and small molecules that are needed for

biosynthesis (Fig). This pathway is essential for providing ribose for incorporation into the nucleotides of DNA, RNA, ATP, NAD^+, and $NADP^+$. Several variations in the pentose phosphate pathway are possible, depending on the need for NADPH, ATP, and small precursor molecules for incorporation into macromolecules.

In one version of the pentose phosphate pathway, glucose is eventually converted into ribulose –5-phosphate and carbon dioxide, a process that requires the use of one ATP molecule and results in the generation of two NADPH molecules. When a large amount of reduced coenzyme is required, the glucose molecule can be completely metabolized to carbon dioxide, with the production of 12 molecules of reduced coenzyme NADPH. This series of reactions really involves a cyclic pathway in which glucose 6-phosphate is broken down and resynthesized, providing a large amount of reduced coenzymes needed by microorganisms during times of active growth. When the cell requires both NADPH and ATP, phosphoglycerate can be converted to pyruvate, with NADPH generated during the initial steps of the pentose phosphate pathway and ATP generated as a result of the oxidation of the pyruvate.

Methylglyoxal pathway

In some bacteria (Escherichia coli and related enteric bacteria, some Clostridium species, and Pseudomonas species) the methylglyoxal pathway operates as an alternate to the Embden-Meyerhof pathway when the cell experiences conditions of low phosphate concentration (Fig.). When phosphate is the rate limiting reagent, this pathway converts dihydroxyacetone phosphate to methyl-glyoxal and then to pyruvate. This bypasses the phosphorylation step that converts glyceraldehydes-3-phosphate to 1,3-bisphosphoglycerate, yet still produces pyruvate, which can be further metabolized to generate ATP. Overall, the methylglyoxal pathway consumes two ATP molecules rather than generating ATP.

Fig.

8)Tricarboxylic acid cycle

In the second phase of the respiratory metabolism of glucose and other substrates, acetyl-CoA (from β-oxidation of fatty acids or via pyruvate from carbohydrate or protein catabolism) is fed into the tricarboxylic acid cyle (TCA cycle). This results in the production of carbon dioxide, water, reduced coenzymes, and ATP.

To enter the TCA cycle, which is also known as the citric acid cycle or the Krebs cycle, pyruvate molecules generated during glycolysis or protein catabolism first react with CoA in a reaction catalyzed by the pyruvate dehydrogenase complex. Pyruvate dehydrogenase is a multi-enzyme complex that has 48 polypeptides. The decarboxylation of pyruvate, which is coupled with the conversion of the coenzyme NAD^+ to reduced NADH, forms acetyl-CoA and carbon dioxide:

Pyruvate + NAD^+ + CoA → Acetyl-CoA + NADH + CO_2

The acetyl-CoA formed from pyruvate decarboxylation can feed its acetyl group into the TCA cycle (Fig).

Fig.

In the first step of the TCA cycle, acetyl-CoA reacts with oxaloacetate to form citrate and release CoA. Through a series of reactions involving carboxylic acids, the TCA cycle then regenerates oxaloacetate. During the TCA cycle, two reactions liberate carbon dioxide: the conversion of the 6-carbon compound isocitrate to the 5-carbon compound α-ketoglutarate, and the subsequent conversion of α-ketoglutarate to succinyl –

CoA (succinate is a 4-carbon compound). Reduced coenzyme NADH is generated during three reactions of the TCA cycle. The coenzymeflavin adenine dinucleotide (FAD) is also reduced to $FADH_2$ during the conversion of succinate to furmate.

Only one of the exergonic reactions of the TCA cycle, the conversion of succinyl-CoA to succinate, is directly coupled with the generation of a high energy phosphate-containing compound. In this reaction for some bacteria, ATP is formed in a substrate level phosphorylation by transfer of energy form the high energy succinate-CoA bond to ADP and P_i. In eukaryotic cells, a different reaction occurs within the mitochondria in which guanosine triphosphate (GTP) is synthesized from guanosine diphosphate (GTP) is synthesized from guanosine diphosphate (GDP) and p_i. Some bacteria similarly form GTP rather than ATP in this metabolic reaction. GTP is the energy source used in some specific cellular reactions, most importantly during the synthesis of protein at the ribosomes. The energy stored within GTP is equivalent to that stored within ATP, and the high energy phosphate group of GTP can be transferred to ADP to form ATP by the reaction:

$$GTP + ADP \rightarrow GDP + ATP$$

For energy accounting purposes, the GTP generated in this reaction will be treated as if it is all transformed to ATP.

An important aspect of the TCA cycle is that the reaction intermediates are reused. The two carbon atoms that originated from acetyl-CoA are completely oxidized to CO_2. The other carbon atoms of the reactants are conserved as the cycle repeats itself and picks up another two carbons from a new acetyl-CoA molecule.

The net reaction of TCA cycle, starting with the pyruvate generated by the glycolysis of glucose can be summarized as:

$$2 \text{ pyruvate} + 2 ADP + 2P_i + 2FAD + 8NAD^- \longrightarrow 6 CO_2 + 2 ATP +$$

2 $FADH_2$ + 8 NADH

At the end of the TCA cycle, all of the carbon from the original glucose molecule has been converted to carbon dioxide. Assuming the pyruvate that fed into the TCA cycle was generated by the Embden-Meyerhof pathway and two from the TCA cycle. Ten reduced coenzyme molecules will be generated in the form of NADH (two from the Embden-Meyerhof pathway and eight from the TCA cycle), while two reduced coenzyme molecules in the form of $FADH_2$ will come from the TCA cycle.

In addition to its role in the overall respiratory generation of ATP, the TCA cycle also plays a central role in the flow of carbon through the cell. It supplies organic precursor molecules to many biosynthetic pathways, as is discussed in Chapter 5. In fact, many microorganisms oxidize only part of their substrate molecules for the production of ATP, using the remainder for biosynthesis. Because some of the intermediates in the TCA cycle are siphoned out of it for use I biosynthesis, they must be resynthesized to maintain TCA cycle activity. Therefore the reduced coenzymes generated by the TCA cycle and glycolysis can be used either for generating ATP, or to synthesize the reduced coenzyme NADPH for use in biosynthesis.

Fig.

9)Oxidative phosphorylation

In the third and final phase of the respiratory metabolism of glucose and other substrates, reduced coenzymes generated earlier in glycolysis and the TCA cycle are reoxidized. This process is called oxidative phosphorylation. The electrons and protons that are released in this process are transported through a series of membrane-bound carriers to establish a proton

gradient (protonmotive force) across a membrane. Electron transport ends with the reduction of a terminal electron acceptor.

10)Electron transport chain

During oxidative phosphorylation, electrons from the reduced coenzymes NADH and $FADH_2$ are transferred though a series of membrane-bound carriers that form an electron transport chain. This transfer of electrons involves a series of oxidation-reduction reactions of the membrane-bound carrier molecules, which leads to a terminal acceptor, such as oxygen, that is reduced and ATP is synthesized by chemiosmosis. The reduced coenzymes are reoxidized in this process and can be reused in cellular metabolism as electron acceptors.

An important aspect f oxidation-reduction reactions of the lectron transport chain is their relationship to the free energies of chemical reactions. Oxidation-reduction reactions can be written a two district reactions called half reactions: in one the reduced substrate gains an electron; in the other one the oxidized substrate loses an electron. The greater the difference in voltage between the half reactions of oxidation-reduction reactions, the greater the free energy of the reaction, and hence the greater the energy that may be channeled into the generation of ATP. Reduction potential (E'_o) is related to free energy according to the equation:

$$\Delta G^{o'} = -nF\Delta E'_o$$

Where $\Delta G^{o'}$ is the standard free energy change at pH 7.0, n is the number of electrons transferred, F is the Faraday constant (23,000 cal/volt), and $\Delta E'_o$ is the difference between the potentials of the two half reactions involved in an oxidation-reduction reaction. For example, based on E'_o values of –0.32 volt for the half reaction $NAD^+/NADH$ and +0.82 for the half reaction ½ O_2/H_2O, the oxygen-linked oxidation of NADH to NAD^+ has an $\Delta E'_o = 1.14$, which is equivalent to a free energy for this reaction of –52.4 kcal. This particular exergonic reaction

NADH→ NAD^+ is very important in the generation of cellular energy by respiration.

The carriers in the electron transport chain participate in a series of reactions with increasing reduction potential difference ($\Delta E'_o$) between that the primary electron donor and the terminal electron acceptor (Fig). Flavoproteins (containing flavin mononucleotide) and iron-sulfur proteins (non-heme) iron proteins) transfer hydrogen from NADH to coenzyme Q (quinone). Electrons from coenzyme Q reduce a series of cytochromes, usually beginning with cytochrome c or cytochrome b. Cytochromes contain a central iron ion, which can be cycled between the oxidized ferric state (Fe^{3+}) and the reduced ferrous state (Fe^{2}+). Ultimately, electrons are passed to a cytochrome alcytochrome oxidase (cytochrome o) complex and then to O_2 in aerobic respiration or to an alternate inorganic (e.g., nitrate or sulfate) or low molecular weight organic (e.g., fumarate) final electron acceptor in anaerobic respiration.

Fig.

The transport of electrons from reduced coenzymes to a terminal electron acceptor can be blocked by various agents, resulting in the inability of a cell to generate ATP and therefore resulting in the death of the cell. For example, cyanide can bind to the iron of certain cytochromes, blocking their ability to transfer electrons and turn over oxygen. Likewise, carbon monoxide can bind to the terminal cytochrome, blocking the reduction of oxygen.

Within the electron transport chain, some carriers transport hydrogen atoms (an electron plus a proton), whereas others transport only electrons (Table). Flavoproteins and

quinines are hydrogen atom carriers. Cytochromes and non-heme iron proteins are electron carriers. Different cells have different specific carriers but the general series of electron transfers is from NADH to a flavoprotein, to a non-heme iron-protein, to a quinone, to cytochromes, and then to the terminal electron acceptor.

Table Components of Electron Transport Systems

Component	Type of Molecule	Function	Component	Type of Molecule	Function
NADH dehydrogenase	Protein, enzyme	Transfers H^+ and e^- from NADH	Non-heme iron sulfur proteins	Iron-sulfur containing proteins	e^- donor and acceptor
Falvoproteins	Flavin containing protein	H^+ acceptor; e^- donor	Quinones Cytochromes	Heme containing protein	H^+ acceptor; e^- donor and acceptor

In eukaryotic cells, the electron transport chain is located within the inner mitochondria membrane. In mitochondria, two protons are transferred from NADH of a flavoprotein, which expels the protons across and outside the inner membrane as electrons are transferred to a non-heme iron protein (Fig). The reduced non-heme iron transfers its electron to the quinone (coenzyme Q) and two protons are picked up from the cytoplasm to form reduced coenzyme Q ($CoQH_2$). The $CoQH_2$ transfers its electrons to a cytochrome b-cytochrome C_1 complex, and protons are expelled outside the membrane. The electrons then pass from the cytochrome b-cytochrome c_1 complex to cytochrome c and then to cytochrome a, or cytochrome a_3. In the final step of the pathway, the electrons from the cytochrome-a complex are used to reduce O_2 to H_2O.

Fig.

Eukaryotic mitochondrial and bacterial electron transport chains have several distinguishing features. In eukaryotes, the mitochrondrial electron transport chains are linear. Bacterial electron transport chains are usually branched, with the branching point at coenzyme Q (quinone) or cytochrome, which means the pathway can utilize an alternate cytochrome. In addition, the components of the electron transport chain found within a particular species of bacterium can vary, depending on the environmental conditions in which the cell is growing (Fig). For example, in Escherichia coli the electrons from NADH are transferred to flavoprotein, non-heme iron, and coenzyme Q. Then, depending on environmental conditions, the electron is transferred to a distinct cytochrome (either $b_{550,}$ cytochrome o, and oxygen, or to cytochrome b_{550}, cytochrome d, and oxygen). Cytochrome o is used under high oxygen concentrations and cytochorme d is used under low oxygen concentrations. In the absence of oxygen, another cytochrome b can transfer electrons to nitrate to complete the electron transport chain.

Fig.

When nitrate serves as the terminal electron acceptor during anaerobic respiration, the products of its reaction can also serve as terminal electron acceptors. This establishes a series of anaerobic respirations where nitrate is reduced to produce nitrite, nitrite is reduced to produce nitrous oxide, and nitrous oxide is reduced to molecular nitrogen (Fig). This denitrification process returns N_2 to the atmosphere. Similarly, when sulfate acts as s terminal electron acceptor a series of reactions can eventually produce hydrogen sulfide and water. The equation for this reaction is:

$H_2 + SO_4^{2-} \longrightarrow H_2S + 2H_2O + 2OH^-$

Fig.

11)Chemiosmotic ATP generation

The transfer of electrons from the reduced coenzyme to the terminal electron acceptor and the coupled transfers of protons establishes the proton gradient across the membrane that powers the formation of ATP. The electron carriers of the electron transport chain are asymmetrically distributed through the membrane, and the movement of protons across the membrane, as a result of electron transport, forms an electrochemical proton gradient that is used for generating ATP. In bacteria, this electrochemical gradient is established across the cytoplasmic membrane, whereas for eukaryotic cells it is formed across the inner mitochrondrial membrane.

The orientation of the carriers in the bacterial cytoplasmic membrane is such that proton carriers transport toward the outside of the cell and electron carriers transport toward the inside. At each junction in the chain of a hydrogen atom carrier and an electron carrier, one or more protons (H^+) are, transported out of the cell. It is unclear as to the exact number of protons that are expelled across the membrane during electron transport from NADH to O_2. As many as 10 protons may be transported across a membrane for each NADH molecule. A portion of the chemical energy released by the net reaction of the electron transport chain (oxidation of the primary electron donor by the terminal electron acceptor) is thereby trapped in the form of a protonmotive force that can be used to generate ATP or perform other work. The proton motive force measured across the cytoplasmic membrane of Escherichia coli is sufficient to generate the formation of the high energy phosphate bond of ATP.

The generation of ATP by chemiosmosis depends on the fact that protons cannot simply diffuse back across the membrane but can only recross it via a specific proton channel, such as the one established by a membrane-bound adenosine triphosphatase (ATPase). Electron transport can be uncoupled fromm ATP generation by agents such as dinitrophenol, which allows leakage of protons across a membrane and hence destroys the proton gradient and its associated protonmotive force.

ATPase is a multicomponent enzyme system containing two major polypeptide complexes called F_o and F_1 (Fig). The F_oF_1 complex couples the synthesis of ATP with proton diffusion. The F_1 polypeptides sit on the inner surface of the membrane, whereas the F_o polypeptides are embedded inn the membrane. F_o forms a channel across the membrane through which protons flow to F1. Then F_1 catalyzes the synthesis of ATP from ADP + P_i. F_1 is also capable of catalyzing the conversion of ATP to ADP + P_i.

As a consequence of the protonmotive force established by the electron transport chain, three ATP molecules can be synthesized for each NADH molecule oxidized to NAD^+, and two ATP molecules can be made for each $FADH_2$ molecule oxidized to FAD. The difference between the amount of ATP that can be generated from NADH compared to $FADH_2$ occurs because the oxygen-linked oxidation of NADH liberates 52.4 kcal/mole compared to only 42 kcal/mole for the oxygen-linked oxidation of $FADH_2$. This results in the transport of fewer protons (H^+) across the membrane for use in chemiosmotic ATP synthesis. For a bacterial cell, the 10 NADH molecules generated during glycolysis and the TCA cycle, therefore, could be used to synthesize 30 ATP molecules during oxidative phosphorylation, and the $2FADH_2$ molecules generated during the TCA cycle can generate 4 ATP molecules.

The chemiosmotic synthesis of ATP during oxidative phosphorylation is in addition to the ATP formed during glycolysis and he TCA cycle. Thus the overall reaction for the respiratory metabolism of glucose by a bacterial cell using the

Embden-Meyerhof pathway of glycolysis can be expressed as follows:

$$\text{Glucose} + 6O_2 + 38\ ADP + 38\ P_i \longrightarrow 6\ CO_2 + 6H_2O + 38\ ATP$$

The production of 38 ATP molecules from glucose is a theoretical maximal yield. For example, it may occur in the bacterium paracoccus dentrificans, which has an electron transport chain that can yield 3 ATPs for each NADH and 2 ATPs for each $FADH_2$. However, the electron transport chains of other bacteria, such as Escherichia coli, may produce only 2 ATPs for each NADH and 1 ATP for each $FADH_2$. As a result, such bacterial cells produce only 26 ATPs from the respiratory metabolism of each glucose molecule.

Anaerobic respiration, which occurs in some bacteria, often yields less ATP than aerobic respiration, producing only about one third the ATP made by aerobes. This is because a complete tricarboxylic acid cycle does not function in the absence of molecular oxygen and also because there is less of a free energy difference between NADH and nitrate or sulfacte than there is between NADH and molecular oxygen. Therefore only two ATPs are made for each NADH in anerobic respiration rather than three ATPs made during aerobic respiration.

In the mitochondria of eukaryotic cells, the overall respiratory metabolism can produce only 36 ATP molecules per glucose. This is because glycolysis takes place in the cytoplasm of a eukaryotic cell. The transport of the two NADH molecules produced during glycolysis into the mitochondrion, where the tricarboxylic acid cycle and oxidative phosphorylation occur, requires active transport that consumes ATP. One ATP is consumed per NADH entering the mitochondrion from the cytoplasm. Therefore the NADH formed in glycolysis produces a net gain of only 2 ATPs compared to the 3 ATPs produced from the NADH formed in bacteria. Thus only 36, rather than 38, ATP molecules can be produced from each glucose molecule in eukaryotic cells.

Although in all mitochondria and most bacterial cells carrying out respiration a protonmotive force (based on a proton gradient) is used for the chemiosmotic generation of ATP, some bacterial cells use a sodium ion potential for this purpose. For example, the bacterium Vibrio alginolyticus couples the electron transport chain with the translocation fo sodium ions across the cytoplasmic membrane when it is growing in alkaline environments. When growing at neutral or acidic conditions V. alginolyticus transports protons across the cytoplasmic membrane. Therefore V.alginolyticus is able to couple either a protonmotive force or an electrochemical gradient based on sodium ions with ATPase for the chemiosmotic generation of ATP. This bacterium illustrates the diversity of metabolic strategies evolved by bacteria that enables them to grow under highly diverse conditions.

1.3.2.3 Fermentation

In fermentation, an organic substrate acts as an electron donor and a product of that substrate acts as an electron acceptor. Both the electron donor and the acceptor are internal to the organic substrate in a fermentation pathway, meaning that the eventual acceptor is derived from the original substrate. There is no net change in the oxidation state of the products relative to the starting substrate molecule in fermentation pathways. The oxidized products are exactly counterbalanced by the reduced products, and thus the required oxidation-reduction balance is achieved. Coenzymes that are reduced at the beginning of a fermentation pathway are reoxidized later in the pathway, so that they are in fact not consumed in the process.

Fermentation yields less ATP per substrate molecule than respiration. This is because in fermentation the organic substrate molecule must serve as both the internal electron donor and internal electron acceptor. Thus the carbon and hydrogen atoms of the organic substrate cannot be fully oxidized to carbon dioxide and water but are simply rearranged into a form containing less chemical energy than the organic substrate had when the reactions began. In respiration the carbon and hydrogen atoms of the substrate molecule are completely

oxidized to carbon dioxide and water, with the accompanying release of far more free energy, much of which becomes trapped within ATP. The ΔG^o for the complete oxidation of glucose to carbon dioxide and water is –686 kcal/mol. By contrast, when glucose is only partially oxidized during fermentation to two molecules of the fermentation product lactic acid, the ΔG^o value is only –58 kcal/mol. This dramatic difference makes it clear why far less ATP is generated by the fermentation of glucose than by its complete respiration.

Because fermentation generates fewer ATP per molecule of substrate than respiration, more substrate molecules must be metabolized during fermentation than during respiration to achieve equivalent growth. So from the viewpoint of both bioenergetics and utilization of available organic nutrient sources, respiration is more beneficial than fermentation. Cells that have the ability to perform both types of catabolic metabolism will generally use the energetically more favourable respiration pathway when conditions permit. They will rely on fermentation only when there is no available external electron acceptor that they can use. Even though they are energetically less favourable than respiration pathways, fermentation pathways can occur in the absence of air because there is no requirement for oxygen or another electron acceptor to achieve a balance in the oxidation-reduction reaction. The organic substrate provides both the electron donor and the acceptor needed to achieve this balance.

The synthesis of ATP in fermentation is due to substrate level phosphorylations and is largely restricted to the amount formed during glycolysis. Oxidative phosphorylation and chemiosmotic generation of ATP do not occur in fermentation. Because they do not require oxygen, all fermentation pathways are anaerobic, and microorganisms that generate their energy by fermentation carry out anaerobic metabolism, even if the organism is growing in the presence of molecular oxygen.

Obligately fermentative bacteria, such as Streptococcus species, do not use oxidative phosphorylation to generate ATP, but they do have an F_oF_1-ATPase system in their cytoplasmic

membranes. In such bacteria, ATP is used to pump protons through the F_oF_1 complex in the reverse direction. In this process, ATP generated by substrate level phosphorylation in a fermentation pathway is converted to ADP and P_i by the ATPase, and the energy of this reaction is coupled with the export of protons from the cell. The F_oF_1 – ATPase system thereby generates a protonmotive force across the cytoplasmic membrane. This maintains the intracellular pH at the appropriate value and provides a mechanism for driving processes that depend on the protonmotive force across the membrane, such as the active uptake of sugars and other substances, the export of Na^+ and Ca^{2+}, and the rotation of flagella.

A complete fermentation pathway begins with a substrate, includes glycolysis, and terminates with the formation of end products (table). Considering the actual way in which ATP is generated in fermentative bacteria, the initial metabolic steps of the fermentation pathway are identical to those of a respiration pathway. The metabolic pathway for carbohydrate fermentation, for example, begins with glycolysis. If a cell uses the Embden-Meyerhof glycolytic pathway for the fermentation of glucose, it generates two pyruvate molecules, two reduced coenzyme NADH molecules, and two ATP molecules for each molecule of glucose. N general, the two ATP molecules formed during glycolysis represent the total energy yield of the fermentation pathway, although some bacterial fermentation pathways subsequently generate additional ATP. The remainder of the fermentation pathway is usually concerned with reoxidizing the coenzyme.

Table Types of Fermentative Metabolism

Fermentation Pathway	End products*
Homolactic acid	Lactic acid
Hetrolactic acid	Lactic acid + ethanol + CO_2
Ethanolic	Ethanol + CO_2
Propionic acid	Propionic acid + CO_2
Mixed acid	Ethnol + aceic acid + lactic acid + succinic acid + formic acid + H_2 + CO_2
Butanediol	Butanediol + CO_2
Butyric acid	Butyric acid + butanol + acetone + CO_2

Amino acid	Acetic acid + NH_4^+ + CO_2
Methanogenesis	CH_4 + CO_2

In fermentation, the reoxidation of NADH to NAD^+ depends on the reduction of the pyruvate molecules formed during glycolysis, which balances the oxidation-reduction reactions. This happens in different ways for bacterial, archaeal, and eukaryotic cells and produces various end products, depending on which pathway is used. The different fermentation pathways generally are named for the characteristic end products that are formed.

1)Lactic acid fermentation

In the lactic acid fermentation pathway, pyruvate is reduced to lactic acid, with the coupled reoxidation of NADH to NAD^+. This fermentation pathway is carried out by bacteria that, by virtue of their fermentation end products, are classified as lactic acid bacteria. Two important genera of lactic acid bacteria are Streptococcus (Gram-positive cocci that tend to form chains) and Lactobacillus (Gram-positive rods that tend to form chains).

2)Homolactic fermentation

When the Embden-Meyerhof pathway of glycolysis is used in the lactic acid fermentation pathway, the overall pathway is a homolactic fermentation because the only end product formed is lactic acid (Fig). The overall lactic acid fermentation pathway can be expressed as follows:

Glucose + 2 ADP + $2P_i$ → 2 lactic acid + 2 ATP

Homolactic fermentation is carried out by Streptococcus, Pediococcus, Lactococcus, Enterococcus, and various Lactobacillus species.

The homolactic acid fermentation pathway is important in the dairy industry. It is the pathway responsible for souring

milk and is used in the production of numerous types of cheese, yogurt, and various other dairy products.

`Steptococci living on tooth surfaces in the oral cavity (month) produce lactic acid by the homolactic acid pathway. The lactic acid is held against the tooth by dental plaque and gradually eats through the enamel of the tooth, creating caries (cavities). Even though they can grow in the mouth, Streptococcus species are metabolically obligate anaerobes using only fermentative metabolism.

Lactobacillus species occur in the human digestive tract and aid in the digestion of milk. These species are the initial colonizers of the intestinal tract. Some adults lack the ability to digest the carbohydrates in milk and therefore suffer disease symptoms (lactose intolerance) if they consume milk. Lactobacillus acidophilus is added to various commercial milk products (acidophilus milke) to aid individuals who are unable to digest milk products adequately. The enzymes produced by L. acidophilus convert milk sugars to products that do not accumulate and cause gastrointestinal problems.

3)Phototrophic metabolism

Microorganisms with a photoautotrophic metabolism obtain their energy supplies directly from the energy of the sun, which they use to drive the production of ATP. The conversion of light energy from the sun into chemical energy within ATP occurs by a process known as photophosphorylation. This process is initiated when light energy excites a photoreactive molecule, a pigment molecule able a to absorb particular wavelengths of sunlight. This absorption of the sun's energy causes release of an electron from the photoreactive pigment molecule. The electron is then transferred through a series of membrane-bound carriers, which are collectively known as a photosystem. Electron transfer by the photosystem drives the formation of a proton gradient across the membrane, which in turn serves to drive the formation of ATP by chemiosmosis.

Algae and cyanobacteria (formerly known as blue-green algae) have two photosystems. These photoautotrophic microorganisms carry out oxygenic photosynthesis, meaning that they produce oxygen in addition to ATP and reduced coenzymes as a result of their photoautotrophic metabolism. During oxygenic photosynthesis, H_2O is split to serve as a source of electrons needed in reduction actions and oxygen is liberated as a product.

Other photoautotrophic bacteria carry out oxygenic photosynthesis in which ATP is produced but oxygen is not. They do not use H_2O as an electron donor for generating reducing power but use altemate electron donors such as H_2 or H_2S. These anaerobic photoautotrophic bacteria have only one photosystem. The differences between the oxygenic photosynthetic algae and cyanobacteria and theanoxygenic photoautotrophic bacteria lie in the nature of their photosynthetic pigments, the structural arrangement of the pigments in the cell, and the oxidation-reduce mechanisms with which the cells balance their biochemical reactions.

4)Absorption of light energy

Photoautotrophic microorganisms have pigment molecules, including chlorophylls or bacteriochlorophylls, associated with their specialized photosynthetic membranes (Table). These pigment molecules trap light energy to initiate the process that results in the conversion of some of that energy into chemical energy. The general strategy of this process involves the initial capture of the light by light-harvesting “antennae” pigments, which then transfer the photons of light to a “photochemical reaction center” (which is just a particular pigment molecule) where the process of electron flow begins. Ultimately, this electron flow results in the chemiosmotic synthesis of ATP.

Pigments of various colors permit different photoautotrophic microorganisms to tap light energy of different wavelengths. These pigments include red, orange, and yellow carotenoids (molecules that have a cyclic ring with a side chain of alternating single and double carbon-carbon bonds) and green

chlorophylls (molecules with porphyrin rings containing magnesium) (Fig). Photoautotrophic microorganisms are often stratified within lakes, with the stratification pattern determined by the particular wavelengths of light that each type of microorganism can absorb. The anoxygenic photoautotrophic bacteria, such as the purple sulfur bacteria, absorb light of longer wavelengths and live well below the water's surface. Algae, cyanobacteria, and prochlorobacteria absorb light of shorter wavelengths and live nearer the water's surface.

Cyanobacteria, prochlorobacteria, and algae possess chlorophyll a as the predominant reaction center pigment and chlorophylls, carotenoids, or phycobiliproteins and other accessory molecules as antenna pigments. The prochlorobacteria synthesize chlorophyll b in addition to the chlorophyll a.

The green and purple photoautotrophic bacteria posses bacteriochlorophylls, generally a or b, and other accessory pigments, including carotenoids, that absorb light energy. Most of the bacteriochlorophyll molecules function as accessory or light-harvesting antenna pigments that absorb light and pass photons to reaction center pigments. Carotenoids also function as accessory antenna pigments. Some of the green photoautotrophic bacteria produce vesicles called chlorosomes that are filled with antenna pigments, including bacteriochlorophylls c, d, or e, bacteriochlorophyll a, and carotenoids. Chlorosomes serve as light-harvesting complexes in addition to the photosynthetic pigments that are found in the cytoplasmic membrane.

The reaction center bacteriochlorophylls and chlorophylls are directly involved in the photochemical oxidation-reduction reactions of photosynthesis. These bacteriochlorophylls and chlorophylls emit electrons when they absorb light energy. The primary photoreaction center in the purple bacteria is bacteriochlorophyll $P_{870.}$ In the green bacteria, it is bacteriochlorophyll $P_{840.}$ In the heliobacteria, it is bacteriochlorophyll P_{798}. The cyanobacteria and algae have two photoreaction centers: one with chlorophyll P_{680} and the other

with chlorophyll P_{700}. The subscript numbers refer to the wavelengths at which the particular bacteriochlorophyll or chlorophyll molecules maximally absorb light.

Tab.

Fig.

The electrons emitted by the varying bacteriochlorophylls or chlorophylls have differing energy levels. All have sufficient energy to generate a protonmotie force to drive the formation of ATP. Most have adequate energy to also drive the direct reduction of $NADP^+$ to NADPH. However, the electrons released by bacteriochlorophyll P_{870} of the purple photoautotrophic bacteria do not directly lead to the formation of NADPH. Additional energy input from the protonmotive force by reverse electron flow is required to drive the formation of NADPH in the purple photoautotrophic bacteria.

A few nonphotosynthetic, heterotrophic bacteria also possess bacteriochlorophylls. Erythrobacter longus and Protomonas species contain bacteriochlorophylls that stimulate their aerobic growth in the light. The function of bacteriochlorophyll and its evolutionary significance in these species is unclear.

5)Oxygenic photosynthesis

The cyanobacteria, prochlorobacteria, and the algae – like green plants – have two photosystems that are involved in the generation of ATP and NADPH. Each photosystem has its own photoreaction center. Photosystem I has a reaction center with chlorophyll a P_{700}, and photosystem II has a reaction center composed of a modified chlorophyll a P_{680}. Photosystems I and II are normally liked into a unified pathway, the Z pathway of

oxidative photophosphorylation, that generates ATP and reduced coenzyme for biosynthesis (Fig). The operation of the Z pathway requires two separate photoacts, that is, the absorption of light energy at two different photoactivation centers.

Fig.

In photosystem II, which is a noncyclic photophosphorylation pathway, electrons are transferred in one direction (unidirectionally) through a series of membrane-bound electron carriers. The electron flow is initiated when chlorophyll a P_{680} absorbs light energy, causing an energetically excited state that results in the release of an electron. The P_{680} chlorophyll becomes oxidized as a result of the electron release. This oxidation reaction is balanced by the splitting of water to form oxygen, hydrogen ions, and the electrons that are donated to the oxidized P_{680} chlorophyll to reduce it back to its original state. Because oxygen is produced, the process is called oxygenic photosynthesis.

Electrons from photosystem II are transferred through a series of membrane-bound carriers to the P_{700} chlorophyll reaction center molecule of photosystem I. The overall process that transfers an electron from an excited P_{680} chlorophyll molecule of photosystem II to the P_{700} chlorophyll molecule of photosystem I establishes a sufficient proton gradient across the membrane to synthesize one molecule of ATP. The electron transport chain then continues when a molecule of P_{700} absorbs light energy, initiating the electron transfer sequence of photosystem I. Each electron that is transferred from photosystem II balances an electron ejected from the excited P_{700} molecule of photosystem I. The electrons transferred through photosystem I are normally eventually used to reduce the coenzyme $NADP^+$ to NADPH, providing an essential source of reducing power for biosynthetic metabolic reactions.

The movement of electrons through the entire Z pathway is normally noncyclic, with a unidirectional electron flow from the electron donor H_2O to the electron acceptor $NADP^+$, but electrons can also flow cyclically through photosystem I. When this occurs, reduced coenzyme NADPH is not generated but ATP is synthesized. At low light intensities, many cyanobacteria can carry out non-oxygen-evolving photosynthesis, during which photosystem I follows this cyclic photophosphorylation pathway. No oxygen is generated because photosystem Ii is inoperative and therefore is not splitting water to generate oxygen. When only photosystem I is active, the cyanobacteria derive their reducing power from the oxidation of hydrogen sulfide, which is coupled with coenzyme reduction. When cyanobacteria utilize hydrogen sulfide as a reducing agent in this process, they form elemental sulfur granules that are deposited outside of the cells.

6)Anoxygenic photosynthesis

In anoxygenic photosynthesis, light energy is captured and used to generate ATP but oxygen is not produced. In the anaerobic green and purple photosynthetic bacteria and the heliobacteria, there is only one photosystem, known as photosystem I or cyclic oxidative photophosphorylation (Fig). An electron is initially removed from a bacteriochlorophyll molecule as a result of light excitation-thus oxidizing it-and ultimately returns to that molecule to reduce it. The excitation of the bacteriochlorophyll by absorbtion of light energy causes it to emit an electron. When the electron is emitted from the bacteriochlorophyll it is transported to a primary electron acceptor and then passes along an electron transport chain. A series of carriers takes it back to reduce the bacteriochlorophyll molecule from which it came. Thus there is no need for an external donor or acceptor of electrons. The bacteriochlorophyll molecule acts as an internal electron donor and acceptor mediating the cyclic flow of electrons around the photosystem. In essence, the energy of light drives electrons around the cycle repeatedly, with some of the energy being used to drive the synthesis of ATP. This ATP can be made because during the passage of electrons through the carriers of photosystem I, four protons are picked up from the cytoplasm of the cell; two of them

are used to reduce an oxidized carrier, known as the secondary quinone carrier, and two are extruded to the outside of the membrane. This causes a proton gradient and associated protonmotive force to be set up, which is used to generation ATP via chemiosmosis through membrane-bound ATPase.

Fig.

In the purple bacteria, photons (light energy) are initially absorbed by the antenna pigments and transferred to the photochemical reaction center pigments, which consist of four bacteriochlorophyll molecules, two molecules of bacteriopheophytin (bacteriochlorophyll that lacks magnesium atoms) and two ubiquinone molecules. Two of the bacteriochlorophyll molecules of the reaction center behave as a pair, and the initial interaction of the photon from the antenna pigments produces an excited singlet state in this pair as an electron is released from these molecules. The electron is rapidly transferred to the bacteriopheophytin and then to a primary ubiquinone. In the last step of the reaction center sequence, the electron is passed from the primary quinone to a secondary quinone. The reduced secondary quinone can transfer its electron to a cytochrome bc_1 complex. The cytochrome bc_1 complex transfers the electron to cytochrome C_2 that, in turn, passes the electron back to the oxidized bacteriochlorophyll pair.

Not all anoxygenic photoautotrophic bacteria contain this identical pathway of cyclic electron flow and electron carriers. Variations in different bacteria include (1) different numbers of reaction center pigment molecules, (2) substitutions of bacteriochlorophyll b for bacteriochlorophyll a, and (3) substitutions of menaquinones for ubiquinones. Reaction center bacteriochlorophylls absorb maximally at 840 nm in the green bacteria and at 798 nm in the heliobacteria.

Cyclic oxidative photophosphorylation generates ATP without generating reduced coenzymes. The anaerobic photosynthetic bacteria, however, require the reduced coenzyme NADH for biosynthetic reactions. To generate NADH, phototrophic anoxygenic bacteria utilize reduced inorganic compounds such as H_2S, H_2, or organic acids as electron donors. When bacteria utilize an organic compound such as malate as an electron donor, growth is said to be photoheterotrophic (photoorganotrophic). The electrons from the initial oxidation of bacteriochlorophyll are used to reduce NAD^+ to NADH and the electrons from the external organic electron donor can then be used to rereduce the oxidized bacteriochlorophyll molecule.

The flow of electrons from the external electron donor to form the reduced coenzyme may pass through photosystem I in a noncyclic pathway. In the purple sulfur and green nonsulfur photosynthetic bacteria, the reduction potential of the primary electron donor is not sufficient to reduce NAD^+. In these bacteria, reverse electron-flow up the electron transport chain driven by energy from the electrochemical potential of the membrane is used to drive the formation of reduced coenzyme.

1.3.2.4 ModelQuestions

1)Explain different strategies forgenerating cellular energy

2)Describe how the energy is produced by fermentation

1.3.2.5 Reference books

Lesson1.3.3

ROLE OF MICROORGANISMS IN CARBON & SULFUR CYCLE

Objective

Objective

Carbon,and Sulfur are the elements that are mainly forms the biomolecules in living systems.In the present chapter the chemical reactionsinvolved in the recyclisation process of the above elements in biotic and abiotic environments were dissussed

1.3.3.1 Introduction

1)Biogeochemical cycling

All living organisms carry out chemical transformations that influence their environment. Many of these chemical changes are a consequence of oxidation-reduction reactions that occur during microbial metabolism. Changes in the chemical forms of various elements can lead to the physical translocations of materials, sometimes mediating transfers between the atmosphere (air), hydrosphere (water), and lithosphere (land). These chemical and physical changes result in global cycling of substances.

Biogeochemical cycling is the movement of materials via biochemical reactions through the global biosphere. The biosphere is the portion of Earth and its atmosphere in which living organisms occur. The activities of microorganisms within the biosphere have a direct impact on the quality of human life. Without the essential biogeochemical cycling activities of microorganisms, all forms of life, including humans, could not exist.

1.3.3.2 Role of microorganisms in carbon cycle

Carbon is actively cycled between inorganic carbon dioxide and the various organic compounds that compose living organisms. The carbon cycle primarily involves the transfer of carbon dioxide and organic carbon between the atmosphere, where carbon occurs principally as inorganic CO_2, and the hydrosphere and lithosphere, which contain varying concentrations of organic and inorganic carbon compounds. In the lithosphere and hydrosphere, carbon dioxide reacts with water to form carbonate and bicarbonate, which are the principal inorganic forms of carbon found there.

1)Microbial metabolism of inorganic and organic carbon

The autotrophic metabolism of photosynthetic and chemolithotrophic organisms is responsbible for primary production: the conversion of inorganic carbon dioxide to organic carbon. After carbon is fixed (reduced) into organic compounds. It can be transferred from population to population within the biological community, supporting the growth of many heterotrophic organisms. The respiratory and fermentative metabolism of heterotrophic organisms returns inorganic carbon dioxide to the atmosphere, completing the carbon cycle. This represents the decay portion of the carbon cycle. The combination of carbon fixation by autotrophs and decomposition by heterotrophs cycles carbon through ecosystems.

Production of methane by a specialized group of methanogenic archaea represents a shunt to the normal cycling of carbon because the methane that is produced cannot be used by most heterotrophic organisms and thus is lost from the biological community to the atmosphere. Normally, fossil fuels, such as coal and petroleum, are not actively cycled through the activities of microorganisms. Burning of fossil fuels adds CO_2 to the atmosphere, which has led to a general rise in the concentration of atmpspheric CO_2 and a resultant warming of global temperatures, a phenomenon known as the greehouse effect. Agricultural practices that result in soil erosion and logging that results in deforestation are important contributors

to global warming through loss of organic carbon from within living biomass or soil organic matter that results in increased CO_2 production and release into the atmosphere.

The carbon dioxide converted into organic carbon by the primary producers, the autotrophs, in an exosystemm represents the gross primary production (total amount of organic matter produced) by the biological community in a given habitat. Part of the gross primary production is converted back to carbon dioxide by the respiration of the primary producers, and only the remaining organic carbon in the form of biomass and soluble metabolites the net primary production – is available for heterotrophic consumers in terrestrial and aquatic habitats. The oxidative metabolism of the biological community removes organic carbon and the energy stored in such compounds from the ecosystem and thus represents a decay of the energy stored within a given habitat. If the net primary production is greater than the community respiration, organic matter accumulates within the ecosystem. If, on the other hand, respiratory activities are greater than the net primary production, organic matter must be added from an external source or the community in that ecosystem will decline.

Most ecosystems depend on the photosynthetic fixation of carbon dioxide, that is, the input of organic matter by photosynthetic organisms, including plants, algae, and photosynthetic bacteria. The thermal rift areas of the deep ocean regions near the Galapagos Islands represent an interesting exception because the ecosystems associated with these areas are based on the input of organic carbon by sulfur-oxidizing chemolithotrophic bacteria that grow in the warm, hydrogen sulfide-rich waters that enter the ocean through thermal vents (Fig.). These organisms generate ATP and reduced coenzymes by oxidizing hydrogen sulfide and use the ATP and reduced coenzymes to drive the reduction of CO_2 via the Calvin cycle.

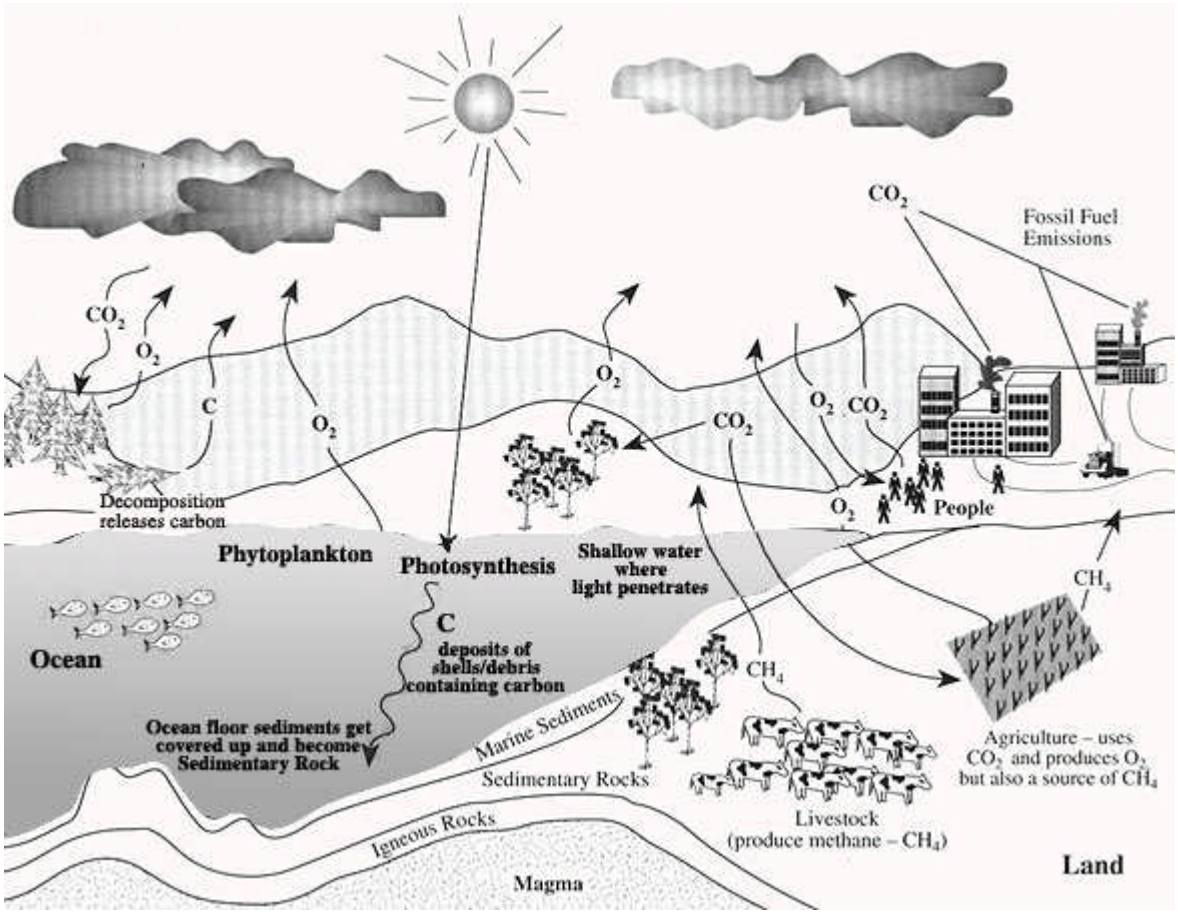
2)Trophic relationships

Feeding relationships between organisms establish the trophic structure, that is, the routes by which energy and materials are transferred within an ecosystem. This movement of carbon and energy through an ecosystem occurs in steps from one trophic level to another. Each step is called a trophic level (feeding level). When one organism consumes another, carbon and energy are transferred to the next higher trophic level. The carbon and energy in organic compounds that are formed by primary producers, move through the biological community of an ecosystem in this manner. Energymoves through the system in one direction while carbon is cycled. Only a portion of the energy is transferred, usually about 10%, to the next higher trophic level.

Transfer of energy stored in organic compounds between the organisms in the community forms a food web, an integrated feeding structure (Fig). At the base of the food web are the primary producers, which form the organic matter for the system. Grazers are organisms that feed on primary producers. In phytoplankton-based food webs, algae and cyanobacteria are the primary food source for grazers. In detrital food webs, microbial biomass produced from growth on dead organic matter (detritus) serves as a primary food source for grazers. The grazers, in turn, are eaten by predators, which in turn may be preyed on by larger predators. In this manner, carbon and stored energy are moved to the higher levels of the food web. Respiration causes some of the carbon and energy to be lost during each transfer.

The overall feeding relationships establish a pyramid of biological populations in the food web (Fig). The pyramid shape occurs because only a small portion of the energy stored in any trophic level is transferred to the next higher trophic level. Normally, 85% to 90% of the energy stored in the organic matter of a trophic level is consumed by respiration during transfer to the next trophic level and enters the decay portion of the food web. Since only 10% of the energy is transferred to each successively higher trophic level, the higher the trophic level, the smaller its biomass.

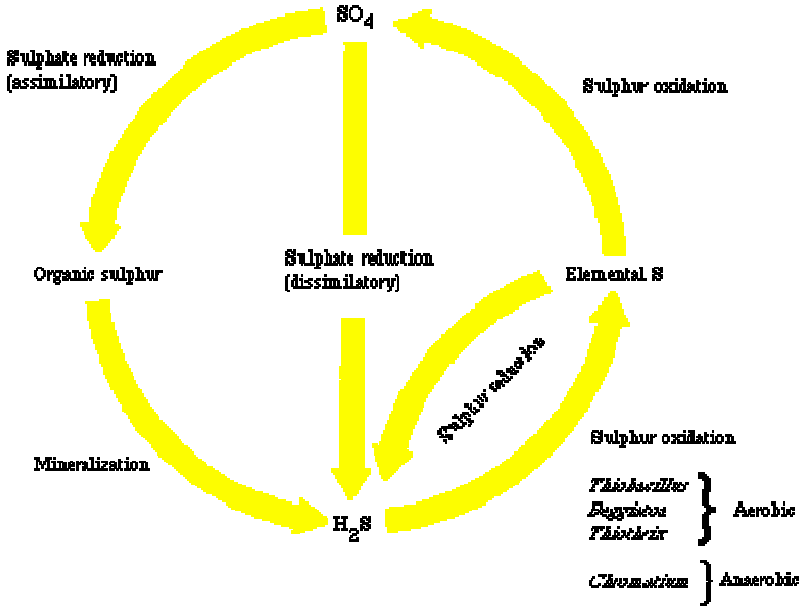
The decay portions of food webs are dominated by microorganisms (Fig). Microbial decomposition of dead plants and animals and partially digested organic matter is largely responsible for the conversion of organic matter to carbon dioxide and the reinjection of inorganic CO_2 into the atmosphere. The trates of organic matter mineralization depend on various factors, including environmental conditions – such as pH, temperature, and oxygen concentration – and the chemical nature of the organic matter. Some natural organic compounds, such as lignin, cellulose, and humic acids, are relatively resistant to attack and decay only slowly. Various synthetic compounds, such as DDT, may be recalcitrant, that is, completely resistant to enzymatic degradation. We depend on the activities of microorganisms to decompose organic wastes and, when microbial decomposition is ineffective, organic compounds accumulate. This is evidenced by the environmental accumulation of plastic materials that are recalcitrant to microbial attack. Many modern problems relating to the accumulation of environmental pollutants reflect the inability of microorganisms to degrade rapidly enough the concentrated wastes of industrialized.



1.3.3.3 Role of microorganisms in sulfur cycle

Sulfur can exist in various oxidation sates within organic and inorganic compounds, and oxidation-reduction reactions-mediated by microorganisms-change the oxidation states of sulfur within various compounds. Microbial transformations of sulfur establish the sulfur cycle (Fig). Microorganisms are capable of removing sulfur from organic compounds. Under aerobic conditions, the removal of sulfur (desulfurization) from organic compounds results in the formation of sulfate, whereas under anaerobic conditions hydrogen sulfide is normally produced from the mineralization of organic sulfur compounds (Fig). Hydrogen sulfide may also be formed by sulfate-reducing bacteria that utilize sulfate as the terminal electron acceptor during anaerobic respiration. Hydrogen sulfide can accumulate

in toxic concentrations in areas of rapid protein decomposition, is highly reactive, and is very toxic to most biological systems. It can react with metals to form insoluble metallic sulfides.



The predominant source of hydrogen sulfide in different habitats varies. In organically rich soils, most of the hydrogen sulfide is generated from the decomposition of organic sulfur-containing compounds. In anaerobic sulfate-rich marine sediments. Most of the hydrogen sulfide is generated from the dissimilatory reduction of sulfate by sulfate-reducing bacteria, such as members of the genus Desulfovibrio. Anaerobic sulfate reduction is important in corrosion processes and in the biogeochemical cycling of sulfur.

1)Use of hydrogen sulfide by autotrophic microorganisms

Although hydrogen sulfide is toxic to many microorganisms, the photosynthetic sulfur bacteria use it as an electron donor for generating reduced coenzymes during their metabolism. The anaerobic photosynthetic bacteria often occur on the surface of sediments, where there is light to support their activities and a supply of hydrogen sulfide from dissimilatory

sulfate reduction and anaerobic degradation of organic sulfur-containing compounds. Some photosynthetic bacteria deposit elemental sulfur as an oxidation product, whereas others form sulfate.

Some bacteria, including members of the genera Beggiatoa and Thiothrix, generate ATP by oxidizing hydrogen sulfide. These bacteria deposit elemental sulfur granules within the cell, which in the absence of hydrogen sulfide can be further oxidized to sulfate. Most Beggiatoa and Thiothrix species are not true chemolithotrophs and, although energy is apparently derived from the oxidation of hydrogen sulfide, these organisms require organic carbon for growth. Only a few marine species are autotrophs that obtain their carbon from carbon dioxide and generate cellular energy based on the oxidation of hydrogen sulfide. Chemolithotrophic members of the genus Thiobacillus oxidize sulfur as their source of energy. Thiobacillus species are used in bioleaching processes for mineral recover. Some Thiobacillus species are acidophilic and grow well at 2 to 3 pH. The growth of such species can produce sulfate from the oxidation of elemental sulfur, leading to the environmental accumulation of sulfuric acid.

2)Acid mine drainage

Acid mine drainage is a consequence of the metabolism of sulfur and iron-oxidizing bacteria. Coal in geological deposits is often associated with pyrite (FeS_2) and when coal mining activities expose pyrite ores to atmospheric oxygen, the combination of autoxidation and microbial sulfur and iron oxidation produces large amounts of sulfuric acid. When pyrites are mined as part of an ore recovery operation, oxidation may produce large amounts of acid. The acid draining from mines kills aquatic life and renders the water it contaminates unsuitable for drinking or for recreational uses. At present, approximately 10,000 miles of waterways in the United States are affected in this manner, predominantly in the states of Pennsylvania, Virginia, Ohio, Kentucky, Indiana, and Colorado. Strip mining is a particular problem with acid mine drainage because this method of coal recovery removes the overlying soil

and rock, leaving a porous rubble of tailings exposed to oxygen and percolating water. The problem of strip mining can be alleviated by covering with soil so as to reduce the availability of oxygen. Oxidation of the reduced iron and sulfur in the tailings produces acidic products, causing the pH to drop rapidly and preventing the reestablshment of vegetation and a soil cover that would seal the rubble from oxygen. A strip-mined piece of land continues to produce acid mine drainage until most of the sulfide is oxidized and leached out; recovery of this land may take 50 to 150 years.

The overall reaction for the oxidation of pyrite can be summarized as:

$$2\ 7\ H_2O\ FeS_2 + 7.5\ O_2 + 7\ H_2O \longrightarrow 2\ Fe(OH)_2 + 4H_2SO_4$$

The sulfuric acid produced accounts for the high acidity and the precipitated ferric hydroxide accounts for the deep brown color of the effluent. The mechanism of pyrite oxidation in acid mine drainage is quite complex. At neutral pH, oxidation by atmospheric oxygen occurs rapidly and spontaneously; below pH 4.5. autoxidation is slowed drastically. In the pH range of 4.5 to 3.5, the acidophilic bacteria of the genus Thiobacillus oxidize the reduced iron sulfide in the pyrite. The rate of microbial oxidation of FeS_2 is several hundred times greater than the rate of spontaneous oxidation, and although pyrite oxidation begins spontaneously, microbial oxidation of sulfur and iron is responsible for the continued production of high levels of acid mine drainage.

1.3.3.4 Modelquestions

1)Explain the role of microorganisms carbon cycle

2) Explain the role of microorganisms in sulfur carbon cycle.

1.3.3.5 Reference books

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Lesson 1.3.4

NITROGEN CYCLE AND REGULATION OF NITROGENASE

1.3.4.1 OBJECTIVE:

Nitrogen is the principlecomponent of proteins .The present chapter deaqls with the mechanismand regulatory points of nitrogen cycle and regulation ofnitrogenase and nif genes

1.3.4.1 Introduction

Nitrogen is a principle element present in all biomolecules.Eventhough the amont of nitrogen in atmosphere is more than 70% the living organisms cant utilize it directly .Mostly it will be taken into the body as nitrite or nitrate form, which will be formed by the action of different soil microbes.When the organisms die the nitrogen will once again reach the soil in the form of ammonia where it converts into nitrogen.

1.3.4.2 Role of microorganisms in nitrogen cycle

Nitrogen can exist in various oxidation states. Molecular nitrogen, the most abundant substance in the atmosphere, is not directly usable by most organisms; only a few bacteria use molecular nitrogen directly. Microorganisms utilize other forms of nitrogen such as NH_4^+, and NO_3^-, as well as organic nitrogen-containing compounds such as amino acids and proteins. The conversions of nitrogen compounds, primarily by microorganisms, change the oxidation states of nitrogenous compounds and establish a nitrogen cycle. As a result of the biogeochemical cycling of nitrogen, known as the nitrogen cycle, nitrogen moves from the atmosphere through the biota (soil and aquatic habitats).

1)Nitrogen fixation

The natural ability of organisms to convert atmospheric nitrogen to ammonia is called nitrogen fixation. This process provides fixed forms of nitrogen, such as ammonium ions, that can be used by other organisms. Other than the industrial chemical fixation of molecular nitrogen using the Haber-Bosch process to form nitrogen fertilizers, the natural biological fixation of nitrogen (conversion of N_2 to ammonia or organic nitrogen) is restricted to a very limited number of bacterial and archaeal species. No eukaryotic microorganisms, plants, or animals use atmospheric nitrogen directly; plants, or animals use atmospheric nitrogen directly; plants, animals, and most microorganisms depend on the availability of fixed forms of nitrogen for incorporation into their cellular biomass.

2)Free-living Nirtogen-fixing bacteria

In terrestrial habitats, the microbial fixation of atmospheric nitrogen is carried out by free-living bacteria and by bacteria living in symbiotic association with plants. Azotobacter species, which are free-living nitrogen fixers, have exceptionally high

respiratory rates far in excess of those of all other aerobic-bacteria, and this may prvent molecular oxygen from reaching and inactivating th oxygen –sensivitive nitrogenase. Azotobacter species also produce resting cells known as cysts that are quite resistant to desiccation but not to heat. Free-living, nitrogen-fixing members of the genera Azotobacter, Azomonas, and Derxia are common in temperate regions in neutral or alkaline soils and waters. These bacteria tend to be sensitive to low pH. In tropical regions, Beijerinckia species, which are more acid tolerant, are the prevalent nitrogen-fixing, free-living soil microorganisms. Frankia and other actinomycetes are also important symbiotic and free-living nitrogen-fixing bacteria in various terrestrial ecosystems.

Azospirillum lipoterum and Azotobacter paspali are nitrogen-fixing soil bacteria associated with the rhizophere of some tropical grasses. These bacteria use the organic compounds in root exudates as the energy source to support nitrogen fixation. Such nitrogen fixation within the rhizosphere is important for supporting the growth of rice. Azaspirillum also occurs in the rhizosphere of corn but does not appear to contribute significant concentrations of nitrogen to corn.

In aquatic habitats, cyanobacteria, such as Anabaena and Nostoc, are very important in determining the rates of nitrogen fixation.

The ability of microorganisms to fix nitrogen is readily detected by the acetylene reduction assay. The assay is based on the fact that the nitrogenase system also catalyzes the reduction of acetylene which, like molecular nitrogen, has a triple bond. The reduction of acetylene forms ethylene, which is easily detectable by gas chromatography. Consequently, many additional free-living bacteria have been shown to be capable of fixing atmospheric nitrogen. Most of these free-living, nitrogen-fixing bacteria exhibit nitrogen-fixing activities only at oxygen levels well below 0.2 atm. Such conditions frequently occur in subsoil and sediment environments. Although the amount of nitrogen fixed per hectare by free-living soil bacteria is considerably lower than the amount fixed by symbiotic nitrogen-

fixing species, the widespread distribution of the free-living bacteria in soil makes a significant contribution to the input of nitrogen to terrestrial habitats.

b)Nitrogen-fixing symbiosis of rhizobia and leguminous plants

Symbiotic nitrogen fixation by Rhizobium or Bradyrhizobium is most important in agricultural fields, where these bacteria live in association with leguminous crop plants. Rhizobium and Bradyrhizobium species are Gram-negative rod-shaped bacteria that form an association with leguminous plants; Rhizobium species are fast growing and Bradyrhizobium species grow slowly,. Rhizobium and Bradyrhizobium species generally exhibit rates of nitrogen fixation that are two to three orders of magnitude higher than those accomplished by free-living, nitrogen-fixing soil bacteria.

The highest rates of nitrogen fixation occur when nitrogen-fixing bacteria establish mutualistic relationships with plants. The nitrogen-fixing symbiotic relationship between members of the bacterial genera Rhizobium and Bradyrhizobium and leguminous plants is extremely important for maintaining soil fertility. These bacterial species invade the roots of suitable host plants, leading to the formation of nodules. Whithin nodules, Rhizobium and Bradyrhizobium are able to fix atmospheric nitrogen. Bradyrhizobium species nodulate soybeans, lupines, cowpeas, and various tropical leguminous plants. Rhizobium species nodulate alfalfa, peas, clover, and numerous other leguminous plants.

The symbiotic associations between nitrogen-fixing Rhizobium and Bradyrhizobium species and leguminous plants is very important to the bacterial and plant symbiotic partners, both of which benefit greatly. The leguminous plant derives great nutritional benefit from the fixed forms of nitrogen that it receives from the action of the bacterial nitrogenase (plants cannot directly obtain any nutritional benefit from molecular nitrogen). The rhizobia and bradyrhizobia obtain nutritional benefit from the organic acids or TCA cycle intermediates

supplied by the plants through their photosynthetic conversion of carbon dioxide to organic compounds.

The interaction between these microorganisms and a leguminous plant involves (1) attraction of the bacteria to the plant roots by amino acids secreted by the plant; (2) binding of the bacteria to receptors (lectins) on the plant root; (3) activity of plant growth substances, leading to curling and branching of the rootlets; (4) entry of bacteria into the root hairs; (5) development of an infection thread; (6) transformation of the plant cells to form a tumorous growth; (7) multiplication of bacteria within the nodule; and (8) transformation of the invading bacteria into distorted (pleomorphic) forms.

Nodulin genes essential for infection of the plant root and nodule formation by symbiotic nitrogen-fixing bacteria are divided into two classes. The first class includes genes that specify the biochemical composition of the bacterial cell surface, such as genes determining the synthesis of exopolysaccharides (exo genes), lipopolysaccharides (Ips genes), capsular polysaccharides or K antigens, and β-1,2-glucans (ndv genes). Mutations in these genes disturb the infection process to various degrees. The exo and lps genes may play a role in determining host specificity but this has yet to be firmly established.

The second class of genes consists of the nodulation (nod or nol) genes (Table). Inactivation of the nodulation genes can result in various plant phenotypes, such as the absence of nodulation (Nod^-), a delayed but effective nodulation (Nod^d Fix^+), or changes in the host range. Some of the nod genes appear to be interchangeable for nodulation function between different species and biovars (variants) and are designated therefore as common nod genes. Other nod genes are involved in the nodulation of a particular host and hence are called host-specific nod (hsn) genes.

Table Some Features of nod Gene Products

Nod Protein	Sequence Homology
NodA	Unknown

NodB	Deacetylase
NodC	Chitin synthases
NodD	Transcription actiyator, LysR family
NodE	β-Ketoacyl synthase
NodF	Acyl carrier protein
NodG	Alcohol dehydrogenase, β-ketoacyl reductase
NodH	Sulfotransferase
NodJ	Capsular polysaccharide secretion proteins
NodK	Unknown
NodL	Acetyltransferase
NodM	D-Glucosaming synthase
NodN	Unknown
NodO	Hemolysin
NodP	ATP-sulfurylase
NodQ	ATP-sulfurylase and APS kinase
NodS	Methyltransferase (Ac)
NodT	Transit sequences
NodU	Unknown
NodV	Sensor two-component regulatory family
NodW	Regulator, two-components regulatory family
NodX	Acidic exopolysaccharide encoded by exoZ

NodY	Unknown
NodZ	Unknown

In most Rhizobium species studied to date, the nod genes reside on large symbiotic plasmids (pSym) that also carry nif and fix nitrogen-fixing genes. The nif and fix genes include the structural genes fro nitrogenase. In Rhizobium loti and Bradyrhizobium and Axorhizobium spp., the symbiosisrelated genes are localized on the bacterial chromosome.

The common nod ABC genes have been found in all Azorhizobium, Rhizobium, and Bradyhizobium isolates studied so far. These genes have been called common nod genes because they are structurally conserved and functionally interchangeable between Rhizobium, Azorhizobium, and Bradyrhizobium species without altering the host range.

Rhizobium and Bradyrhizobium species have nif genes that are homologous to the 20 nif genes of Klebsiella. In Bradyrhizobium japonicum the nif and fix genes occur in the bacterial chromosome, whereas the nif genes in Rhizobium meliloti occur on a very large plasmid. Additionally, all nitrogen-fixing bacteria have ntr genes (nitrogen regulatory genes) that respond to levels of fixed forms of nitrogen and control the expression of nitrogen fixation genes. The ntr system is a complex two-component regulatory system.

At least ten different rhizobial nif genes have been identified in Rhizobium meliloti and Bradyrhizobium japonicum (Table; Fig). These bacteria have fix genes that are involved in nitrogen fixation but are not homologous to the Klebsiella nif genes. The fix genes form a heterogeneous group with varied functions, including the development and metabolism of bacteroids.

Table Functions Assciated with Rhizobia Genes for Nitrogen Fixation

Gene	Product and Function
NifH	Fe proteinof nitrogenase
NifD	α subunit of MoFe protein of nitrogenase
NifK	β subunit of MoFe protein of nitrogenase
NifE	Involved in FeMo cofactor biosynthesis
NifN	Involved in FeMo cofactor biosynthesis
NifB	Involved in FeMo cofactor biosynthesis
NifS	Cysteine desulfurase
NifW	Function unknown; required for full activity of FeMo protein
NifX	Function unknown
NifA	Positive regulator of nif, fix and other genes
Fix ABCX	Unknown function; required for nitrogenase activity; Fix X shows similarity of ferredoxins
Fix NOQP	Membrane – bound cytochrome oxidase
FixGHIS	Redox process – coupled cation pump
FixLJ	Oxygen-responsive two-component regulatory system involved in positive controlof fixK (Rm, Bj, Ac) and nifA (Rm)
FixK/fixK$_2$	Positive regulator of fixNOQP (Rm, Bj, Ac) nifA (Ac) rpoN, and"nitrate respiration" (Bj); negative regulator of nifA and fixK (Rm)
Rm fixK'	Reiterated, functional copy of fixK
Bj fixK	Function unknown

FixR	Function unknown
NrfA	Regulation of nifA

c)Nitrogen-fixing symbioses with nonleguminous plants

In addition to the symbiotic relationship between Rhizobium and Bradyrhizobium with leguminous plants, various other bacterial species, including cyanobacteria and actinomycetes, enter into similar mutualistic relationships with a restricted number of other types of plants.

Anabaena species, for example, form associations with plants in which the bacteria fix nitrogen. Anabaena is a cyanobacterium that establishes a symbiotic relationship with the water fern Azolla. Anabaena forms specialized cells called heterocysts, both in symbiotic association with Azolla and when free living, in which nitrogen fixation occurs. Only the noncyclic photosynthetic pathway is operative in heterocysts. The oxygen-producing photosystem II is is inactive in heterocysts, which is important because the enzymes responsible for nitrogen fixation (nitrogenases) are oxygen labile.

Rhizobium, for example, ca fix nitrogen in association with Trema, a tree found in tropical and subtropical regions. Likewise, the actionmycetes Frankia alni infects the roots of trees, leading to the formation of nodules. Frankia species are actinomycetes (filamentous bacteria) that form septated hyphae and numerous nomotile spores; Frankia species form associations with various nonleguminous plants, including various woody shrubs and small trees. Such an actionomycete-type nitrogen-fixing symbiosis is especially important with

angiosperms. The productivity of many forests depends on such nitrogen-fixing symbioses. In Frankia, a part of the hyphae becomes differentiated into specialized nitrogen-fixing cells called vesicles. Frankia is also capable of forming differentiated vesicles and fixing nitrogen when it is living free of a plan.

2)Ammonification

Many microorganisms, as well as plants and animals, convert organic amino nitrogen to ammonia; this process is known as ammonification. Deaminases play an important role in this process of ammonification, which transfers nitrogen from organic to inorganic forms. Microbial decomposition of urea, for example, results in the release of ammonia, which may be returned to the atmosphere of may occur in neutral aqueous environments as ammonium ions. Ammonium ions can be assimilated by various organisms, continuing the transfer of nitrogen within the nitrogen cycle.

3)Nitrification

Although many organisms are capable of ammonification, relatively few are capable of nitrification, the process in which ammonium ions (oxidation level = -3) are initially oxidized to nitrite ions (oxidation level = +3) and subsequently to nitrate ions (oxidation level = +5). Nitrification is an example of aerobic respiration. The oxidation of ammonia to nitrite and the oxidation of nitrite to nitrate, the two steps of nitrification, are energy yielding processes from which chemolithotrophic bacteria derive needed energy. The metabolism of the chemolithotrophic nitrifying bacteria changes the oxidation levels of the ammonium and nitrite ions when these ions serve as electron donors for chemiosmotic generation ATP.

Table Genera of Nitrifying Bacteria

Genus	Chemical Conversion	Habitat

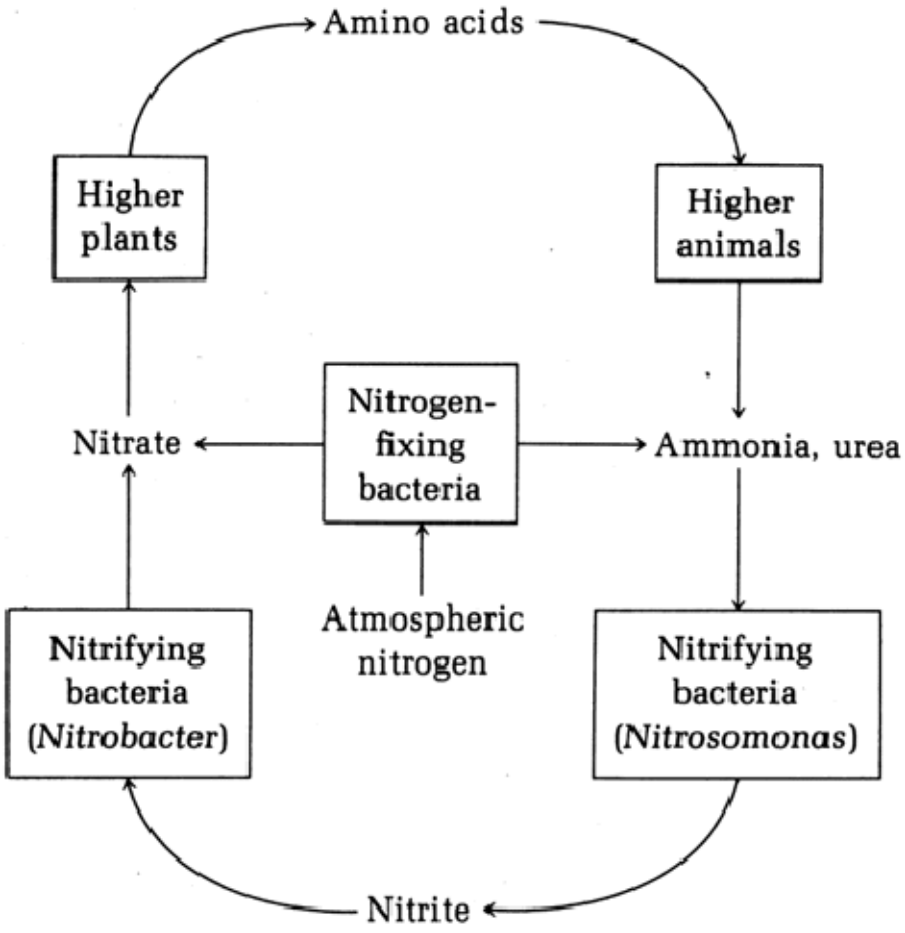
Nitrosomonas	Ammonia to nitrate	Soils, freshwater, marine
Nitrosospira	Ammonia to nitrate	Soils
Nitrosococcus	Ammonia to nitrate	Soils, freshwater, marine
Nitrosolobus	Ammonia to nitrate	Soils
Nitrobacter	Nitrate to nitrate	Soils, freshwater, marine
Nitrospina	Nitrite to nitrate	Marine
Nitrococcus	Nitrite to nitrate	Maine

Relatively low amount of ATP are generated by the oxidation of inorganic nitrogen compounds. Therefore large amounts of inorganic nitrogen compounds must be transformed to generate sufficient ATP to support the growth of these chemolithotrophic bacteria. The oxidation of approximately 35 moles of ammonia is required to support the fixation of 1 mole of carbon dioxide. The oxidation of approximately 100 moles of nitrite is required to support the fixation of 1 mole of carbon dioxide. As a consequence of the high amounts of nitrogen that must be transformed to support the growth of chemolithotrophic bacterial populations, the magnitude of the nitrification process is typically very high, whereas the growth rates of nitrifiers are generally relatively low compared to those of other bacteria.

The two steps of nitrification, the formation of nitrite from ammonium and the formation of nitrate from nitrite, are carried out by different microbial populations (Tab). For the most part, the oxidative transformations of inorganic nitrogen compounds in the nitrification process are restricted to several species of autotrophic bacteria. In addition to the chemolithotrophic nitrifying bacteria, some heterotrophic bacteria and fungi are

capable of oxidizing inorganic nitrogen compounds but the rates of heterotrophic nitrification are normally four orders of magnitude lower than those of autotrophic nitrification. In soils, Nitrosomonas often is the dominant bacterial genus involved in the oxidation of ammonia to nitrite and Nitrobacter often is the dominant bacterial genus involved in the oxidation of nitrite to nitrate. Several other autotrophic bacteria, including ammonia-oxidizing members of the genera Nitrosospira, Nitrosococcus, and Nitrosolobus and nitrite-oxidizing members of the genera Nitrospira and Nitrococcus, are also important nitrifiers in different ecosystems. Many of the nitrifying bacteria contain extensive internal membrane networks that are probably the sites of nitrogen oxidation.

The transfer of nitrate and nitrite ions from surface soil to groundwater supplies is critical for two reasons: (1) it represents an important loss of nitrogen from the soil, where it is needed to support the growth of higher plants, and (2) high concentrations of nitrate and nitrite in drinking water supplies pose a serious human health hazard. The use of nitrification inhibitors in combination with the application of ammonium nitrogen fertilizers can minimize the nitrate leaching problem and at the same time support better soil fertility and increased plant productivity.



4)Nitrite ammonification

Some bacteria, particularly Clostridium species, reduce nitrite to ammonium ions in a process called nitrite ammonification. Although involved in ATP generation, the process is not an example of anaerobic respiration. In nitrite ammonification, electrons from NADH are used to reduce nitrite rather than to reduce an organic compound. Consequently, the organic products of fermentation are more completely oxidized and the yield of ATP via substrate level phosphorylation can be greater. Denitrification, nitrite ammonification, does not remove nitrogen from the soil. In fact, much of the nitrate added to soils is reduced-to ammonia by fermentative bacteria rather than to N_2 by denitrifiers.

5)Denitrification

Denitrification, the conversion of fixed forms of nitrogen to molecular nitrogen, is another important process in the biogeochemical cycling of nitrogen that is mediated by microorganisms. Some aerobic bacteria can use nitrate in place of oxygen as a final electron acceptor, reducing nitrate as a result of anaerobic respiration. Some bacteria, such as E. coli, are only able to reduce nitrate to nitrite but various other bacteria can carry out the two subsequent anaerobic respirations by which nitrite ion is reduced to nitrous oxide gas (N_2O) and subsequently to molecular nitrogen (N_2). The process is called denitrification when N_2O or N_2 is produced. Some species of Pseudomonas, Moraxella, Spirillum. Thiobacillus, and Bacillus are capable of denitrification. Nitrous oxide formation occurs preferentially in habitats with high nitrate concentrations and / or low pH values. Formation of molecular nitrogen is favored when there is an adequate amount of organic matter to supply energy. Dissimilatory nitrate reductase, the enzyme involved in initiation of the denitrification process, is inhibited by oxygen, and denitrification process, is inhibited by oxygen, and denitrification generally occurs under anaerobic conditions. The return of nitrogen to the atmosphere by the denitriication process completes the nitrogen cycle.

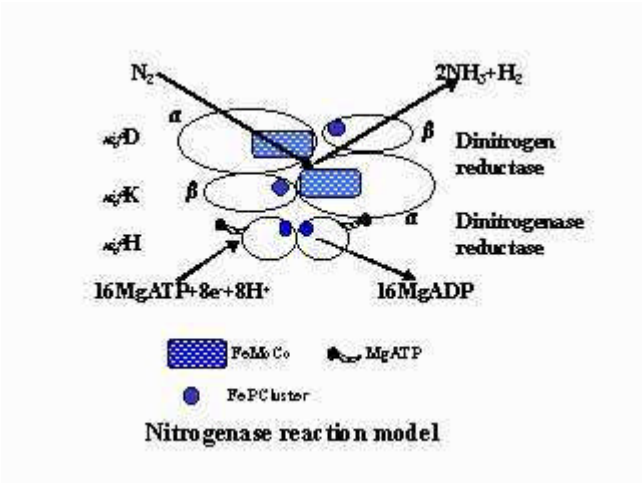
1.3.4.3 Regulation of Nitrogenase

The fixation of atmospheric nitrogen depends on the nitrogenase enzyme system (Fig). In this enzyme system, composed of nitrogenase and nitrogenase reductase, electrons are transferred through ferredoxin or flavodoxin to nitrogenase reductase, electrons are transferred through ferredoxin or flavodoxin to nitrogenase reductase and then to nitrogenase, where they are used to reduce N_2 and H^+ to NH_2 and H_2 according to the equation:

$$N_2 + 8e^- + 8H^+ + 16MgATP \longrightarrow 2NH_2 + H_2 + 16MgADP + 16\ P_i$$

The nitrogenase enzyme system has two coproteins, a MoFe protein containing molybdenum plus iron and a Fe protein

containing iron only. The active site of nitrogenase, where reduction of nitrogen actually occurs, is associated with an iron- and molybdenum-containing cofactor (FeMoco). The production of H_2 that accompanies the reduction of nitrogen adds to the ATP requirements the reduction of nitrogen adds to the ATP requirements of nitrogen fixation. Evolution of hydrogen accompanies biochemical nitrogen fixation. Only some strains of Rhizobium and Bradyrhizobium have hydrogenase and can utilize the hydrogen; other nitrogen-fixing bacteria wastefully evolve hydrogen gas. Nodulated root systems evolving hydrogen often are colonized by hydrogen-oxidizing Acinetobacter strains that grow on the hydrogen released by nitrogen-fixing bacteria.



Nitrogenase reaction model

Nitrogenase is very sensitive to oxygen and is irreversibly inactivated on exposure to even low concentrations. Nitrogen fixation therefore often is restricted to habitats in which nitrogenase is protected from exposure to molecular oxygen. Nitrogenase for example, is protected in the root nodule system, where some bacteria fix nitrogen, and by the red pigment leghemoglobin, which supplies oxygen to the organisms for respiration without denaturing the nitrogenase. Nitrogenase is also inhibited by high concentrations of ATP, but large amounts of ATP are required to drive the electron transfer reactions catalyzed by the nitrogenase enzyme system. The fixation of atmospheric nitrogen requires a high energy input

(approximately 30 ATP/N_2 fixed) and in terrestrial ecosystems largely depends on the availability of relatively high concentrations of organic matter for use in the respiratory generation of ATP. In addition to nitrogen reduction, the nitrogen ase complex forms one H_2 for every N_2 reduced and ca also reduce other substrates such as acetylene to ethylene.

Nitrogenase is a complex, oxygen-labile enzyme composed of dinitrogenase (MoFe protein) and dinitrogenase reduce (Fe protein). Dinitrogenase is composed of two dissimilar polypeptides, $\alpha_2\beta_2$. The α polypeptides are encoded by nifD and the βpolypeptides by nifk genes. The dinitrogenase protein contains two active metalloclusters: the P cluster containing 8 iron and 7 to 8 sulfur atoms (Fe_8S_{7-8}) and iron,-molybdenum cofactor (FeMoco) containing 7 iron, 9 surfur, one molybdenum atom, and one molecule of homocitrate (Fe_7S_9 Mo-homocitrate). The P cluster acts as an intermediate electron acceptor and probably transfers the electron to the FeMoco cluster. The FeMoco cluster functions as the site of nitrogen reduction.

The dinitrogenase reductase protein (Fe protein) consists of two identical polypeptides, γ_2, encoded by the nifH gene. Each polypeptide contains two iron atoms. The four Fe atoms are organized into an Fe_4S_4 cluster. The main function of the Fe protein is to bind and hydrolyze MgATP and to transfer electrons from the Fe_4S_4 cluster to the P cluster of the MoFe protein. Both proteins are folded in such a way as to bring the active centers of each in close proximity.

During enzymatic catalysis, electrons are sequentially transferred one at a time from the iron centers of dinitrogenase reductase to the ironmolybdenum cofactors of dinitrogenase. The electrons are ultimately transferred to the substrate, nitrogen, thus reducing it. Multiple rounds of electron transfer must occur before nitrogen is reduced to ammonia and at least two Mg-ATP molecules are hydrolyzed for each electron that is transferred. The Mg-ATPs bind to the Fe protein, dinitrogenase reductase, but ATP is not hydrolyzed unless the Fe protein is complexed to the MoFe protein, dinitrogenase.

Several enzymes are involved in the transfer of electrons to dinitrogenase reductase; at least twenty adjacent genes are involved in nitrogen fixation in Klebsiella pneumoniae, a free-living bacterium that fixes nitrogen under conditions of reduced oxygen concentration. Klebsiella nif genes are organized into eight operons that occupy about 24kb of DNA.

Some methanogens (archaea) fix atmospheric nitrogen and their nif genes have a high degree of homology to those of free-living nitrogen-fixing bacteria. In Methanococcus thermolithotrophicus there are two nifH genes that are less similar to each other than they are to sequences of many bacterial nifH genes. Overall nif genes of bacteria and archaea appear to have evolved from the same common ancient ancestor.

1.3.4.4 Model questions

1)Write a detail account on different steps involved in nitrogen cycle
2)Write about the regulation of nitrogenase and nif gene

1.3.4.5 Reference books

Shlegel, Microbiology, Cambridge University press

Atlas, Principles of Microbiology, McGraw-Hill Science Publishers

Jogdand, S.N. (2003) Gene Biotechnology, Himalayan Publishing House, Mumbai

Lesson 1.4.1

Introduction to Immune System

1.4.1.1 Objective :

The main objective of this chapter is give a detailed description of the immune system

1.4.1.2 Intraduction

The immune system is a complex network of specialized cells and organs that has evolved to defend the body against attacks by "foreign" invaders. When functioning properly it fights off infections by agents such as bacteria, viruses, fungi, and parasites. When it malfunctions, however, it can unleash a torrent of diseases, from allergy to arthritis to cancer to AIDS.

The immune system evolved because we live in a sea of microbes. Like man, these organisms are programmed to perpetuate themselves. The human body provides an ideal habitat for many of them and they try to break in; because the presence of these organisms is often harmful, the body's immune system will attempt to bar their entry or, failing that, to seek out and destroy them.

The immune system, which equals in complexity the intricacies of the brain and nervous system, displays several remarkable characteristics. It can distinguish between "self" and "non self." It is able to remember previous experiences and react accordingly; thus, once you have had chicken pox, your immune system will prevent you from getting it again. The immune system displays both enormous

diversity and extraordinary specificity; not only is it able to recognize many millions of distinctive non self molecules, it can produce molecules and cells to match up with and counteract each one of them. And it has at its command a sophisticated array of weapons.

The immune system is composed of many interdependent cell types that collectively protect the body from bacterial, parasitic, fungal, viral infections and from the growth of tumor cells. Many of these cell types have specialized functions. The cells of the immune system can engulf bacteria, kill parasites or tumor cells, or kill viral-infected cells

The organs of the immune system are stationed throughout the body.They are known as lymphoid organs because they are concerned with the growth, development, and deployment of lymphocytes—white blood cells that are key operatives of the immune system.The organs involved with the immune system are called the lymphoid organs, which affect growth, development, and the release of lymphocytes (a certain type of white blood cell). The blood vessels and lymphatic vessels are important parts of the lymphoid organs, because they carry the lymphocytes to and from different areas in the body. Each lymphoid organ plays a role in the production and activation of lymphocytes.

The lymphoid system consists of primary lymphoid organs or central lymphoid tissue (bone marrow, thymus) and secondary lymphoid organs or peripheral lymphoid tissue (lymph nodes, spleen, mucosa-associated lymphoid tissue(1.4.1.1)

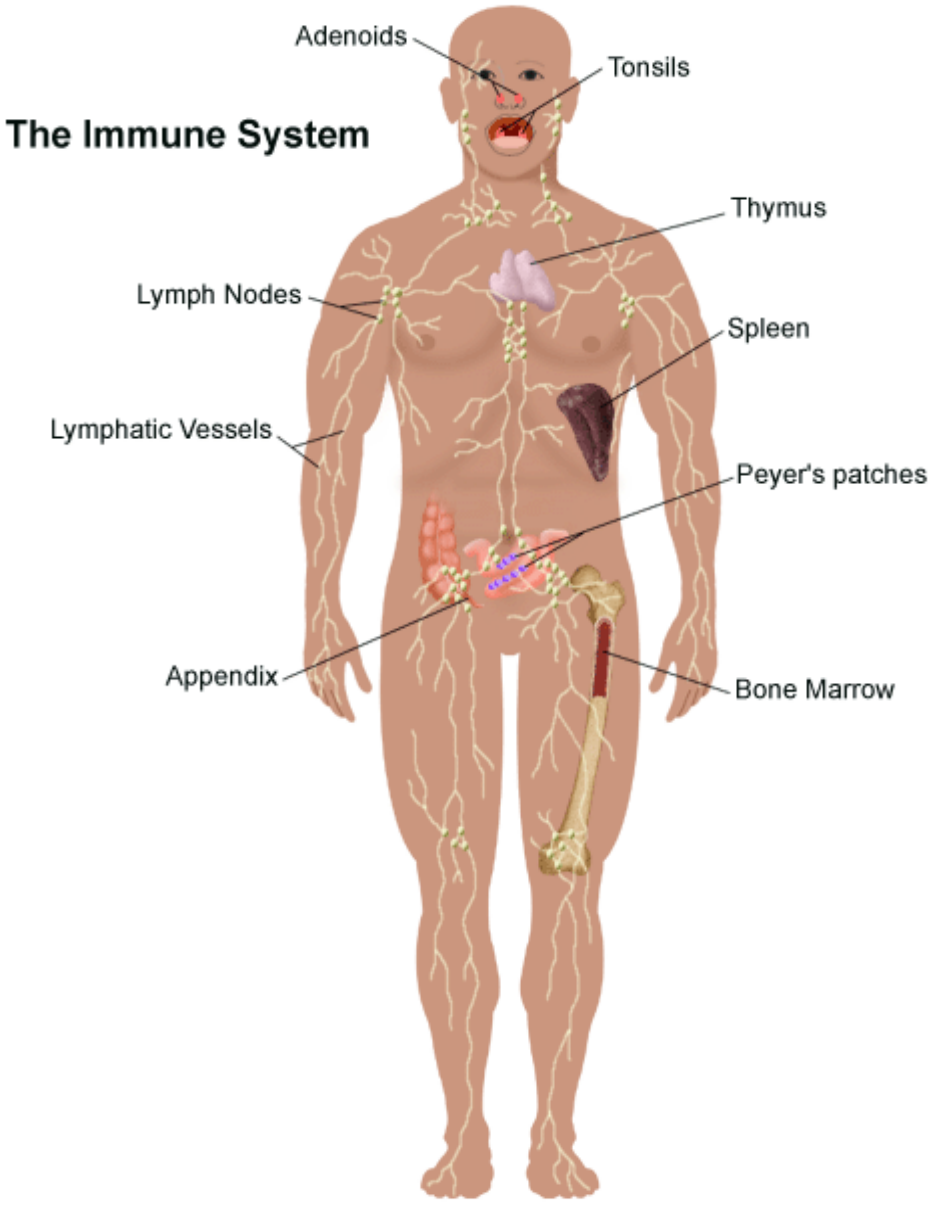


Fig 1.4.1.1 Organization of the immune system in the human body

1.4.1.3 The Lymphoid System

The body uses the lymphoid system to enable lymphocytes to encounter antigens and it is here that adaptive immune responses are initiated. The lymphoid system consists of primary lymphoid organs or Central lymphoid tissue (bone marrow, thymus) and Secondary or Peripheral lymphoid tissue (lymph nodes, spleen, mucosa-associated lymphoid tissue

1.4.1.3.1 Primary lymphoid organs or Central lymphoid tissue:

The bone marrow and the thymus constitute the primary lymphoid organs. Both B-lymphocytes and T-lymphocytes are produced from stem cells in the bone marrow. B-lymphocytes mature in the bone marrow while T-lymphocytes migrate to the thymus and mature there. After maturation, both B-lymphocytes and T-lymphocytes circulate through and accumulate in secondary lymphoid organs

1.4.1.3.1.1 Bone Marrow :

All the cells of the immune system are initially derived from the bone marrow. They form through a process called hematopoiesis. During hematopoiesis, bone marrow-derived stem cells differentiate into either mature cells of the immune system or into precursors of cells that migrate out of the bone marrow to continue their maturation elsewhere. The bone marrow produces B cells, natural killer cells, granulocytes and immature thymocytes, in addition to red blood cells and platelets:

Bone marrow is a loosely-organized grouping of cells located in central soft tissue portion of bones (surrounded by the calcified matrix) throughout the body Other cells found here include hematopoietic stem cells and progenitor cells, as well as immature and mature forms of all blood cells Hematopoietic stem cells (HSC) present in the bone marrow are responsible for development of all blood cells after about the seventh month of gestation in humans B lymphocytes, granulocytes, monocytes and erythrocytes all develop to maturity in the bone marrow before they are released into the bloodstream for transport to other locations in the body Pro T

lymphocytes (immature T cells) are released into the bloodstream before final maturation as a matter of course ... in contrast to granulocytes and erythrocytes, which may be released into the bloodstream in immature forms in times of great need

1.4.1.3.1.2 Thymus-

It is a bi lobed organ in the upper anterior thorax right above the heart.Each lobe is surrounded by a capsule and divided into multiple lobules by fibrous septa or trabeculae. Each lobule consists of an outer cortex and an inner medulla. The thymus is the site of T cell maturation. Cellular Organization of the Thymus The cortex contains a dense collection of pre T lymphocytes (thymocytes), The lighter staining medulla is more sparsely populated with lymphocytes. The thymus has a rich vascular supply and efferent (exiting) lymphatic vessels that drain into mediastinal (tissue between two lungs) lymph nodes. The thymus has no afferent lymphatic supply. Thus the traffic of pre T cells to the thymus is via the blood. The differentiated T cells leave through the efferent lymphatics or the venous drainage (by the medullary vein). Lymphocytes can recirculate back into the thymus from the blood. Both the cortex and medulla of the thymus are crisscrossed by a 3 dimensional network of stromal cells composed of thymic epithelial cells, interdigitating dendritic cells, and macrophages which make up the framework of the organ and contribute to thymocyte maturation. Many of these stromal cells physically interact with the developing thymocytes. These stromal cells produce thymic hormones and express high levels of MHC molecules. In the medulla are structures called Hassall's corpuscles which are composed of tightly packed concentric whorls of epithelial cells that may be remnants of degenerating cells so are believed to be sites of cell destruction.

Function of the Thymus
Precursors that are committed to the T cell lineage called thymocytes enter the thymic cortex via blood vessels and mature there to become antigencommitted, immunocompetent T cells. Large numbers of primitive T cells enter the thymus and cells that might recognize self

antigens do not survive whereas cells whose receptors are specific for foreign antigens are stimulated to mature and to start expressing surface markers. These selection processes are critical for the ability of the immune system to discriminate between self and nonself. It is estimated that in mice about 50 X 106 immature cells enter the thymus everyday and fewer than 1 x 106 mature cells leave. Although 98% of the thymocytes generated each day die no widespread damage is seen indicating that death is occurring by apoptosis rather than necrosis. The thymus undergoes physiologic involution with aging, so that by puberty it is difficult to locate. Maturation and selection of T cells continues well into adult life suggesting that either the remnants of the thymus are adequate for performing these functions or extrathymic T cell
maturation can also occur. However, no such extrathymic sites of T cell development have been identified.

The development of T cells can be considered as a series of discrete phases.
Thymic epithelial cells secrete several hormonal factors necessary for the differentiation and maturation of T lymphocytes. Thymocyte progenitors enter the thymus in the subcapsular region. At this stage they express neither the antigen receptor nor either of the two coreceptors CD4 and CD8 (the cell surface markers for T helper and T cytotoxic cells respectively) and are known as double negative cells. As double negative cells the thymocytes proliferate and begin the process of gene rearrangement that culminates in the expression of the preT cell surface receptor along with the co receptors CD4 and CD8 to produce double positive cells. As the cells mature they move deeper into the thymus. These cells then rearrange the T receptor genes to generate antigenic diversity and become sensitive to peptide:MHC complexes. After developing thymocytes begin to express antigen binding receptors, they are subjected to a selection process so that only T cells recognizing antigenic peptides in the context of self-MHC molecules are released from the thymus. Thymic stromal cells which express high levels of class I &II MHC molecules play a role in this selection process.The first step is positive selection- Any developing thymocytes that are unable to recognize self-MHC molecules are not selected and are thought to be eliminated by programmed cell death (apoptosis). The second step is negative selection- potentially self reactive thymocytes with a high affinity receptor for self antigen plus self-MHC are eliminated. By means of positive and negative selection in the thymus, tolerance to self antigens is achieved by eliminating T cells that are self reactive and only those cells whose receptor

recognizes an MHC molecule plus foreign antigen are allowed to mature.(fig 1.4.1.2)

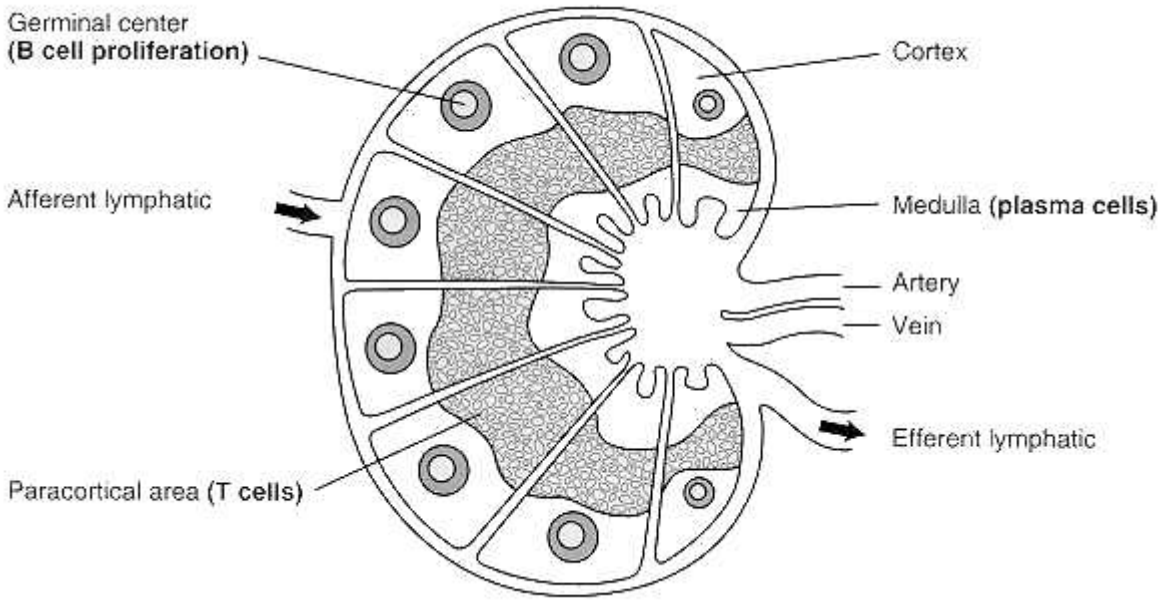


Fig 1.4.1.2 Cross section of the Thymus

1.4.1.3.2 Secondary lymphoid Organs or Peripheral Organs

Adaptive immune responses require antigen-presenting cells, such as macrophages and dendritic cells, and ever changing populations of B-lymphocytes and T- lymphocytes. These cells gather to detect antigens in secondary lymphoid organs. The secondary lymphoid organs include highly organized lymphoid organs such as lymph nodes and the spleen, as well as less organized accumulations of lymphoid organs scattered strategically throughout the body. These latter include the tonsils, the appendix, the Peyer's patches in the lining of the small intestines (part of the gut-associated lymphoid tissue or

GALT), lymphoid tissue beneath the mucous membranes of the bronchi (bronchial-associated lymphoid tissue or BALT), and aggregates of lymphatic tissue throughout the mucous membranes (mucosa-associated lymphoid tissue or MALT) and beneath the skin (skin-associated lymphoid tissue or SALT).

1.4.1.3.2.1 Lymph Nodes:

Lymphonodes are small nodular aggregates of lymphoid tissue situated along
lymphatic channels throughout the body. These nodes are clustered at junctions of the lymphatic vessels, they are the first organized lymphoid structure to encounter most antigens that enter the tissue spaces.

Each node is surrounded by a fibrous capsule that is pierced by numerous
afferent (arriving) lymphatics which empty the lymph into a subcapsular
sinus. Morphologically a lymph node can be divided into 3 roughly concentric
regions: the cortex, paracortex, and medulla. The node consists of cortex in which there are aggregates of cells constituting the **follicles** some of which contain central areas called **germinal centers**. Follicles without germinal centers contain predominantly mature, resting B cells that have apparently not been stimulated with antigens. The germinal centers develop in response to antigen stimulation. Following infection or introduction of other antigen into the body, the lymph leaving a node through its single efferent lymphatic vessel is enriched with Abs newly secreted by medullary plasma cells and also has a 50 fold increase of lymphocytes than the afferent lymph. The increase in lymphocytes is due in part to lymphocyte proliferation within the node in response to antigen but bloodborne lymphocytes can also migrate into the node by crossing a post capillary venule. Because antigenic stimulation can increase migration 10 fold, during an
active immune response the influx of cells can be seen as swelling of the
nodes.(fig 1.4.1.3)

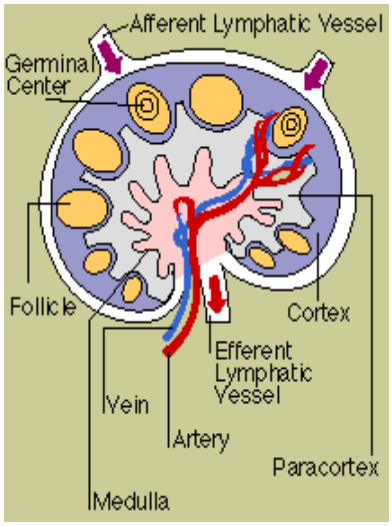


Fig 1.4.1.3 Cross section of Lymph node

1.4.1.3.2.2 SPLEEN:

The spleen is an organ weighing about 150 gm in adults located in the left upper quadrant of the abdomen. It is supplied by a single artery which pierces the capsule at the hilum and divides into progressively smaller branches that remain surrounded by protective and supporting fibrous trabeculae. The spleen is similar in structure to a large lymph node. It has, however, special features not seen in the lymph nodes. It is a vascular organ, having a large arterial blood supply. On entering the spleen, the blood flow enters a meshwork of dilated blood vessels, or "sinuses", which lie between large masses of lymphocytes. The walls of the sinuses contain phagocytes that are capable of engulfing dead cells and foreign particles in the blood and removing them from the general circulation Like the lymph nodes, the spleen is an important source of antibodies, however, to a greater extent than the lymph nodes, the spleen is concerned with the removal of abnormal or normally worn out ("dying") red blood cells from the circulation by destroying them

The spleen is encased in a thick connective-tissue capsule. Inside, the mass of splenic tissue is of two types, the red pulp and the white pulp, which do not separate into regions but intermingle and are distributed throughout the spleen. The white pulp is lymphoid tissue that usually

surrounds splenic blood vessels. The red pulp is a network of channels (sinuses) filled with blood, and it is in the red pulp that most of the filtration occurs.

The white pulp of the spleen contains such typical lymphoid elements as plasma cells, lymphocytes, and lymphatic nodules, called follicles in the spleen. Like the lymph nodes, it reacts to microorganisms and other antigens that reach the bloodstream. Phagocytic cells in both red and white pulp serve to remove foreign material from the blood and initiate an immune reaction that results in the production of antibodies. Germinal centers in the white pulp are sites of lymphocyte production.

The red pulp has a specialized role in addition to filtration. It is the body's major site of the destruction of red blood cells, which normally have a life span of only 120 days. Degenerate red cells are removed from the circulation in the spleen, and the hemoglobin that they contain is degraded to a readily excretable pigment and an iron molecule that is recycled; i.e., used to produce new hemoglobin elsewhere.

In some species the spleen also acts as a reservoir for blood during periods of inactivity. When such an animal is aroused for defense or flight, the capsule of the spleen contracts, forcing additional blood reserves into the circulation. The human spleen probably does not have this capability(1.4.1.4)

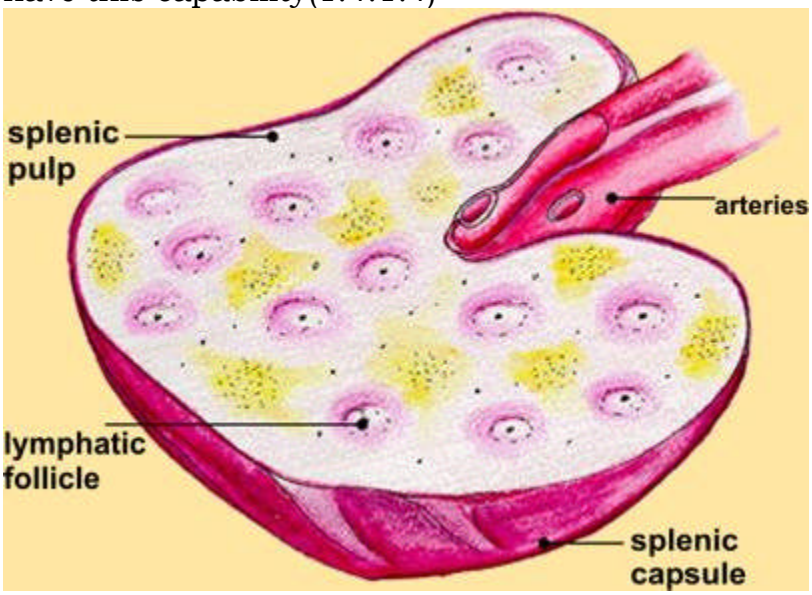


Fig 1.4.1.4 Cross section of the spleen

1.4.1.3.2.3 Other Tissues

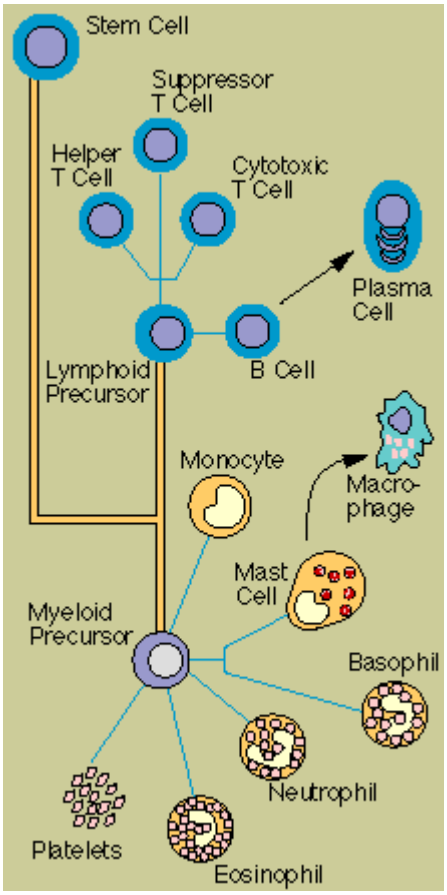
Lymphocytes are found either scattered or in aggregates in many tissues. Some of these collections are anatomically well organized and have unique properties. The mucous membranes lining the digestive, respiratory, and urogenital system have a combined surface area of about 400 m^2 and are the major sites of entry for most pathogens. The defense of these vulnerable membrane surfaces is provided by organized lymphoid tissues known collectively as mucosal associated lymphoid tissue
(MALT). The **mucosal immune system** consists of aggregates of lymphocytes, and other accessory cells located beneath the mucosal epithelium. These include the payer's patches in the lamina propria of the small intestine, lymphoid follicles in the appendix, tonsils in the pharynx, and sub mucosal lymphoid follicles throughout the upper airways and bronchi. The functional importance of MALT in the body's defense is attested to by its large population of Antibody producing plasma cells-the number far exceeds that of plasma cells in the spleen, lymph nodes, and bone marrow combined.

The **cutaneous immune system** consists of immune cells in the dermis. It is the site of immune responses to topically applied antigens. An important Ag presenting cell in the skin is the epidermal **Langerhans cell** which has been postulated to be related to the dendritic cells of the lymphoid organs.

1.4.1.4 The Cells of the Immune System

The main function of the immune system is to defend the body against a wide variety of pathogenic infectious agents with vastly differing natures, i.e. viruses, bacteria, fungi, protozoa and parasitic worms. The complexity of this task requires a sophisticated repertoire of mechanisms for the recognition of, and defence of the body against, these pathogens. This is achieved by an array of cells (and molecules which they secrete) which are dispersed throughout the body and collectively constitute the immune system.

Most of the major cell types of the immune system are derived from progenitors (stem cells) in the bone marrow. Many of the mature cells circulate in the bloodstream and are dispersed throughout tissues of the body, while some also congregate in specialized lymphoid tissues. Furthermore, in order to generate effective immunity, the various cell types cooperate with each other by means of direct interactions between cell surface molecules and via the molecules that they secrete(1.1.5)



1.4.1.4.1 Lymphocytes

The three major types of lymphocytes are called B cells, T cells and NK (natural killer) cells. They arise from lymphoid progenitors in the bone marrow: mammalian B cells fully develop here, whereas T cell precursors migrate to the thymus for selection and maturation. The bone marrow and thymus are thus known as primary lymphoid organs. Mature B and T cells circulate in the bloodstream and lymphatic system, spending some time in the secondary lymphoid tissues, i.e. the spleen, lymph nodes and mucosa-associated lymphoid

tissues (MALT). Two morphological types of resting lymphocytes can be distinguished: B cells and the majority of T cells are small lymphocytes with a thin rim of cytoplasm surrounding the nucleus, whereas NK cells and some T cells are larger, have more cytoplasm and distinct cytoplasmic granules, and are known as large granular lymphocytes (LGLs)

B and T lymphocytes are entirely responsible for adaptive or acquired immunity, i.e. the ability to recognize each pathogen in a specific way and to mount a faster and bigger response on repeated exposure to a particular pathogen (immunological memory). This is because B and T cells express surface receptors which specifically bind to materials that are foreign to the body (known as antigens). The receptors of a single lymphocyte are identical to each other and recognize a single antigen. However, millions of different antigen receptors are collectively expressed by the whole population of lymphocytes in the human body, thus conferring the ability to recognize a great many foreign antigens. A lymphocyte can be activated when it binds an antigen for which its receptors are specific, causing it to become an enlarged, dividing lymphoblast. Some of the progeny differentiate into short-lived effectors of the immune response while others become long-lasting memory cells which will be reactivated if there is subsequent exposure to the same antigen

1.4.1.4.1.1 B lymphocytes

B-lymphocytes refer to lymphocytes that are produced in the bone marrow and require bone marrow stromal cells and their cytokines for maturation. During its development, each B-lymphocyte becomes genetically programmed through a series of gene-splicing reactions to produce an antibody molecule with a unique specificity - a specific 3-dimensional shape capable of binding a specific epitope of an antigen

The B cells constitute 5–15% of human blood lymphocytes. The main function of a B cell is to secrete soluble recognition molecules called antibodies which specifically bind to an antigen recognized by that B cell. These antibodies (also known as immunoglobulins) are, in fact, the secreted form of a B cell's surface antigen receptors and bind to exactly the same antigen (fig1.4.1.6) A B cell will only produce antibodies when it has been activated by binding antigen; this activation process also usually requires help from T cells. The

activated B cell undergoes multiple divisions and some of the resulting cells differentiate into antibody-secreting cells. These are known as plasma cells, and they possess copious rough endoplasmic reticulum involved in antibody synthesis

During activation, B cells can undergo two types of genetic changes that modify the nature of the antibodies they produce. First, they can change their antigen-binding properties by a process called somatic mutation, so that some B cells (and their antibodies) bind more strongly to their specific antigen. Secondly, they can change their immunoglobulin class (which is initially IgM and IgD) to produce antibodies with different biological effector functions (IgG, IgA or IgE). The main structural difference between antibodies of different classes is in the nonantigen-binding portion of the molecules, called the Fc region, which is constant in structure between antibodies of the same class produced by different B cells. Various cell types of the immune system which do not themselves have antigen-specific receptors, and are therefore considered to be components of the innate immune system, express receptors which bind to the Fc region of antibodies. The Fc receptors thus enable these cells to bind antigens via interaction with antibodies specific for the antigens.(1.4.1.7) The binding of an antibody with its antigen on the one hand, and with an Fc receptor on the other, are both high-affinity interactions: this is therefore a very efficient mechanism for targeting antigens to cells of the immune system. An important aspect of antigen recognition by B cells is that these lymphocytes, and the antibodies they produce, bind to antigens in their natural or native form, i.e. as they occur as constituents of pathogens

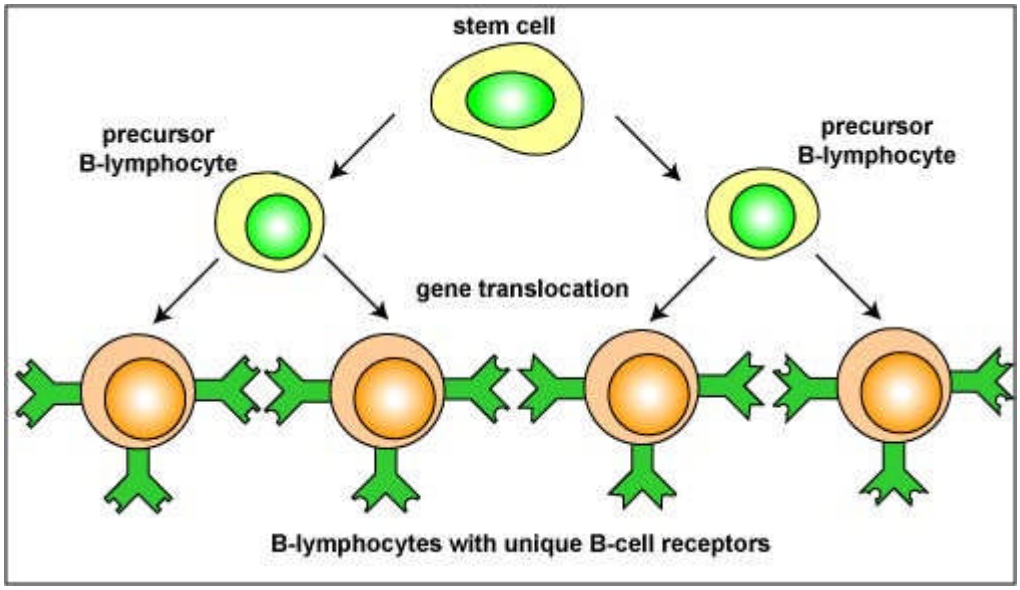

stem cell
precursor
B-lymphocyte
precursor
B-lymphocyte
gene translocation
B-lymphocytes with unique B-cell receptors

Fig 1.4.1.6

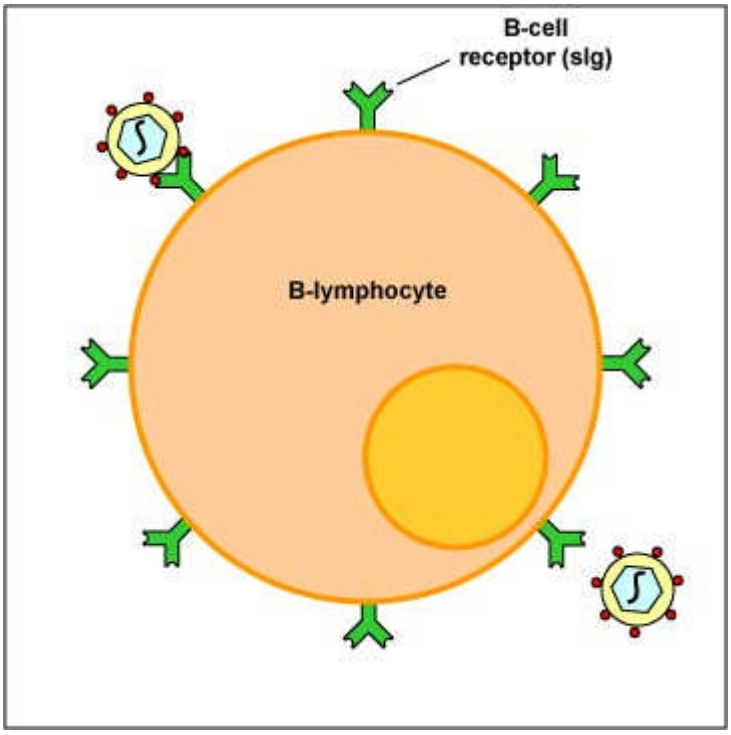

B-cell
receptor (sIg)
B-lymphocyte

Fig 1.4.1.7

1.4.1.4.1.2 T lymphocytes

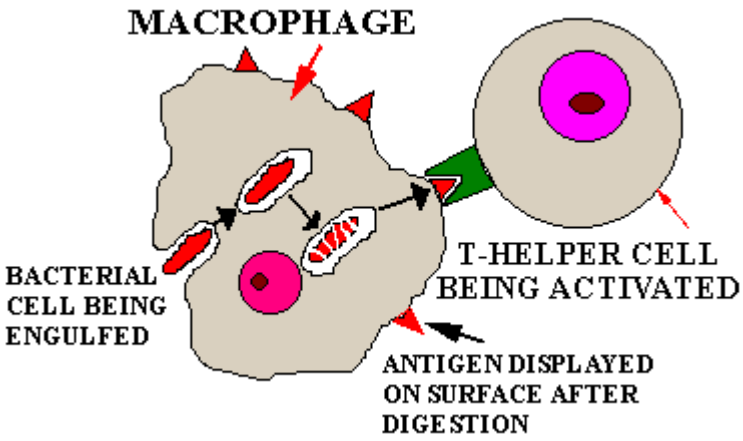
About 70% of human blood lymphocytes are T cells. The main functions of T lymphocytes are to exert effects on other cells, either regulating the activity of cells of the immune system or killing cells that are infected or malignant. Like B lymphocytes, T cells have surface antigen receptors, but there is no secreted form of these equivalent to antibodies. Furthermore, T cells cannot recognize antigens in their native forms, but only when they are presented on the surface of antigen-presenting cells (APCs). The antigen receptors of most T cells (αβ T cells) are composed of two polypeptides called α and β chains, and they interact with peptides derived from the degradation (processing) of foreign antigenic proteins. These peptides are bound to molecules of the major histocompatibility complex (MHC) on the surface of APCs The interaction between the T-cell antigen receptors and the peptide–MHC complexes binds a T cell to the surface of an APC, thus targeting the T cell to exert effects on the APC. There are two types of MHC molecules, called class I and class II, which present antigen peptides to αβ T cells expressing the surface proteins CD8 or CD4, respectively. This is because CD8 binds to MHC class I and CD4 binds to MHC class II

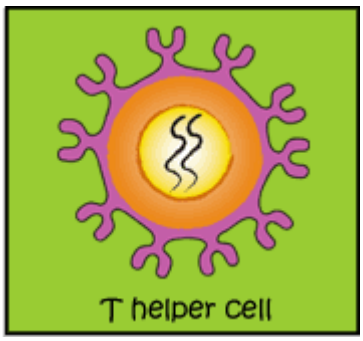
CD4+ T lymphocytes

The main function of CD4-expressing T cells is to help other cells of the immune system to mediate immune responses: for this reason they are called helper T (T_H) cells For example, T_H cells help B cells to become activated and differentiate into plasma cells, or help macrophages to become more effective at killing bacteria. Macrophages and B cells, together with dendritic cells, are known as professional APCs because they normally express MHC class II molecules when activated, whereas most other cells of the body do not. This targets CD4+ T_H cells to interact with these cells, thereby

giving the appropriate focusing of their activity. Indeed, the binding of T_H cells to professional APCs is a mutual interaction in which the APCs activate the T cells as well as vice versa The activities of T_H cells involve not only direct interactions between cell surface molecules, but also the effects of secreted regulatory proteins known as cytokines. Different T cells develop different profiles of cytokine production: T_H1 cells secrete cytokines (e.g. interleukin 2 and interferon γ) that mainly promote cell-mediated immunity by cytotoxic T cells and macrophages, whereas the cytokines produced by T_H2 cells (e.g. IL-4 and IL-10) primarily stimulate antibody production by B cells. Some cytokines also downregulate immune responses by suppressing the activity of cells of the immune system. For example, T_H1 and T_H2 cells are mutually inhibitory by virtue of the cytokines they produce, and some T lymphocytes (called T_H3 cells) produce transforming growth factor β, which is generally immunosuppressive

Fig 1.4.1.7

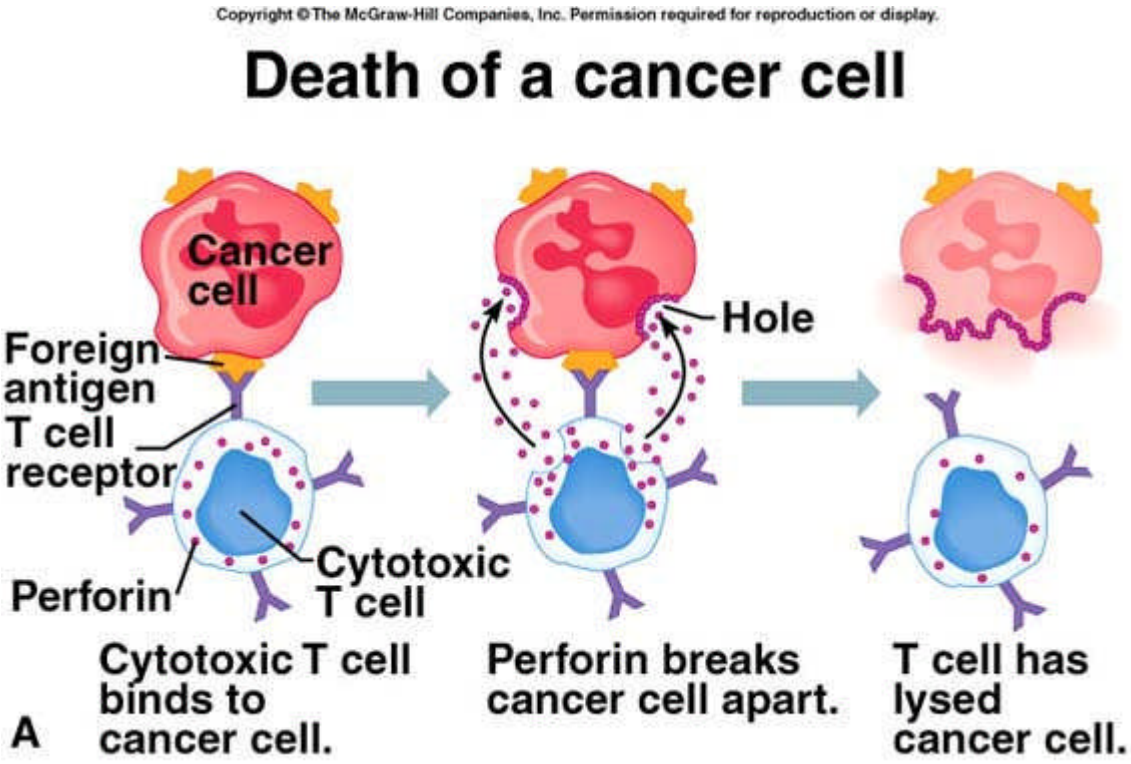




CD8+ T lymphocytes

The main function of CD8-expressing T cells is to kill cells that have become infected or malignant: for this reason they are known as cytotoxic (T_C) cells For example, in virally infected cells, some of the newly synthesized viral proteins are processed into peptides which associate with MHC class I molecules and are presented on the cell surface. These cells then become targets for CD8+ T_C cells with receptors specific for the viral peptides. Once the T_C cells have bound to the infected cells, they have several mechanisms by which they can kill their targets. The T_C cells secrete proteins stored in granules in their cytoplasm. These include perforins, which form pores through the surface membranes of the target cells, and granzymes, which enter the target cells through the perforin pores to activate caspase enzymes involved in apoptosis. T_C cells also express a surface molecule called Fas ligand and a cytokine called tumour necrosis factor. These can induce apoptosis by binding to their respective receptors on target cells In contrast to the limited expression of MHC class II molecules, most cells normally express MHC class I molecules and are thus potential targets for T_C cells if they become infected or malignant.

Fig 1.4.1.8



1.4.1.4.1.3 Natural Killer Cells

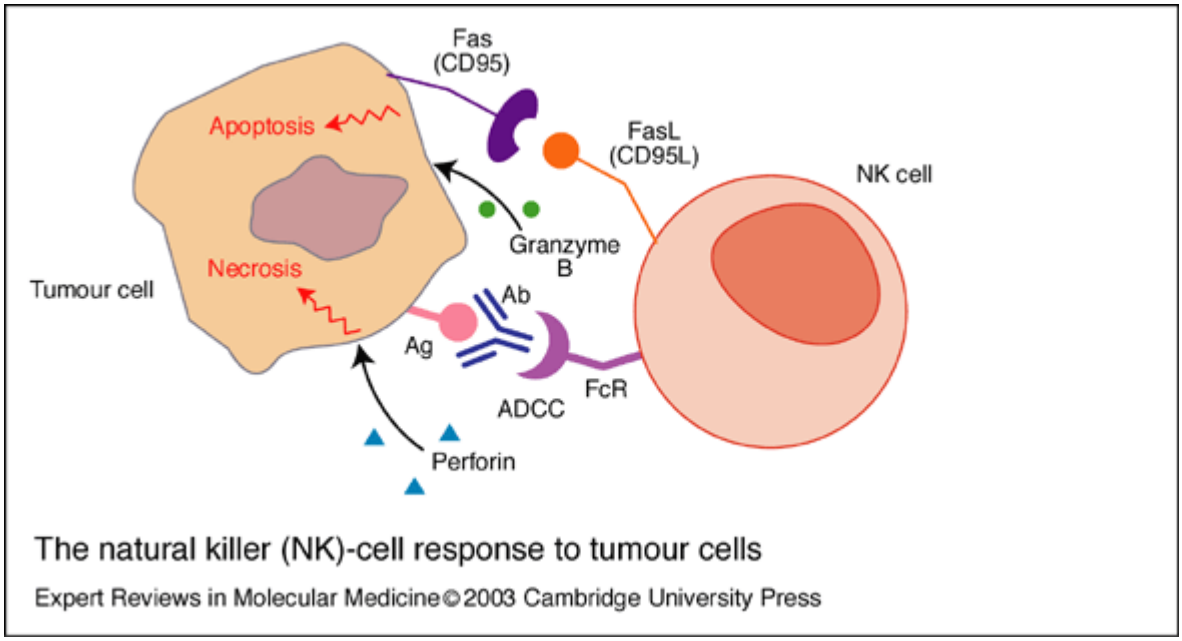
Natural killer (NK) cells constitute up to 15% of human blood lymphocytes. Together with γδ T cells and about 50% of CD8+ T cells they are known as large granular lymphocytes because, compared with most T and B lymphocytes, they have more cytoplasm and contain prominent granules. In contrast to all T and B cells, NK cells do not express antigen-specific receptors and do not possess the adaptive property of memory cell development: they are therefore considered to form part of the innate immune system. However, like T_C lymphocytes, their main function is to kill infected cells and tumour cells using similar mechanisms to those of T_C cells to induce apoptosis of their targets

Since they lack antigen receptors, NK cells do not recognize specific antigens on the surface of a target cell. Instead, they detect molecular changes in the surface of a cell which are indicative of that cell being abnormal and therefore a potential threat to the body. In particular, they kill cells with reduced expression of MHC class I molecules, as

can result from viral infection or malignant transformation. NK cells express surface ligands for MHC class I known as killer inhibitory receptors (KIRs) because their binding to MHC class I on the surface of a potential target cell inhibits the cytotoxic activity of the NK cell. This prevents NK cells from killing normal tissue cells with normal levels of MHC class I expression. However, when they interact with infected or malignant cells with reduced expression of MHC class I the lack of KIR engagement allows activation of the cytotoxic mechanisms. A variety of NK cell surface molecules can be involved in the interactions with targets cells which lead to killing, including CD2, CD16, CD69 and lectins (sugar-binding proteins). In addition, NK cells bear Fc receptors for IgG, so that killing can result from interaction with antibodies specifically bound to antigens on a target cell surface: this is called antibody-dependent cellular cytotoxicity (ADCC).

Since they do not require activation by specific antigen in order to mediate their effects, NK cells are effective killers of infected cells during the early stages of a viral infection (thus demonstrating their 'natural' cytotoxicity) and help to limit the spread of the infection within the body until virus-specific T_C lymphocytes become active. Indeed, NK cells are activated by interferon α, which is produced by virally infected cells, and are themselves a source of interferon γ, which helps to promote cell-mediated immunity. All large granular lymphocytes (NK cells, γδ T cells and some CD8+ T cells) can be activated by the T_H1-derived cytokine interleukin 2 (IL-2) to exhibit enhanced antigen nonspecific cytotoxicity; these are called lymphokine-activated killer (LAK) cells

Fig 1.4.1.9



The natural killer (NK)-cell response to tumour cells

Expert Reviews in Molecular Medicine©2003 Cambridge University Press

1.4.1.4.2 Granulocytes or Polymorphonuclear (PMN) Leukocytes –

Another group of white blood cells is collectively referred to as granulocytes or polymorphonuclear leukocytes (PMNs). Granulocytes are composed of three cell types identified as neutrophils, eosinophils and basophils, based on their staining characteristics with certain dyes. These cells are predominantly important in the removal of bacteria and parasites from the body. They engulf these foreign bodies and degrade them using their powerful enzymes.

1.4.1.4.2.1 Dendritic Cells

Dendritic cells are so called because, when they are mature, their cytoplasm extends into transient spiny dendrites and sheet-like veils. This provides a large surface area for their main function of antigen

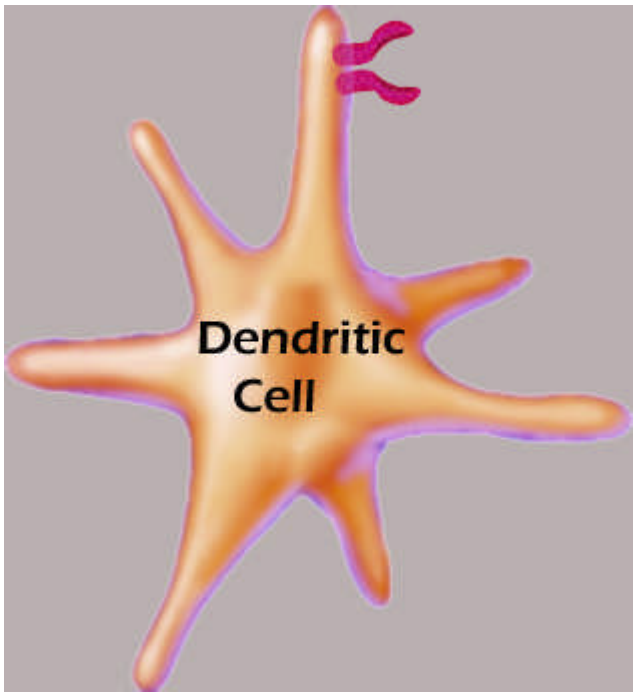
presentation to T lymphocytes. Indeed, they are the most potent APCs for T cells, expressing ten to a hundred times more antigen peptide–MHC complexes than the other professional APCs (B cells and monocyte/macrophages).

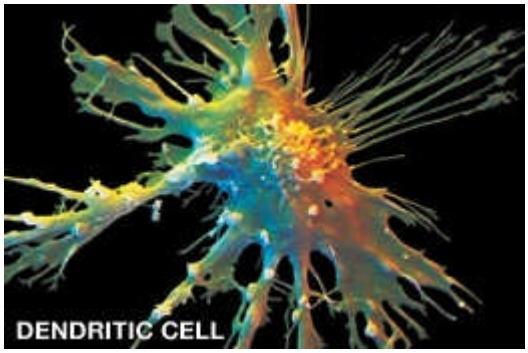
All dendritic cells are derived from bone marrow stem cells, but appear to be heterogeneous, with various precursors (including monocytes) differentiating into dendritic cells when stimulated by appropriate combinations of cytokines. Immature dendritic cells are found in tissues throughout the body (e.g. Langerhans cells in the skin epidermis) and are very efficient at capturing and processing antigens: they can ingest particulate antigens (phagocytosis), engulf material in the surrounding fluid (macropinocytosis), and take up (by receptor-mediated endocytosis) sugar-bearing antigens which bind to surface lectins or antigen–antibody immune complexes which bind to Fc receptors. The internalized antigens are degraded into peptides and some of these associate with cytoplasmic MHC molecules followed by transportation to the cell surface for presentation.

The dendritic cells which have captured and processed antigen mature into potent APCs as a result of their interaction with antigen and stimulation by cytokines and certain microbial products (e.g. lipopolysaccharide). This strong T-cell stimulatory capacity of the mature dendritic cells is due not only to their high levels of antigen peptide–MHC, but also to their expression of numerous costimulatory adhesion molecules and cytokines. The antigen-laden dendritic cells migrate to secondary lymphoid tissues (lymph nodes and spleen) where they form clusters with T and B cells and stimulate antigen-specific immune responses: they present antigen peptides associated with both MHC class II and MHC class I molecules, and so can induce primary activation of both CD4+ T_H cells and CD8+ T_C cells, respectively.

Dendritic cells not only activate T cells specific for antigens of foreign pathogens, but also help to prevent T cell reactivity against the body's own components. If the body does not have this 'self-tolerance', autoimmune diseases can develop. In the thymus, T cell precursors which develop antigen receptors specific for self antigens are eliminated if they interact with thymic dendritic cells expressing these antigens, and similar interactions with mature self-reactive T cells in secondary lymphoid tissues may also help to maintain the ability of the immune system to discriminate between foreign and self components

Fig 1.4.1.10





1.4.1.4.2.2 Monocytes and Macrophages

Monocytes, which constitute 5–10% of mononuclear leucocytes in the blood, differentiate into macrophages when they migrate into tissues. The main functions of macrophages are to phagocytose (i.e. engulf) and destroy particulate material and, by virtue of expressing MHC class II, to present antigens to T_H cells

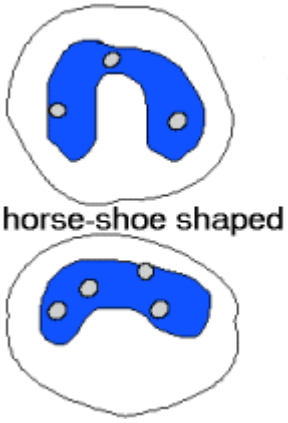
The blood monocytes arise from myeloid progenitors in the bone marrow. Monocytes are larger than most lymphocytes and have a kidney-shaped nucleus: they possess azurophilic lysosomal granules containing lysozyme, acid hydrolases and myeloperoxidase. Macrophages can be resident in tissues for prolonged periods of time where they take on various morphologies, and are known by different names, depending on their tissues of residence, e.g. Kupffer cells in the liver, mesangial cells in the kidney and microglial cells in the brain Macrophages have a variety of surface receptors for binding particulate antigens such as bacteria; these include receptors for certain sugars (e.g. mannose) and for lipopolysaccharide (via interaction with LPS-binding protein). They also have Fc receptors and complement receptors enabling them to bind antigens which have been coated (i.e. opsonized) with IgG antibodies and C3b complement protein, respectively. In addition to the removal of microbes, macrophages are also important in the rapid clearance of tissue cells dying by apoptosis and, for example, have receptors for phosphatidylserine expressed on the outer surface of apoptotic cells

The binding of particulate material to a macrophage triggers phagocytosis: the material is enveloped in cytoplasm, forming an intracellular vesicle called a phagosome. Lysosomes fuse with the phagosome so that their contents can participate in the destruction of the ingested material. This destruction is mediated partly by reactive oxygen species (e.g. hydroxyl radicals and nitric oxide) generated by enzyme activity known as the oxidative or respiratory burst, and also by the digestive lysosomal enzymes

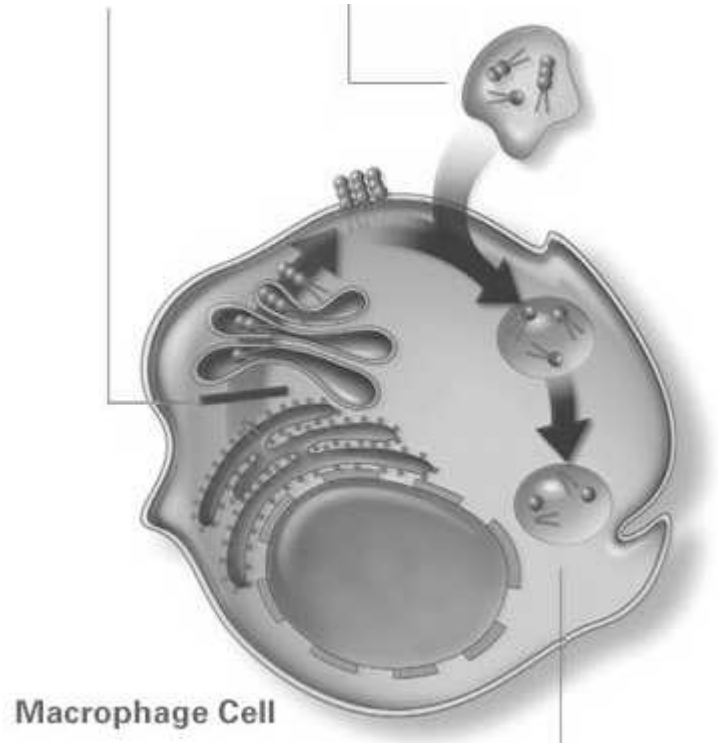
The maximal activation of macrophages to mediate phagocytosis and killing, and also antigen presentation, is stimulated by cytokines, particularly interferon γ. Macrophages are themselves producers of cytokines and inflammatory mediators like prostaglandins and leucotrienes. They are also an important source of some complement proteins

Fig 1.4.1.11

Monocyte


horse-shoe shaped


Macrophage



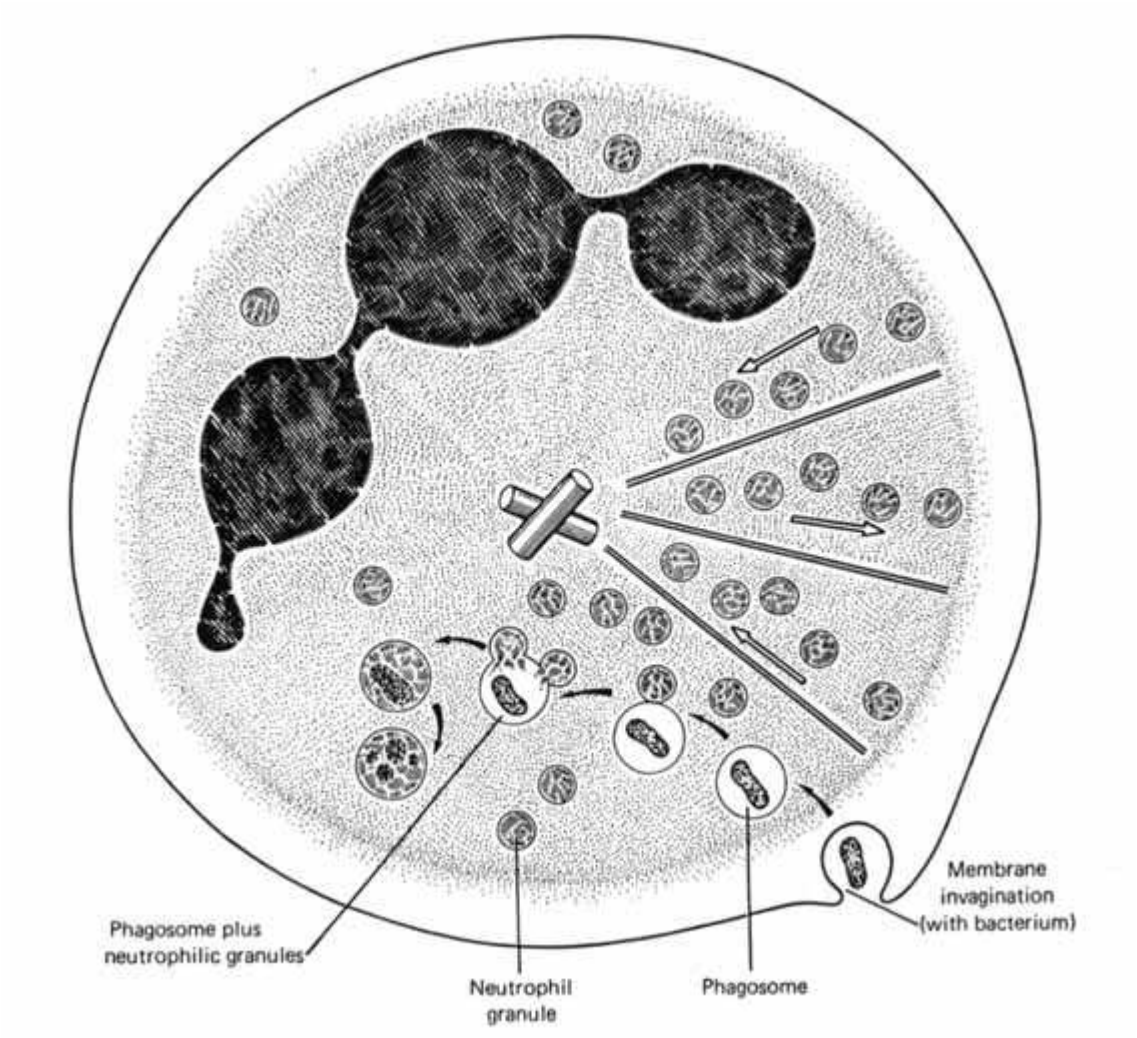
1.4.1.4.2.3 Neutrophils

Granulocytes, so named because of their prominent cytoplasmic granules, comprise the majority of white blood cells (60–70%). They are also known as polymorphonuclear leucocytes because of their multilobed nuclei and are larger than most mononuclear blood cells. Derived from myeloid progenitors in the bone marrow, granulocytes are released at a rate of seven million per minute, but are short-lived (2–3 days). They migrate into tissues, particularly to sites of infection where they are involved in the acute responseNeutrophils account for 95% of granulocytes in the blood. Like macrophages, they contain azurophilic lysosomal granules which, in addition to myeloperoxidase, lysozyme and acid hydrolases, contain other antimicrobial proteins (e.g. defensins and serprocidins). They also possess secondary specific granules which contain the iron-binding protein lactoferrin as well as lytic enzymes.

The main function of neutrophils is the phagocytosis and intracellular digestion of particulate antigens (e.g. bacteria) essentially as described for macrophages. Thus, neutrophils express a similar range of receptors for binding their targets, including Fc receptors (20-fold

more than macrophages) specific for IgG or IgA, and complement receptors for binding opsonized material. They also bring about destruction with reactive oxygen species and the antimicrobial enzymes and proteins. The vast number of neutrophils in the circulation means that they have a prominent role in the acute phase of the response to bacterial infection. They also produce some cytokines as well as prostaglandins and leucotrienes. In contrast to macrophages, neutrophils do not normally express MHC class II and so do not present antigens to T_H cells.

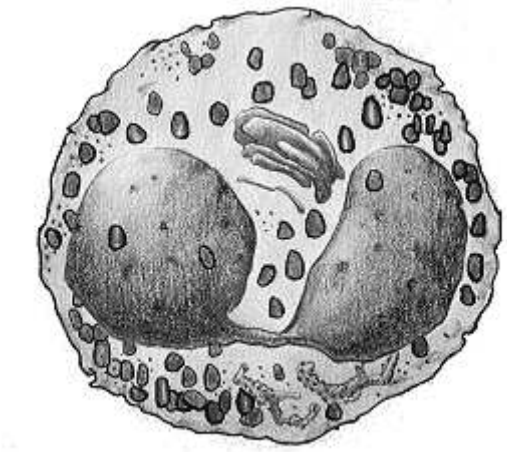
Fig 1.4.1.12



1.4.1.4.2.4 Eosinophils

The granulocytes whose granules stain with acidic dyes are called eosinophils. They comprise 2–5% of white blood cells and have bilobed nuclei. In contrast to the phagocytosis and intracellular digestion normally displayed by neutrophils, eosinophils secrete their granule contents for extracellular digestion of infectious pathogens which are too large to be engulfed (e.g. parasitic worms). Eosinophils have Fc receptors for IgG and IgE antibodies and for C3b, enabling them to bind to opsonized targets. They then secrete their antibiotic granule contents (including major basic protein and eosinophil cationic protein) and reactive oxygen species to bring about damage to the target. Eosinophils also produce cytokines, prostaglandins and leucotrienes, and enzymes which can inhibit the inflammatory products of mast cells (e.g. histaminase and aryl sulfatase).

Fig 1.4.1.13 Eosinophils



1.4.1.4.2.5 Basophils and Mast Cells

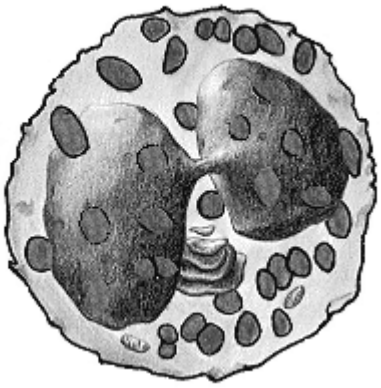
The main products of basophils and mast cells are mediators which promote inflammatory responses. Basophils (so called because their granules stain with basic dyes) are found in the circulation where they constitute less than 1% of white blood cells. Mast cells occur in tissues in two forms – connective tissue mast cells and mucosal mast cells. The latter are the most similar to basophils

There are two main mechanisms for mast cell activation during the course of immune responses, e.g. at a site of infection. Mast cells have high-affinity Fc receptors for IgE and are coated with IgE antibodies which they adsorb from their surroundings. Specific binding of antigen to multiple IgE molecules so that they are crosslinked on the mast cell surface triggers activation. Mast cells can also be stimulated by small peptides called anaphylatoxins (C3a and C5a) produced during complement activation.

The activation of mast cells triggers their release of a wide range of inflammatory mediators; some are stored in cytoplasmic granules and released immediately upon activation (e.g. histamine, heparin and factors which attract neutrophils or eosinophils), whereas others are synthesized *de novo* and are released more slowly (leucotrienes, prostaglandins and platelet-activating factor). Mast cells can also produce various cytokines. The cumulative effect of all these inflammatory mediators (together with those from other cells) produced at a site of infection is to facilitate and encourage the movement of lymphocytes, monocytes, granulocytes and their products (e.g. antibodies and complement proteins) out of the bloodstream and into the underlying tissues where they can fight the infection

Fig 1.4.1.14

Basophils



Mast cells

1.4.1.5 Summary

It has become increasingly clear over the past decade that the antibody and T cell responses of the adaptive immune system are influenced by the cells and soluble products of the innate immune system. Macrophages, neutrophils, and other cells that mediate innate immunity form a first line of defense against pathogen invasion, helping to limit infection until antigen-specific B and T lymphocyte responses are induced. One of the most exciting advances of recent years was the realization that innate responses serve to regulate the onset, duration, magnitude, and character of antibody- and cell-mediated adaptive responses. The demonstration of these important roles for innate immunity and a growing appreciation of the underlying mechanisms at the molecular level provide opportunities for new vaccine strategies. Thus, innovative approaches will target cells of the innate immune system as well as T and B lymphocytes to generate potent immunity of the appropriate type to protect against acute infection and stimulate clearance during chronic infection.

Many of the cells involved in both the innate and adaptive immune systems circulate throughout the body. Lymphocytes also home to specialized areas of the spleen and lymph nodes where they multiply and become potent effector cells upon encounter with pathogenic antigens. They then migrate to areas of infection. Increasingly sophisticated experimental approaches are being used to identify the molecular guideposts and signals that direct traffic for the appropriate localization of immune cells, and these trafficking signals are now being studied as targets for therapeutic intervention.

Considerable progress has been made in defining structures and functions of molecules important to immune responses. Molecular mechanisms by which specific antigen receptors are assembled and expressed on T and B cell surfaces, and lymphocyte selection mechanisms that ensure an antigen-reactive repertoire ready to fight disease while maintaining self-tolerance, have been identified. Although questions remain, enormous progress has been made in identifying and characterizing the intracellular molecules that control the effector functions elicited by antigen binding. These effector functions are critical for combating infection, and also are responsible for allergic reactions, autoimmune attacks, and transplant rejection. Notable advances in characterizing these mechanisms include a more thorough understanding of the secreted, cytolytic mediators that kill tumor cells or infected cells, as well as the cell surface and intracellular proteins involved in controlling cell death. The orchestration of particular types of immune responses by different T

cell subsets and cytokines has also been clarified. These accomplishments provide a foundation for future work that will target individual molecular components to improve vaccine development and therapies for immune-mediated diseases.

It has been known for some time that immune responses in the skin and epithelial surfaces of the body, such as lung and intestine, may differ from systemic immune responses in important ways. Because these surfaces are the most common sites of entry for allergens and infectious pathogens, it is important to understand how immunity is controlled at these sites. Although considerable progress was made in recent years, renewed emphasis on this area of research is warranted, and the genetic, biochemical, and cellular tools are now available for more definitive studies in the next decade of research. The rewards of such efforts will include novel vaccine strategies as well as immunomodulatory regimens to control allergic responses.

1.4.1.6 Model Questions

Essay questions

1.What is an Immune system and explain the structure of the immune system?

2. What are the different kinds of the immune cells which take part in immune response and add a note on the different types of the cells?

3.What are the primary lymphoid organs and explain they structure and function ?

4. Explain the structure of the lymphode node and add a note on its function

Short Questions

1. Thymus

1. Dendritic cells

3. Macrophage

4. T lymphocyte

5. B lymphocyte

1.4.1.7 Key woards

1. Bone marrow
2. Spleen
3. Lymphocyte
4. Thymus
5. Lymph node
6. T Lymphocyte
7. B lymphocyte
8. Macrophage
9. Dendritic Cells

1.4.1.7. Refrences

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1.4.2. STRUCTURE Of ANTIGEN AND ANTIBODY

1.4.2.1. Objective

The objective of the lesson is to know what an antigen is and what factors govern the immunogenicity of antigen and what an adjuvant and hapten is ? and also the chapter gives the information of the structure of the antibody and its functions

1.4.2.2. Introduction

Substances capable of inducing a specific immune response are called antigens. The molecular properties of the

antigens and the way in which these properties ultimately contribute to immune activation are central to our understanding of the immune system.

This topic describes some of the molecular features of antigens recognized by B lymphocytes or T lymphocytes (cells) and it also explores the contribution made to immunogericity by the biological system of the host; ultimately the biological system determines whether a molecule that combines with a B or T cell's antigen binding receptor can then induce an immune response.
Antibodies were the first elements of the immune system to be identified. They are antigen-reactive proteins present in an immune serum, called antiserum, and obtained after exposure of the vertebrate host to a given antigen, called an immunogen. By contrast, normal serum, also called preimmune serum, does not contain antibodies specific for that antigen. As the overwhelming majority of antibody activity proved to be associated with the γ-globulin peak seen in fractionation of serum proteins, immune globulins were designated immunoglobulins. Characteristically, antibodies, which are found in the plasma and in extracellular fluids, bind their nominal antigens with exquisite specificity and potentially neutralize their harmful effects

1.4.2.3 ANTIGEN

1.4.2.3.1 Immunogenicity Versus Antigenicity

Immunogenicity and antigencity are related, but distinct. Immunologic and antigenic properties that sometimes are confused. Immunogencity is the ability to induce a humoral and cell mediated immune response.

B cells + antigen → effecter B cells + memory B cells

(Plasma cells)

T cells + antigen → affecter T cells + memory T cells

(es CTLs, T_H)

Although a substance that induces a specific immune response is usually called an antigen, it is more appropriately called an immunogen.

Antigenicity is the ability to combine specifically with the final products of above responses ie antibodies and cell-surface receptors. Although all molecules that have the property of immunogenicity will also have the property of antigenicity, the reverse is not true. Some small molecules, called haptens, are antigenic but are in capable, by themselves, of inducing a specific immune response.

1.4.2.3.2 Factors that Influence Immunogenicity

To protect against infectious disease, the immune system must be able to recognize bacteria, bacterial products, fungi, parasites and viruses as immunogens. The immune system actually recognizes particular macromolecules of an infectious agent, generally either proteins on polysaccharides. Proteins are the most potent immunogens, with polysaccharides ranking second. In contrast, lipids & nucleic acids of an infectious agent generally do not serve as immnogens unless they are complexed with proteins on polysaccharides.

Proteins or polysaccharides are used as immunogens in most experimental studies of humoral immunity. For cell-mediated immunity, only proteins and some lipids and glycolipids serve as immunogens. These molecules are not recognized directly. Proteins must first be processed into small peptides and then presented together with MHC molecules on the membrane of a cell before they can be recognized as immunogens.

Immunogenicity is not an intrinsic property of an antigen but rather depends on a number of properties of the particular biological system that the antigen encounters.

Contribution of the immunogen to immunogenicity

Immunogenicityof an immunogen is determined by four properties :

1. Foreigness

2. Molecular size

3. Chemical composition and complexity

4. Ability to be processed and presented

1 Foreigness

In order to elicit an immune response, a molecule must be recognized as non-self by biological system.

Much of the ability to recognize self-molecules is thought to arise during lymphocyte development by exposure of immature lymphocytes to self components. Any molecule that has not been exposed to immature lymphocytes during this critical period may be later recognized as nonself, or foreign by the immune system. When an antigen is introduced into an organism, the degree of its immunogenicity depends on the degree of foreignness'. greater the foreignness' greater is the immune response

2. Molecular size

There is a correlation between the size of a macromolecule and its immunogenicity. The best immunogens tend to have a molecular weight approaching 100,000 daltons. Generally, substances with a molecular mass less than 5000-10,000 Daltons are poor immunogens, however, a few substances with a molecular mass less than 1000 Daltons have proved to be immunogenic.

3. Chemical composition and heterogeneity

Size and foreigness are not, by themselves sufficient to make a molecule immunogenic, other properties are needed as well. For eg, synthetic homopolymers tend to lack

immunogenicity regardless of their size. Copolymers of sufficient size, if they contain two or more different aminoacids are immunogenic. The addition of aromatic aminoacids such as tyrosine or phenylalanine profoundly enhances the immunogenicity of there synthetic polymers. For eg, a synthetic copolymer of glutamic acid and lysine requires a minimum molecular weight of 30,000-40,000 for immunogenicity. The addition of tyrosine to the copolymer reduces the required minimum molecular weight to 10,000-20,000 and the addition of both phenylanine and tyrosine reduces the minimum molecular weight to 4000. All four levels of protein organization – primary, secondary tertiary and quaternary contributes the structural complexity of a protein and hence effect its immunogenicity.

4. Susceptibility to antigen processing and Presentation

The development of both humoral and cell-mediated immune responses requires interaction of T cells with antigen that has been processed and presented together with MHC molecule. To T_H cells, the antigen must be processed with class II MHC molecules on an antigen presenting cell, To Tc cells, the antigen must be presented with class I MHC molecules on an altered self cell. Macromolecules that cannot be degraded with MHC molecules are poor immunogens.

Large, insoluble macromolecules generally are more immunogenic than small, soluble ones because the larger molecules are more readily phagocytosed and processed. Intermolecular chemical cross-linking, induction of aggregation by heating, and attachment to insoluble matrices have been routinely used to increase the insolubility of macromolecules, thereby facilitating the phagocytosis and increasing their immunogenicity.

1.4.2.3.3 Contribution of the Biological System to Immunogenicity

Even if a macromolecule has the properties that contribute to immunogenicity, its ability to induce an immune response will

depend on certain properties of the biological system that the antigen encounters. These properties include the genotype of the recipient, the dose and route of antigen administration, and the administration of substances, called adjuvants, that increase immune responses.

1.4.2.3.4. Immunogen Dosage & Route of Administration\

Each experimental immunogen exhibits a particular dose response curve, which is determined by measuring the immune response to various doses and by different administration routes. Some combination of optimal dosage and route of administration will induce a peak immune response in a given animals.

An insufficient dose will not stimulate an immune response either because it fails to activate enough lymphocytes or because it induces a non-responsive state. Conversely, an excessively high dose also can fail to induce a response because it causes lymphocytes to enter a non responsive state.

Experimental immunogens are generally administered by routes other than the digestive tract. The following administration routes are common.

Intravenous : into a vein

Intradermal : into the skin

Subcutaneous : beneath the skin

Intramuscular : into a muscle

Intraperitoneal : into the peritoneal cavity

The administration route strongly influences which immune organs and cell populations will be involved in the response.

1.4.2.3.5 Adjuvants

Adjuvants are substances that, when mixed with an antigen and injected with it, enhance the immunogenicity of that antigen. Adjuvants are often used to boost the immune response when an antigen has low immunogenicity on when only small amounts of an antigen are available. For eg, the antibody response of mice to immunization with BSA can be increased five fold or more if the BSA is administered with an adjuvant. Precisely how adjuvants augment the immune response is not entirely known; but they appear to exert one or more of the following effects:

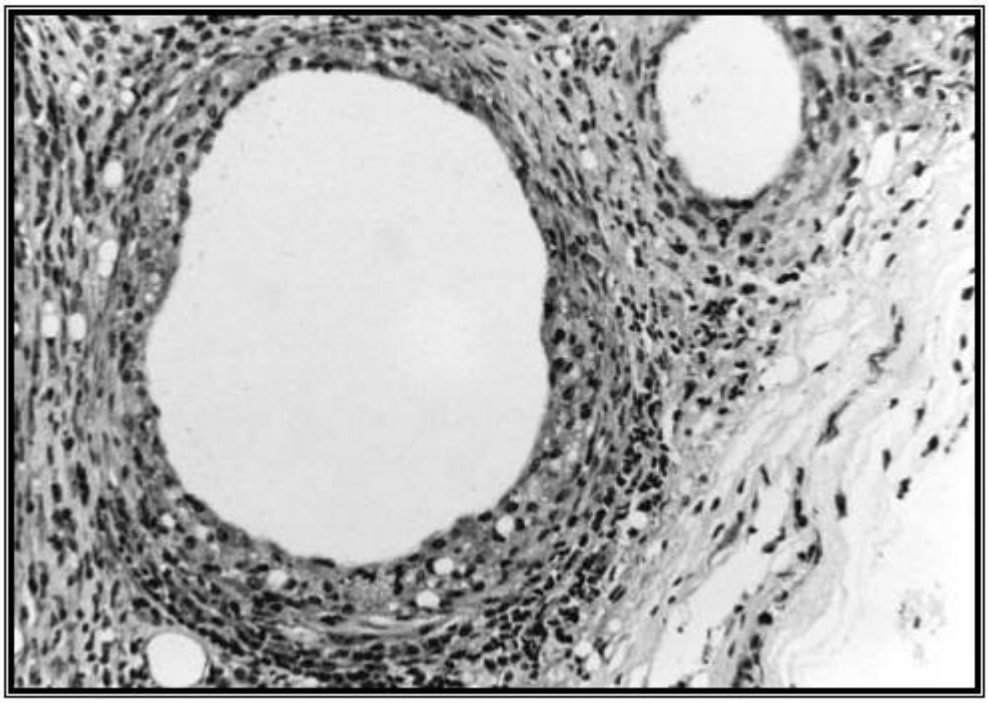
1. Prolong antigen persistence
2. Enhance co-stimulatory signals
3. Induce granuloma formation
4. Stimulate lymphocyte proliferation nonspecifically

Aluminum potassium sulfate (alum) prolongs the persistence of antigen. When an antigen is mixed with alum the salt precipitates the antigen. Injection of this precipitate results in slower release of antigen from the injection site. So that effective time of exposure to the antigen increases from few days without adjuvant to several weeks with adjuvant.

Freund water in oil adjuvant also prolong the persistence of antigen. Freund incomplete adjuvant contains antigen in aques solution, mineral oil and emulsifying agent such as mannide – monooleate which disperses the oil in to small droplets surrounding the antigen. So the antigen is then released slowly from the site of injection.

Freund's complete antigen contains an heat killed mycobacteria in the water-in-oil emulsion. Muramyl dipeptide, a component of mycobacterial cell wall activates macrophages making Freuend complete antigen more potent that in complete one. (Fig1.4.1.1)

Freund's Adjuvant induced Granuloma



1.4.2.3.6 Epitopes

Immune cells do not interact or recognize an entire immunogen molecule, instead lymphocytes recognize discrete sites on the macromolecule called epitopes or antigenic determinants. Thus exposure to a microorganism will generate an immune response to many different epitopes. The antiserum produced will contain different antibodies reactive with each determinant. This will ensure that an individual will be protected from the microorganism by producing a response to at

least a few of the possible determinants. If the host only reacted to the organism as a whole then failure to react to this antigenic determinent site would have dire consequences, ie it would not be able to eliminate the pathogen. Certain antibodies may react with an epitope composed of residues that can also be part of two other epitopes recognized by different antibodies.

A response to antigen involves the specific interaction of components of the immune system, antibodies and lymphocytes, with epitopes on the antigen. The lymphocytes have receptors on their surface that function as the recognition units -→ on B-lymphocytes surface –bound immunoglobulin acts as receptors and an T-lymphocytes the recognition unit is the T-cell receptor. The interaction between an antibody and antigen is governed by the complementarity of the electron cloud surrounding the determinants. The overall configuration of the outer electrons, not the chemical nature of the constituent residues, determines the shape of epitope and its complimentry paratope.

Antigenic determinants have to be topographical ie composed of structures on the surface of molecules or cells, and can be constructed in 2 ways. They may be contained within a single segment of primary sequence but brought together on the surface by the folding of molecule into its native conformation. The formers are known as sequential epitopes and those formed from distant residues are conformational epitopes.

Gell & Benacerraf studied the immune response to protein antigens. The antibodies produced by the immunogen were specific for the form of antigen used for immunization. Antibodies formed against a native antigen react only with native antigen while antibodies produced by immunizing with denatured antigen only bind to the denatured form. However, cell mediated responses with identical specificities can be elicited equally well with either. Thus antigenic structures seen by antibody depend on the tertiary configuration of the immunogen, while epitopes seen by T-cells are defined by primary structure. (Fig !.1.4.1.2)

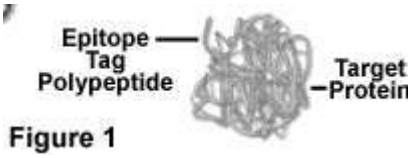


Fig 1.4.1.2 (a) Protein Containing epitope

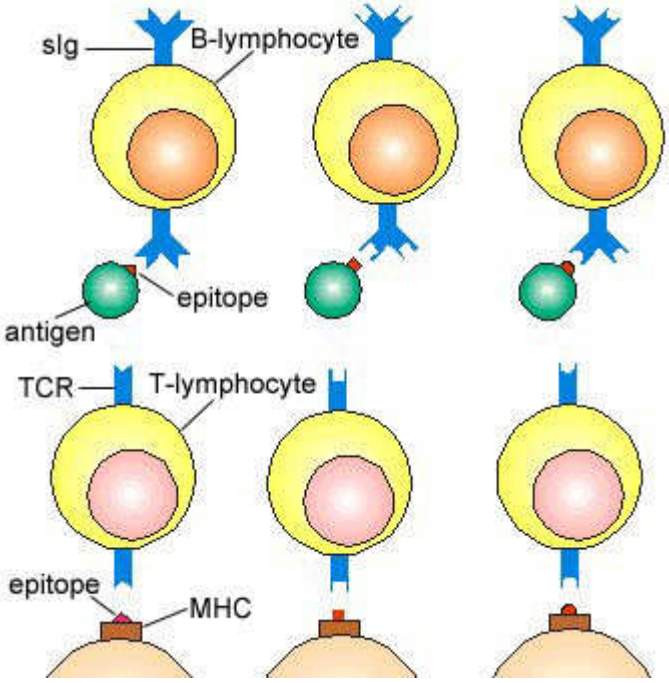


Fig 1.4.1.2(b) Protein Containing epitope

1.4.2.3.7 Antigenic Specificity

Foreignness' of a substance to an animal can depend on the presence of chemical groupings that are not normally found in the animal's body. Arsenic acid, for example, can be chemically introduced into a protein molecule and it acts as an hapten 'ie' a determinant of antigenic specificity of the molecule. This type of chemically defined determinant enabled Karl Landsteiner early in this century to study antigenic specificity in detail. By a slight chemical modification of the antigenic determinant he was able to demonstrate how critical and precise was the fit between antibody and an antigenic determinant.

The ability of antibody or T-cell receptors to form a high affinity interaction with an antigen depends on intermolecular forces which act strongly only when the two molecules come together in a very precise manner. The better the fit the stronger the bond. An antibody molecule directed against a particularly shaped antigenic determinant might be able to react with another similar but not quite identical determinant. This type of cross reaction does occur but the strength of the bond between the two molecules will be diminished in the case of the less well fitting determinant.

A common source of confusion with respect to the specificity of antibodies arises when an antibody to a particular antigen is found to be capable of combining with an apparently unrelated antigen. For example, an antibody that binds to a glucose determinant in antigen x-glucose would be likely to react with the glucose group in antigen y-glucose provided the two determinants are equally accessible. The antibody directed against the glucose determinant is not a non specific type of antibody but is simply reacting with an identical chemical determinant, epitope, in another antigen molecule.

In laboratory practice cross-reactivity is often found between antisera to certain bacterial antigens and antigens present on cell such as erythrocyles. Antigens shared in this way are known as heterophile antigens. Antibodies to such antigens will cross-react with cells or fluids of different species of animal with various microorganisms. The chemical determinants responsible for this cross reactivity are not known but are presumed to be similar or identical groupings, possibly mucopolysacclearide or lipid in nature, present in molecules which are part of the structure of the cells. The best known of the heterophile antigens is the Forssman antigen which is present on the red cells of many species as well as in bacteria such as pneumococci and salmonellae. Another heterophile antigen is found in Escherichia coli and human red cells of blood group B individuals. These Cross-reactive reactions are probably responsible for the generation of antibodies found in individuals of a certain blood group that bind to the red blood cells of individuals of a different blood group. These antibodies

are known as iso haemagglutinins because they are able to bind red blood cells and clump them together ie cause agglutination.

1.4.2.3.8 Haptens

These are the molecules which are antigenic but not immunogenic 'ie' which are incapable by themselves of inducing a specific immune response in other words they lack immunogenecity.

But chemical coupling of these haptens to a large protein called a carrier yields an immunogenic hapten-carrier-coajugate . Animal immunized with such a conjugate produce antibodies specific for (1) the hapten deteriment (2) unaltered epitopes on the carrier protein (3) new epitopes formed by combined parts of the haptan and carried: The haptan by itself cannot function as an antigen. But when multiple haptans coupled with carrier's, the haptan become assessable to the immane system and function as immuno determent.

Fig.

1.4.2.4 ANTIBODIES

1.4.2.4.1. Basic structure of immunoglobulin

Each millilitre of normal serum contains approximately 10^{16} immunoglobulin molecules. Even antibodies specific for the same epitope are heterogeneous. An antiserum raised against a given immunogen contains many different antibodies which bind to the antigen in slightly different fashions, and some of them may cross-react with related antigens and even with antigens exhibiting no obvious structural similarity with the immunogen. Because of this heterogeneity, study of the immunoglobulin structure requires use of homogeneous antibodies produced either by neoplastic plasma cells, termed myeloma cells, or by hybridoma cells. Myeloma cells proliferate and secrete immunoglobulins indefinitely and their products are

referred to as myeloma proteins, found in the serum of diseased subjects and animals.

Rodney porter has proposed a basic four chain structure for the immunoglobulin molecule. According to his proposel, the immunoglobulin molecule is a Y-shaped tetrameric protein characteristically composed of two H and two L polypeptide chains held together by covalent (disulfide) and noncovalent bondsTreatment of immunoglobulins with enzymes and chemical reagents capable of cleaving peptide bonds breaks them up into fragments. Three types of treatment are particularly important in understanding antibody structure. First, agents that cleave disulfide bonds generate two H-chain polypeptides of approximately 50 kDa and two L chains of 25 kDa. Second, papain splits the basic molecule into three fragments of approximately 45 kDa. Two of them retain the antibody ability to recognize the antigen and are referred to as Fab fragments (for 'fragment antigen binding'). Each fragment possesses only one combining site and can bind, but cannot precipitate, the antigen. The third fragment produced by papain digestion can be crystallized from a solution and is therefore referred to as Fc fragment (for 'fragment crystalline'). Finally, treatment with pepsin gives rise to several small fragments and a large fragment, the F(ab)′2 fragment, of molecular weight double that of one Fab fragment, and capable of binding and precipitating the antigen. The Fab is the antigen-binding constituent and the Fc fragment is the anchoring site for proteins of the complement system and for receptors of various effector cellsIn humans, both κ and λ chains of the L chain consist of approximately 250 amino acid residues. By contrast, the different types of H chain vary considerably in length, from 446 amino acid residues for the γ1 chain to 550 residues for the μ chain. This length variability is due essentially to the presence of additional residues at the C-terminal end of the μ and α chains. These extra amino acids participate in polymerization of immunoglobulins.

Each antibody chain is composed of a constant and a variable region. In addition, comparison of antibody amino acid sequences has revealed the existence of homology regions, or domains, each of approximately 110 amino acids. Each domain is a globular structure composed of two layers of polypeptide chains linked by a disulfide intrachain bridge in the centre of the domain. The chains exhibit a β-strand conformation and form two antiparallel β sheets that pack together. An L chain is composed of one variable (V_L) and one constant (C_L) domain. For the H chain, the γ, δ and α chains consist of one

variable (V_H) and three constant (C_H1 to C_H3) domains, and the μ and ε chains exhibit an extra constant domain (C_H4). Carbohydrate chains of variable shape and length, ranging from two sugar residues to a dozen or more units, are almost always present. Simple or branched, the carbohydrate chains normally attach to the Fc portion (the C_H2 domain) of the immunoglobulin. This domain organization appears to be an important feature shared by immunoglobulins from all species studied. The sequence similarity among various domains suggests that they are phylogenetically related, having evolved from a single ancestral gene

Linking the Fab and Fc regions of the immunoglobulin is a short segment of the H chain between the C_H1 and C_H2 domains, known as the 'hinge', with no sequence similarity to any of the other domains. This proline- and cysteine-rich, nonglobular portion of the polypeptide backbone allows segmental flexibility of the Fab arm and Fc region relative to one another. This flexibility is important for antigen binding and effector functions. The cysteine residues form interchain disulfide bonds linking the two H chains together. In addition to these interchain bridges, most immunoglobulins comprise a disulfide bridge between the H and L chain In addition, the μ and ε chains contain an additional constant region domain that replaces the hinge region found in the γ, δ and α chains.

At the amino acid level, the variable region is comprised of three regions of extreme variability which include residues that may contact the antigen by virtue of mutual complementarity. They are called complementarity-determining regions, or CDRs. Interspersed among the CDRs are framework residues that represent approximately 80% of the variable region and are less variable and more evolutionarily conserved. At the three-dimensional level, the three CDRs of each chain converge to form a combining site (also called a paratope) which recognizes the antigenic determinant (also called an epitope). Because of the complexity of macromolecular antigens, each molecule has many different epitopes. Some of them are composed of a single segment of the molecule and are termed continuous or linear epitopes. Others, called discontinuous or conformational epitopes, involve sites distributed in different parts of the molecule, but brought together in the three-dimensional structure

X-ray diffraction crystallographic studies of antibody crystals have revealed that the constant regions of the H and L chains do not contribute to formation of the combining site, and have confirmed the

domain-type organization of the immunoglobulin initially deduced from amino acid sequence analysis. They have provided evidence that antigen–antibody interactions are noncovalent and that the antibody-binding site requires contributions from the H- and L-chain variable domains of the Fab fragment. Amino acids from the CDRs, also called loops, between strands of the β sheets in the variable domains provide the specificity of the interaction. The shape and size of the combining site can vary, depending on the particular antigen–antibody complex analysed, from a shallow groove of 15 × 6 Å in size to a conical pocket of approximately 10 Å in diameter. The interactions occur over large sterically and electrostatically complementarity areas. Hydrophobic determinants of the antigen interact with hydrophobic determinants of the antibody-binding site, charged side-chains interact with side-chains of opposite charge, and proton donors and acceptors are involved in hydrogen bonding. Thus, electrostatic forces between charged amino acid side-chains (salt bridges), hydrogen bonds, van der Waals forces and hydrophobic forces, together with surface complementarity, impart antibody-specific recognition. (Fig 1.4.1.3) & (Fig !.4.1.4)

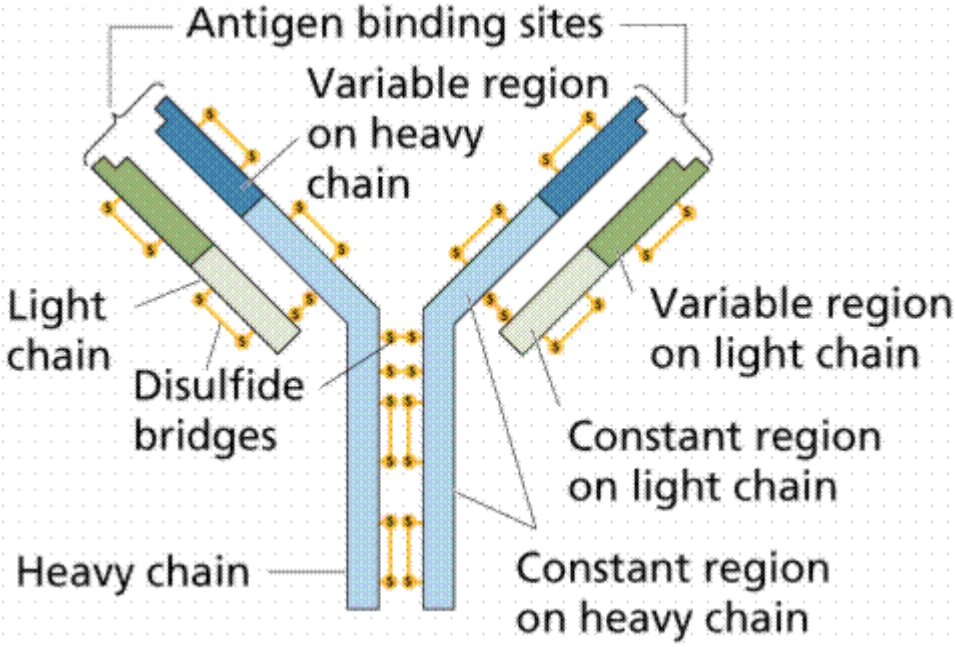


Fig !.4.1.3 Structure of the antibody

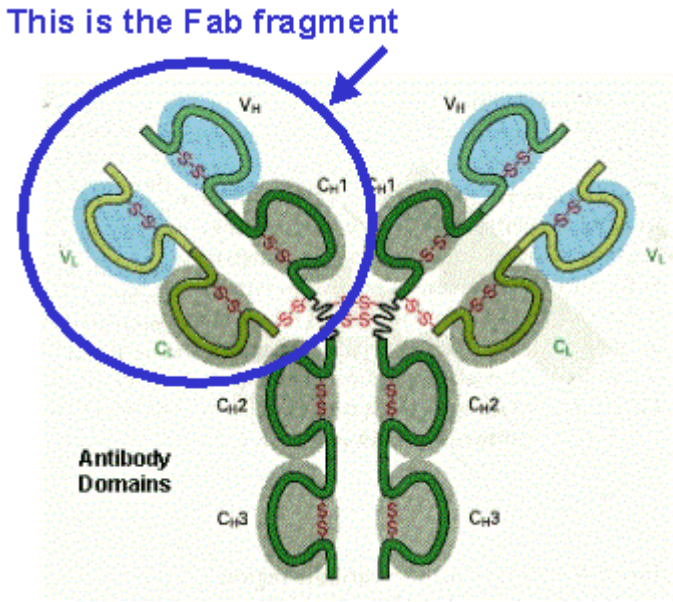


Fig !.4.1.4 Structure of the antibody showing the domains

1.4.2.4.2 Structure and biological properties of immunoglobulin classes

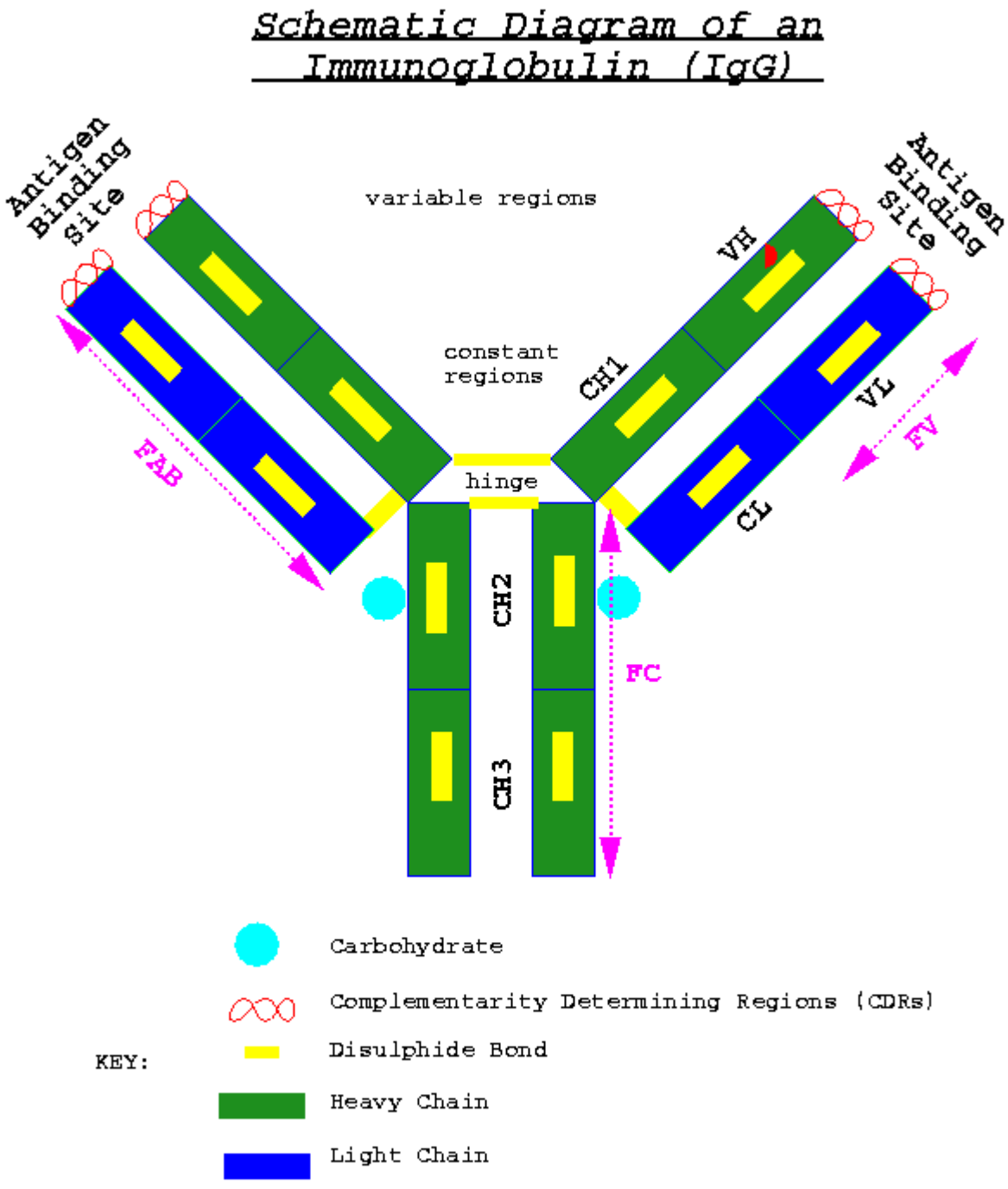
1.4.2.4.2.1 Immunoglobulin G (Ig G)

It is a relatively stable molecule and is composed of four polypeptides chains, Two light chains (either k or λ type) and two heavy chains of gamma (γ) type. The light chain consists of a single constant domain (CL) and a single variable domain (VL). The heavy chain consists of 4 domains namely a single variable domain (VH) and 3 constant domains (CH_1, CH_2 & CH_3). This is the predominant class of immunoglobulin and accounts for approximately 70% of the total immunoglobulin in human serum. Normal serum concentration of Ig G is about 8-16 mg/ml. Ig G is of greatest amount in internal body fluids and is produced particularly during the secondary immune response. The molecular weight of Ig G is 150,000 and its sedimentation coefficient is 75. Ig G has a half life of 25 days. The carbohydrate content of Ig G is about 3%. Under electron microscope, the Ig G looks like a Y

Ig G is the only immunoglobulin that crosses the human placenta and thus offers a passive protection to the new born for about 6-9 months. Ig G activates the classical complement pathway during the antigen antibody reactions. It neutralizes toxins and viruses.By

binding to bacteria, Ig G opsonises them, thereby enhance their phagocytosis and elimination. This process is known as opsonisation

Fig 1.4.1.5 Structure of Immunoglobulin IgG


Schematic Diagram of an Immunoglobulin (IgG)
Antigen Binding site
variable regions
VH
Antigen Binding site
constant regions
CH1
VL
FAB
FV
hinge
CL
CH2
FC
CH3
KEY:
Carbohydrate
Complementarity Determining Regions (CDRs)
Disulphide Bond
Heavy Chain
Light Chain

1.4.2.4.2.2 Immunogloblin Ig A

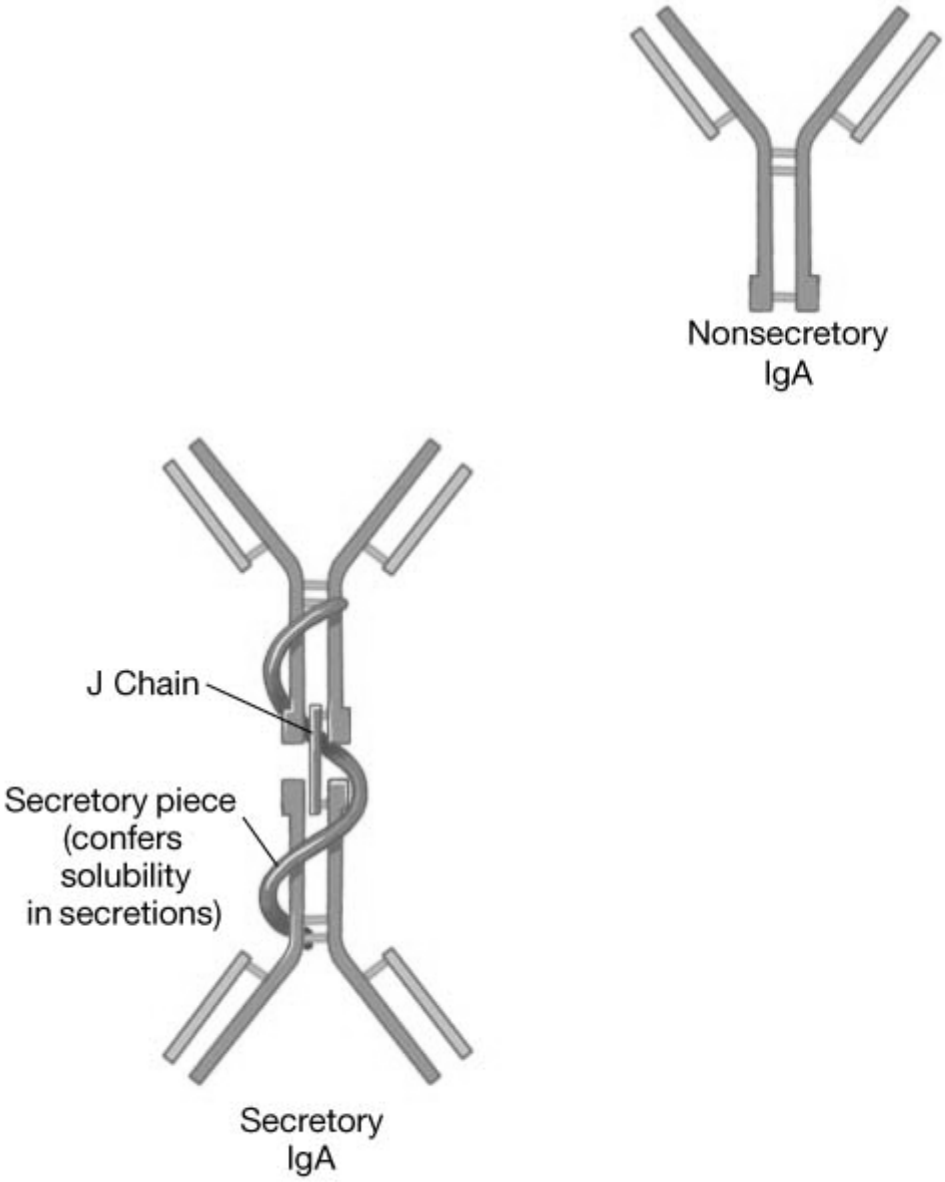
Ig A is found only to a small extent in serum but predominantly found in the extracellular secretions. Ig A is the second in abundance among the immunoglobulins next to Ig G. Two subclasses of Ig A are present namely Ig A_1 & Ig A_2. Ig A_1 has both inter chain and intra chain disulphide linkages. Ig A_2 consists of inter chain disulphide bonding between the heavy and light chains. Instead of disulphide bonds, the two heavy chains are linked together by non – covalent linkages.

Serum Ig A is a monomer having a structure very similar to Ig G. It has 4 polypeptide chains – 2 light & 2 heavy chains. The light chains either k or λ type. The heavy chain is of α type. Serum Ig A has Ig A_1, & Ig A_2.The α chain of Ig A differs from the γ chain of Ig G in having a greater carbohydrate content and the amino acid sequence.

Secretary Ig A occurs as a dimer formed of two molecules of Ig A linked by a secretary component and a joining chain or j chain. Both secretary piece and J chain are proteins. The secretary piece is synthesized by local epithelial cells which synthesized by Ig A dimer against proteolysis. The J-chain is also a polypeptide chain of molecular weight 15,000 daltons.

Secretary Ig A is termed as mucosal paint of mucous membrane. It inhibits the attachment of microbes to the mucosa of gastrointenstinal, respiratory tracts, there by prevents various diseases. Ig A promote phagocytosis and activate bacteriolytic activity. Ig A shows the common functions like agglutination, precipitation, compliment fixation, opsonisation

Fig 1.4.1.6 Structure of the Immunoglobulin IgA And Secretary Immunoglobulin Ig A



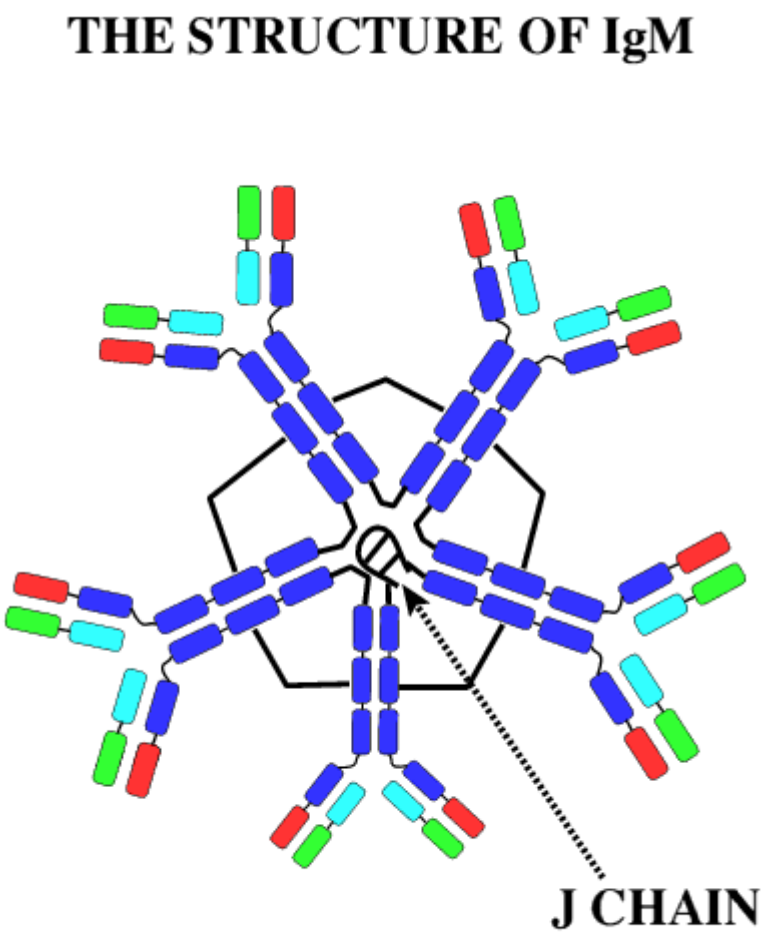
1.4.2.4.2.3 Immunogloblin Ig M

Ig M is a largest immunoglobulin. It is often referred as macroglobulin because of its high molecular weight. They are polymers of usually five molecules (pentamer). The polymerization of these molecules in the presence of J chain. The J chain is holding the 5 subunits together at the FC region and is stabilized by disulphide bond. The serum level of Ig M is very low because of its short half life period. Carbohydrate content is about 10-12%. Sedimentation coefficient is 193. It is the

earliest immunoglobulin to be synthesized by the foctus starting by about the 5th month of the foctal life. It has ten antigen binding sites.

Ig M is the first antibody that occurred in primary immune response but it does not persist for long period because of its short half life. It is therefore an useful indication of recent infection. By having multiple antigen binding sites, it has a high functional affinity for antigens. It also shows properties such as opsinization, compliment fixation, agglutination.

Fig 1.4.1.6 Structure of Antibody IgM

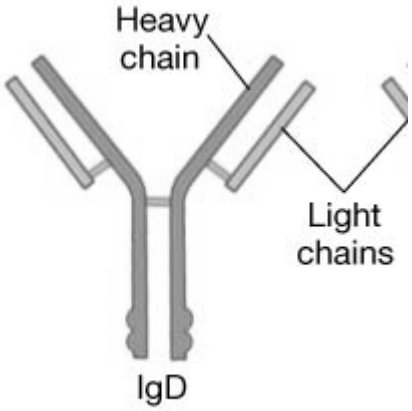


1.4.2.4.2.4 Immunogloblin Ig D

Ig D has typical immunoglobin structure with 2 light chains of kappa or lambda type and 2 heavy chains. The heavy chains are of delta type. Ig D has molecular weight of about 1,80,000 daltons. The average sedimentation coefficient is about 4-63. Ig D is rich in carbohydrate content. Carbohydrate content is about 10-12%.

Very little is known about its functions. It does not mediate any functions. Ig D appears to act as primary receptor for specific antigen on the surface of lymphocytes.

Fig 1.4.1.7

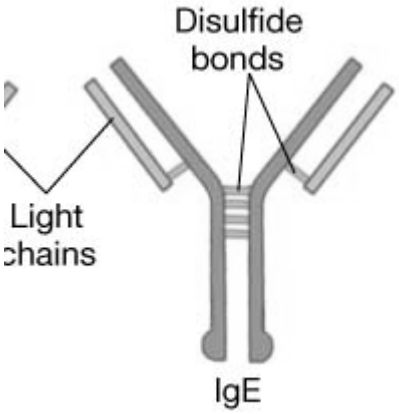


1.4.2.4.2.5 Immunogloblin Ig E

Ig E is a monomer. It has 2 light chains and 2 heavy chains. The light chain is of k or l type. The heavy chain is of E type. The heavy chain has an extra constant region in addition to the usual three. It has molecular weight of 1,90,000 daltons. Ig E are responsible for allergic reactions. Persons with allergic conditions, its level may be 100 times higher than that of normal type. It is carbohydrate content is about 12%. Sedimentation coefficient is 8-9s. It has specific attraction to mast cells which bear receptors for the FC region of the Ig E. Ig E is also named as Reagin antibody

It has protective function, either by acting directly on the parasite or by producing histamine. The release of histamines may result in the destruction of parasites. The Ig E interactions with most cells, results in degranulation of mast cells with the release of histamines

Fig 1.4.1.8



1.4.2.5 Monoclonal Antibodies

Process by which large quantities of antibodies (targeted against a particular antigen X) can be produced.

A mouse is immunized by injection of an antigen X to stimulate the production of antibodies targeted against X. The antibody forming cells are isolated from the mouse's spleen.

Hybridoma Techonology

Mix

- spleen cells from a mouse that has been immunized with the desired antigen with
- myeloma cells.

Use an agent to facilitate fusion of adjacent plasma membranes. Even so, the success rate is so low that there must be a way to **select for** the rare successful fusions. So,

use myeloma cells that have:

- lost the ability to synthesize hypoxanthine-guanine-phosphoribosyltransferase (**HGPRT**).

  This enzyme enables cells to synthesize purines using an extracellular source of **hypoxanthine** as a precursor.

Ordinarily, the absence of HGPRT is not a problem for the cell because cells have an alternate pathway that they can use to synthesize purines.

However, when cells are exposed to **aminopterin** (a folic acid analog), they are unable to use this other pathway and are now fully dependent on HGPRT for survival.

- lost the ability to synthesize any antibody molecules of their own (so as not to produce a hybridoma producing two kinds of antibody molecules).

1. The first property is exploited by transferring the cell fusion mixture to a culture medium - called **HAT medium** because it contains:

- **h**ypoxanthine
- **a**minopterin
- the pyrimidine **t**hymidine

The logic:

- Unfused myeloma cells cannot grow because they lack HGPRT.
- Unfused normal spleen cells cannot grow indefinitely because of their limited life span. However,
- Hybridoma cells (produced by successful fusions) are able to grow indefinitely because the spleen cell partner supplies HGPRT and the myeloma partner is immortal.

2. Test the supernatants from each culture to find those producing the desired antibody.

3. Because the original cultures may have been started with more than one hybridoma cell, you must now isolate single cells from each antibody-positive culture and subculture them.

4. Again, test each supernatant for the desired antibodies. Each positive subculture - having been started from a single cell - represents a clone and its antibodies are monoclonal. That is, each culture secretes a single kind of antibody molecule directed against a single determinant on a preselected antigen.

5. Scale up the size of the cultures of the successful clones.

Hybridoma cultures can be maintained indefinitely:

- in vitro; that is, in culture vessels. The yield runs from 10-60 μg/ml.
- in vivo; i.e., growing in mice. Here the antibody concentration in the serum and other body fluids can reach 1-10 mg/ml. However, animal welfare activists in Europe and in the U.S. are trying to limit the use of mice for the production of monoclonals.

Uses for monoclonal antibodies

Monoclonal antibodies are widely used as diagnostic and research reagents. Their introduction into human therapy has been much slower.

In some in vivo applications, the antibody itself is sufficient. Once bound to its target, it triggers the normal effector mechanisms of the body.

In other cases, the monoclonal antibody is coupled to another molecule, for example

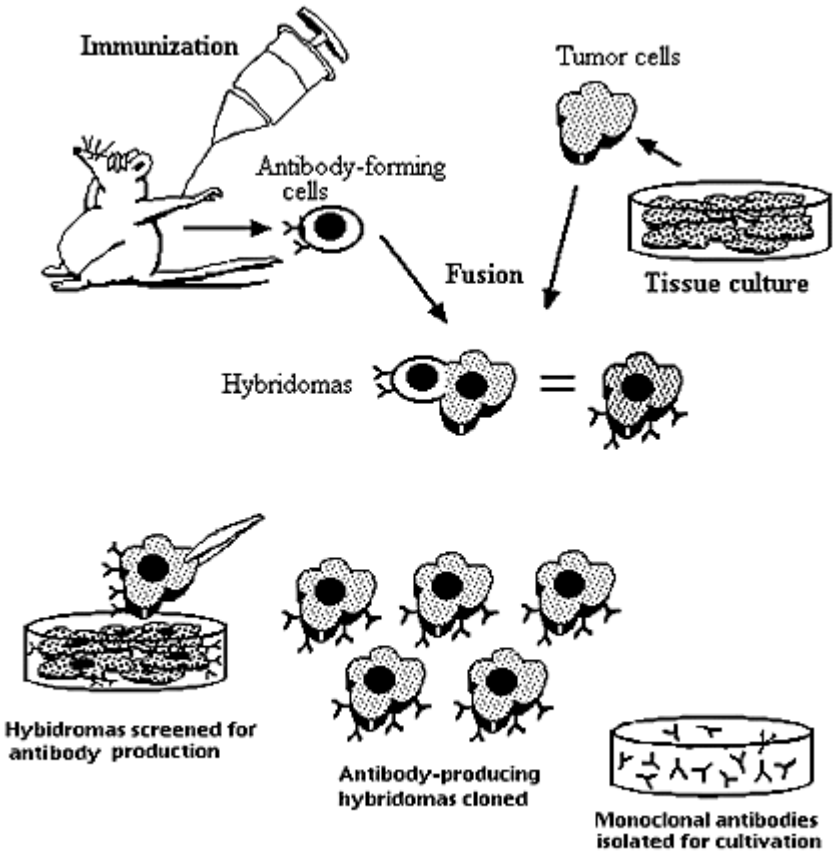
- a fluorescent molecule to aid in imaging the target
- a strongly-radioactive atom, such as Iodine-131 to aid in killing the target.

Monoclonal antibodies are produced by fusing single antibody-forming cells to tumor cells grown in culture. The resulting cell is called a **hybridoma**.

Each hybridoma produces relatively large quantities of identical antibody molecules. By allowing the hybridoma to multiply in culture, it is possible to produce a population of cells, each of which produces identical antibody molecules. These antibodies are called "**monoclonal antibodies**" because they are produced by the identical offspring of a single, cloned antibody producing cell.

Once a monoclonal antibody is made, it can be used as a **specific probe** to track down and purify the specific protein that induced its formation.

Fig 1.4.2.9



Monoclonal Antibody Production

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1.4.2.6. Summary

Immunogenicity is the ability of an antigen to induce an immune response their humoral or cell mediated immune response. The foreignness, molecular size, chemical composition and complexity and avialiability of the antigen to antigen processing and presentation influence the

immuniogenicity of substance and also the biological system that encounters the antigen effects the immunogenicity of antigen. In addition size, route of administration and presence or absence of adjuvants also influences the immunogenicity. Haptan are incomplete antigen which cannot induce immune response but when conjugated to carrier protein can become immunogenic.

The basic structure of an antibody. Molecule consists of two identical light chains and two identical heavy chains which are linked by disulphide bonds. The amino terminal 110 amino acid in each heavy and light chain consists of a variable (V_H and V_L) domain. The reminder of each consists of a single constant domain (C_L) and the heavy chain consists of three or four constant domains (C_H). Based on the type of heavy chain the immunogloblins are classified in to five types as Ig G, Ig M, Ig A, Ig D, Ig E.

1.4.2.7. Key words

1. Antigen
2. Epitope
3. Adjuvant
4. antigenic determinant
5. Haptan
6. B lymphocyte
7. T lymphocyte
8. Immunogenicity
9. Immunogloblin
10. Antibody
11. opsinization
12. Nutrilazation

1.42.8. Model Questions (Essay Questions)

1. What are the different factors which contributes for the immunogenicity of a antigen ?
2. What are adjuvants and what are different kinds of adjuvants and add a note on its importance in immunology?
3. Mention and explain the the importance of antigen in immunology?
4. Define an Antibody and give a detailed explanation of its basic structure
5. What are the different classes of Antibodies and explain their structure and the different kinds of functions

Short Questions

1. Haptan
2. Antigen
3. Adjuvant
4. Immunogenicity
5. Antigenicity
6. immunoglobulin
7. immunoglobulin

1.4.2.9. Reference Books

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2. Immuno biology : Charls Janeway

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H. Lichtman

1.4.3 TYPES OF IMMUNITY

1.4.3.1. Objective

This chapter describes the different kinds of immunity (ie)Natural immunity and Aquaried Immunity

1.4.3.2. Introduction

The immune system is a remarkably versatile defense system that has evolved to protect animals from invading pathogenic microorganisms and cancer.

Immunity- the state of protection from infections disease – has both a less specific and more specific component. The less specific component, innate immunity, provides the first line of defense against infection. In contrast to the broad reactivity of the innate immunity, which is uniform in all members of species, the specific component, adaptive immunity, does not come into play until there is an antigenic challenge to the organism.

i. Innate immunity / Natural immunity

ii. Acquired immunity / Adaptive immunity

1.4.3.4 Innate immunity

Innate immunity can be seen to comprise four types of defensive barriers: anatomic, physiologic, phagocytic, and inflammatory

1. Anatomical barriers
2. Physiological barriers
3. Phagocytory barriers
4. Inflammatory barriers

1.4.3.3.1 Anatomical barriers

Physical and anatomic barriers that tend to prevent the entry of pathogens are an organism's first line of defense against infection. The skin and the surface of mucous membranes are included in this category because they are effective barriers to the entry of most

microorganisms. The skin consists of two distinct layers: a thinner outer layer—the **epidermis**— and a thicker layer—the **dermis.** The epidermis contains
several layers of tightly packed epithelial cells. The outer epidermal layer consists of dead cells and is filled with a waterproofing protein called keratin. The dermis, which is composed of connective tissue, contains blood vessels, hair follicles, sebaceous glands, and sweat glands. The sebaceous glands are associated with the hair follicles and produce an oily secretion called **sebum.** Sebum consists of lactic and fatty acids, which maintain the pH of the skin between 3 and 5; this pH inhibits the growth of most microorganisms. A few bacteria that metabolize sebum live as commensals on the skin and sometimes cause a severe form of acne. One acne drug, isotretinoin (Accutane), is a vitamin A derivative that prevents the formation of sebum. Not only does intact skin prevent the penetration of most pathogens, but its low pH also inhibits most bacterial growth. Breaks in the skin, even small ones, resulting from wounds or abrasion are obvious routes of infection. The skin is penetrated also by biting insects (e.g., mosquitoes, mites, ticks, fleas, and sandflies); if these harbor pathogenic organisms, they can introduce the pathogen into the body as they feed. The protozoan that causes malaria, for example, is carried by mosquitoes, who deposit it in humans when they take a blood meal. Similarly, bubonic plague is spread by the bite of fleas, and Lyme disease is spread by the bite of ticks. The conjunctivae and the alimentary, respiratory, and urogenital tracts are lined by mucous membranes, not by the dry, protective skin that covers the exterior of the body. These membranes consist of an outer epithelial layer and an underlying layer of connective tissue. Although most pathogens enter the body by binding to and penetrating mucous membranes, a number of nonspecific defense mechanisms tend to prevent this entry. For example, saliva, tears, and mucous secretions act to wash away potential invaders and also contain antibacterial or antiviral substances. The viscous fluid called mucus, which is secreted by epithelial cells of mucous membranes, entraps foreign microorganisms. In the lower respiratory tract and the gastrointestinal tract, the mucous membrane is covered by **cilia,** hair like protrusions of the epithelial- cell membranes. The synchronous movement of cilia propels mucus-entrapped microorganisms from these tracts. In addition, nonpathogenic organisms tend to colonize the . epithelial cells of mucosal surfaces. These *normal flora* generally out compete pathogens for attachment sites on the epithelial cell surface and for necessary nutrients. Some organisms have evolved ways of escaping these defense mechanisms and thus are likely to invade the body

through mucous membranes. For example, influenza virus (the agent that causes flu) has a surface molecule that enables it to attach firmly to cells in mucous membranes, preventing the virus from being swept out by the ciliated epithelial cells. Similarly, the organism that causes gonorrhea has surface projections that allow it to bind to epithelial cells in the mucous membrane of the urogenital tract. Adherence of bacteria to mucous membranes is due to interactions between hairlike protrusions on a bacterium, called **fimbriae** or **pili,** and certain glycoproteins or glycolipids that are expressed only by epithelial cells of the mucous membrane of particular tissues For this reason, some tissues are susceptible to bacterial invasion, whereas others are not.(Fig 1.4.3.1)

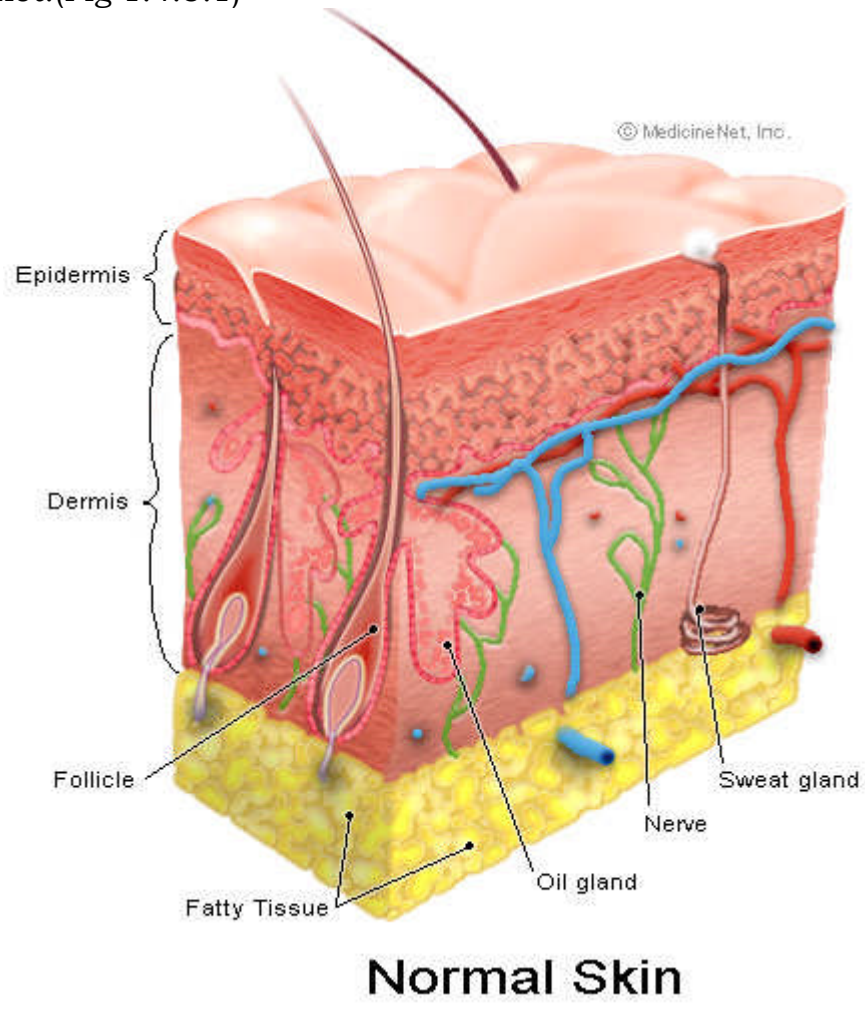

Fig 1.4.3.1

1.4.3.3.2 Physiologic Barriers

The physiologic barriers that contribute to innate immunity include temperature, pH, and various soluble factors. Many species are not susceptible to certain diseases simply because their normal body temperature inhibits growth of the pathogens. Chickens, for example, have innate immunity to anthrax because their high body temperature inhibits the growth of the bacteria. Gastric acidity is an innate physiologic
barrier to infection because very few ingested microorganisms can survive the low pH of the stomach contents. One reason newborns are susceptible to some diseases that do not afflict adults is that their stomach contents are less acid than those of adults.

A variety of soluble factors contribute to nonspecific immunity, among them the soluble proteins lysozyme, interferon, and complement. **Lysozyme,** a hydrolytic enzyme found in mucous secretions and in tears, is able to cleave the peptidoglycan layer of the bacterial cell wall. **Interferon** comprises
a group of proteins produced by virus-infected cells. Among the many functions of the interferons is the ability to bind to nearby cells and induce a generalized antiviral state. **Complement** is a group of serum proteins that circulate in an inactive state. A variety of specific and nonspecific immunologic
mechanisms can convert the inactive forms of complement proteins into an active state that enables them
to damage the membranes of pathogenic organisms, either destroying the pathogens or facilitating their clearance.

1.4.3.3.3 Phagocytic Barriers

Another important innate defense mechanism is the ingestion of extracellular particulate material by **phagocytosis.** Phagocytosis is one type of **endocytosis,** which is the general term for the uptake by a cell of material from its environment. Phagocytosis is the ingestion of particulate material, which may include whole pathogenic microorganisms (Figure 1.4.3.2). In phagocytosis, a cell's plasma membrane expands around the particulate material to form large vesicles
called **phagosomes.** Most phagocytosis is conducted by specialized cells, such as blood monocytes, neutrophils, and tissue macrophages (see Chapter 2). Most cell types are capable of other forms of

endocytosis, such as *receptor-mediated endocytosis* wherein extracellular molecules are internalized after binding by specific cellular receptors, and *pinocytosis,* by which cells take up fluid from the surrounding medium and any molecules contained in it.

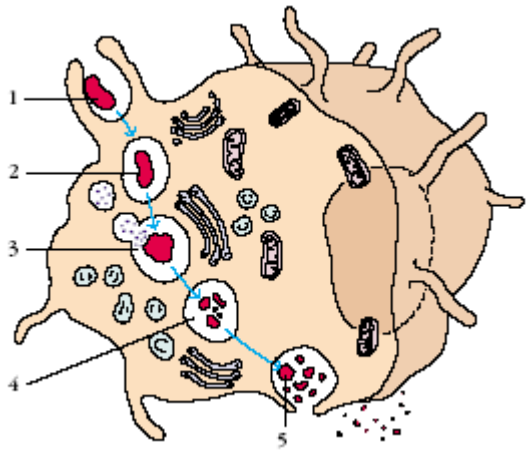

Fig 1.4.3.2 Phagocytosis of bacteria. Schematic diagram of the steps in phagocytosis: (1) attachment of a bacterium (red) to long membrane evaginations, called pseudopodia; (2) ingestion of bacterium, forms a phagosome, which moves toward a lysosome; (3) fusion of the phagosome and lysosome, releases lysosomal enzymes into the phagosome; (4) digestion of ingested material; and (5) release of digestion products from the cell

.

1.4.3.3.4 Inflammatory Barriers

Tissue damage caused by a wound or by an invading pathogenic microorganism induces a complex sequence of events collectively known as the **inflammatory response.** Many of the classic features of the inflammatory response were described as early as 1600 BC, in Egyptian papyrus writings. In the first century AD, the Roman physician Celsus described the “four cardinal signs of inflammation” as *rubor* (redness),

tumor (swelling), *calor* (heat), and *dolor* (pain). In the second century AD, another physician, Galen, added a fifth sign: *functio laesa* (loss of function). The cardinal signs of inflammation reflect the three major events of an inflammatory response (Figure 14.3.3):

1. Vasodilation—an increase in the diameter of blood vessels—of nearby capillaries occurs as the vessels that carry blood away from the affected area constrict, resulting in engorgement of the capillary network. The
engorged capillaries are responsible for tissue redness (*erythema*) and an increase in tissue temperature.

2. An increase in capillary permeability facilitates an influx of fluid and cells from the engorged capillaries into the tissue. The fluid that accumulates **(exudate)** has a much higher protein content than fluid normally released from the vasculature. Accumulation of exudate contributes to tissue swelling **(edema).**

3. Influx of phagocytes from the capillaries into the tissues is facilitated by the increased capillary permeability. The emigration of phagocytes is a complex series of events, including adherence of the cells to the endothelial wall of the blood vessels **(margination),** followed by their emigration between the capillary-endothelial cells into the tissue **(diapedesis** or **extravasation),** and, finally, their migration through the tissue to the site of the inflammatory response **(chemotaxis).** As phagocytic cells accumulate at the site and begin to phagocytose bacteria, they release lytic enzymes, which can damage nearby healthy cells. The accumulation of dead cells, digested material, and fluid forms a substance called pus.
The events in the inflammatory response are initiated by a complex series of events involving a variety of chemical mediators, whose interactions are still only partly understood. Some of these mediators are derived from invading microorganisms, some are released from damaged cells in response to tissue injury, some are generated by several plasma enzyme systems, and some are products of various white blood cells participating in the inflammatory response.

Among the chemical mediators released in response to tissue damage are various serum proteins called **acute-phase proteins.** Its name derives from the fact that it binds to the C-polysaccharide cell-wall component found on a variety of bacteria and fungi. This binding

activates the complement system, resulting in increased clearance of the pathogen either by complement-mediated lysis or by complement-mediated increase in phagocytosis. One of the principal mediators of the inflammatory response is **histamine,** a chemical released by a variety of cells in response to tissue injury.

Vasodilation and the increase in capillary permeability in an injured tissue also enable enzymes of the blood-clotting system to enter the tissue.

These enzymes activate an enzyme cascade that results in the deposition of insoluble strands of **fibrin,** which is the main component of a blood clot. The fibrin strands wall off the injured area from the rest of the body and serve to prevent the spread of infection. Once the inflammatory response has subsided and most of the debris has been cleared away by phagocytic cells, tissue repair and regeneration of new tissue begin. Capillaries grow into the fibrin of a blood clot. New connective tissue cells, called fibroblasts, replace the fibrin as the clot dissolves. As fibroblasts and capillaries accumulate, scar tissue forms

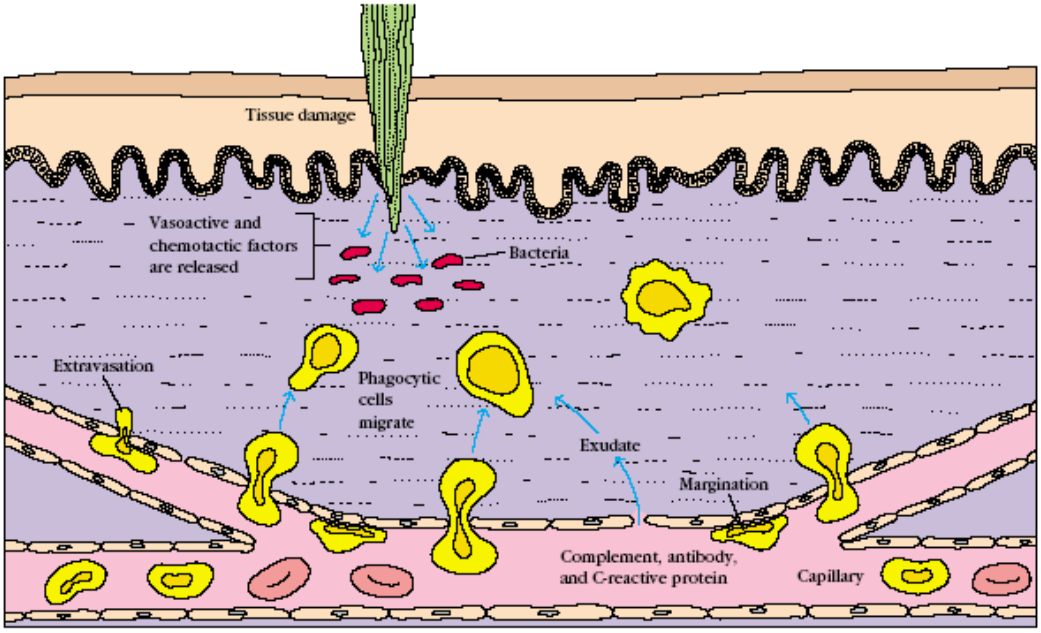

Fig 1.4.3.3
Major events in the inflammatory response. A bacterial infection causes tissue damage with release of various vasoactive and chemotactic factors. Thesefactors induce increased blood flow to the

area, increased capillary permeability, and an influx of white blood cells, including phagocytes and lymphocytes, from the blood into the tissues. The serum proteins contained in the exudate have antibacterial properties, and the phagocytes begin to engulf the bacteria,

1.4.3.4 Collaboration Between Innate and Adaptive Immunity

Innate and adaptive immunity do not operate in total independence of each other. They cooperate in important ways to produce more effective immunity. For example, the encounter between macrophages and microbes can generate "danger"or "warning" signals that stimulate and direct adaptive immune responses. Such encounters can increase the ability of macrophages to display antigen for recognition by antigen-specific T cells. This facilitates the participation ofthe adaptive immune system in the elimination of the pathogen. As well as displaying antigen in a manner that allows it to be recognized by T cells, macrophages stimulated by encounters with microbes also secrete immunoregulatory hormone-like molecules, called cytokines. The particular cytokines
produced direct adaptive immune responses that combat particular intracellular pathogens. These and other signals generated by innate immunity play important roles in triggering lymphocyte responses.

1.4.3.5 Adaptive (Specific) Immunity or Acquired immunity

Adaptive, or specific, immunity is capable of recognizing and selectively eliminating specific foreign microorganisms and molecules (i.e., foreign antigens). Unlike innate immune responses,
adaptive immune responses are reactions to specific antigenic challenges and display four characteristic attributes:

_ Antigenic specificity
_ Diversity
_ Immunologic memory
_ Self/non self recognition

The **antigenic specificity** of the immune system permits it to distinguish subtle differences among antigens. Antibodies can distinguish between two protein molecules that differ in only a single amino acid. The immune system is capable of generating tremendous

diversity in its recognition molecules, allowing it to recognize billions of uniquely different structures on foreign antigens. Once the immune system has recognized and responded to an antigen, it exhibits *immunologic memory*; that is, a second encounter with the same antigen induces a heightened state of immune reactivity. Because of this attribute, the immune system can confer lifelong immunity to many infectious agents after an initial encounter.
Finally, the immune system normally responds only to foreign antigens, indicating that it is capable of *self/non self recognition.* The ability of the immune system to distinguish self from nonself and respond only to non self molecules is essential, Adapative immunity is catagrized in to two types

1. Passive Adaptive Immunity

2. Active Adaptive immunity

Active Adapative and Passive Adapative immunization

Immunity to infectious microorganisms can be achieved by active or passive immunization. In each case immunity can be acquired either by natural processes (usually by transfer from mother to foetus or by previous infection by the organism) or by artificial means such as injection of antibodies or vaccines. The agents used for inducing passive immunity include antibodies from humans or animals, whereas active immunization is achieved by inoculation with microbial pathogens that induce immunity but do not cause disease or with antigenic components from the pathgens.

1.4.3.5.1 Passive immunization

Jenner and Pasteur are recognized as the immunologists who pioneered vaccination, or induction of active immunity but just as much recognition is due to Emil von behring and Hidesburo kitasato for their contributions to passive immunity. These investigators were the first to show that immunity elicited

in one animal can be transferred to another by injecting it with serum from the first.It is catagrized it two types

1. Natural Passive immunity
2. Artificial Passive immunity

Natural passive immunity

Passive immunization, in which preformed antibodies are transferred to a recipient, occurs naturally by transfer of maternal antibodies across the placenta to the developing fetus. Maternal antibodies to diptitheria, tetanus, streptococci, rubeula, rubella, mumps, and poliovirus all afford passively acquired protection to the developing fetus. Maternal antibodies present in colostrums and milk also provide passive immunity to the infants. The antibodies that are transferred are entirely IgG and other Ig such as IgA, IgD, IgE and IgM donot pass the placental barrier. The breast fed babies can absorb these IgA directly from the gastrointestinal tract. This natural system of protection can not operate in bottle fed babies there by breast feeding is advised in human babaies.

Artificial passive immunity

Passive immunization can also be achieved by injecting a recepient with preformed antibodies. In the past, before vaccines and antibiotics became available, passive immunization provided a major defense against various infectious diseases. Despite the risks incurred by injecting animal sera, usually horse serum, this was the only effective therapy for otherwise fatal diseases.

Artificial passive immunity is brought about by using the fallowing serum.

1. Hyper immune serum of human and animal origin.
2. Convolasut serum.

3. Pooled sera from different healthy individuals.

For example, if individuals who have not received up to date active immunization against tetanus suffer a puncture wound, they are given an injection of horse anti serum to tetanus toxin. The preformed horse antibody neutralizes any tetanus toxin produced by clostaidium tetani in the wounds.

Although passive immunization may be the treatment of choice, it should be used only when necessary because certain risks are associated with the injection of preformed antibody. If the antibody was produced in another species, such as horse (serum), the recipient can mount a strong response to the isotypic determinants of the foreign antibody. This antiisotype response can have serious complications. Some individuals for example, produce IgE antibody specific for a passive antibody. Immune complexes of this IgE bound to the passively administered antibody can mediate systemic most cells degranulation. Leading to systemic anaphylaxis. Other individuals produce IgG or IgM antibodies specific for the foreign antibody, which form complement-activating immune complexes. The deposition of these complexes in the tissues can lead to Type II hyper sensitive reactions. Even when human gamma globulin is administered passively, the recepient can generate an anti-allotype response to the human immnoglobulin, although its intensity is usually much less than that of an anti-isotype response.

1.4.3.6 Active immunization

The aim of passive immunization is transient protection or alleviation of an existing condition, the goal of active immunization is to elicit protective immunity and immunologic memory. When the active immunization is successful a subsequent exposure to the pathogenic agent elicits a heightened immune response that successfully eliminates the pathogen or at least prevents disease mediated by its products.

Active immunization can be actieved by natural infection with a microorganism or it can be acquired artificially by administration of vaccines. It is Again catagarized in to two types

1. Natural Active immunity
2. Artificial Active immunity

Natural active immunity

Here immunity is developed by the host irresponse to Ag that enter by natural infection. For example a person attacked by measles or small pox develops natural active immunity as he recovered from the disease. The immunity acquired by this way of infection is also long lasting in many cases.

Life time immunity is got following certain viral infections such as small pox, measles and mumps. The immunity attained after the bacterial disease is commonly less prominent than that attained after viral infection.

Artificial active immunity

In active immunity as the name implies, immune system plays an active role-proliferation of antigen reactive T and B cells results in the formation of memory cells. Active immunization with various types of vaccines has played an important role in the reduction of deaths from infectious diseases, especially among children. Vaccines are prepared by live or killed micro organisms or their products.

→ Live vaccines → Live microbes are attenuated by different methods.

Eg: Sabine vaccine – Poliomyeletis

→ Vaccines prepared with killed microbes

Eg: Salk vaccine – Poliomyeletis

→ Toxoids – Formalin inactivated toxins

Eg: Tettanus toxoid, Diphtheria toxoid

1.4.3.7 Summary

Immunity is the state of protection against foreign organisms or substances. Vertebrates have 2 types of immunity, innate and adaptive.Innate immunity is not specific to any one pathogen but rather constitutes a first line of defense, which includes anatomic, physiologic, endocytic and phagocytic and inflammatory barriers.Innate and adaptive immunity operate in cooperative and inter dependent ways. The activation of innate immune responses produces signals that stimulate and direct subsequent adaptive immune responses.Adaptive immune responses exhibit 4 immunological attributes:Specificity; diversity; memory and self/nonself recognition.The high degree of specificity in adaptive immunity arises from the activities of molecules that recognize and bind specific antigens.The immune system produces both humoran and cell mediated responses. The humoral response is best suited for elimination of exogenous antigens; the cell – mediated response, for elimination of endogenous antigens.While an adaptive immune system is found only in vertebrates, innate immunity has been demonstrated in organisms us different us insects, earthworms and higher plants.

1.4.3.8 Keywords

1.Immunity

2. Native immunity

3. Innate immunity

4.Aquired immunity

5. Adaptive immunity

6.Lysozyme;

7.Phagocytosis

8.Exudates

9.Edema

10.Diapedesis

11.Extravasation

12.Diversity

13.Antigenic-specificity

14.Immunological memory

15.Self / nonself recognition

16.Lymphocytes

17.Antigen presenting cells

18.Humoral immunity

19.Major histocompatibility complex

20.Exogenous antigen

21.Endogenous antigen .

1.4.3.9 Model Questions

Essay Questions

1.What are the different kinds of immunities and explain in detail the adaptive immunity?

2. define Innate immunity and add a note on the Skin as the first line defense barrier?

3.What is immunity and explain the different kinds of immunities?

Short Questions

1. Innate Immunity

2. Active immunity

3. Anatomical Barriers

4. Inflammation

5.Aquired immunity

1.4.3.10 References

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1.5.1COMPLEMENT SYSTEM

1.5.1.1 Objective

The objective of this chapter is to give a detailed explanation of the components of the complement and the activation of the complement

1.5.1.2 Intraduction

The complement system is the major effector of the humeral branch of the immune system. Research on complement began in the 1890's. When Jules Bordet at the institute Pasteur in pairs showed that sheep antiserum to the bacterium vibrio cholera caused lysis of the bacteria and that heating the antiserum destroyed its bacteriolytic activity. Surprisingly, the ability to lyse the bacteria was restored to the heated serum by adding fresh serum that contained no antibodies directed against the bacterium and by itsey was unable to kill it. Bordet correctly reasoned that bacteriolytic activity requires two different substances first, the specific antibacterial antibodies, which survive the heating process, and a second, heat-sensitive component responsible for the lytic activity, Bordet devised a simple test for the lytic activity. The easily detected lysis of antibody-coated red blood cells, caused homolysis, he designated the responsible substance alevin. Bordet's name for this activity did not survive long. Shortly after ward in Berlin, Paul Ehrlich independently carried it as "the activity of blood serum that completes the action of antibody. In causing years, researchers discovered that the action of complement was the result of interactions of a laye and complex group of proteins. The reaction pathways. Interactions, and activities of the components of the complement systems were determined in a series of superiments with proteins that were isolated, purified and then mixed in various combinations to reconstitute the ability to cause lysis. In this way the activity of each component of the complement system was deduced.

1.5.1.3 The functions of complement

Research on complement now includes more than 30 soluble and ceu-bound proteins. Biologic activities of this

system impact both innate and acquired immunity and reach far beyond the original observations of antibody- mediated lysis of bacteria and red blood cells. After initial activation, the various complement components interact, in a highly regulated cascade, to carry out a no. of basic functions.

- → Lysis of cells, bacteria, and uiruses
- → Opsonization, which promotes phagocytosis of particulate antigens.
- → Binding to specific complement receptors on cues of the immune system, triggering activation of immune responses such as inflammation and secretion of immuno regulatory molecules that amplify or later specific immune response.
- → Immune clearence, which removes immune compleues from the circulation and deposits them in spleen and liver.

1.5.1.4 The complement components

The proteins and glycoproteins that compose the complement system are synthesized mainly by liver hepatocytes, although significant amounts of them are also produced by blood monocytes, tissue macrophages, and epithelial cells of gastro intestinal and genitourinary tracts. These components constitute 5% of the serum globulin fraction and most circulate in the serum in functionally inactive forms. Many components are proenzyme or zymogens. Which are inactive until proteolytic cleuage which removes an inhibitory fragment and exposes the active site. The complement – reaction sequence starts with an enzyme cascade. Several of the activated components become inactivated shortly if they do not react with next component in the sequence.

Complement components are designated by numericals, by latter symbols, or by trivial names the peptide fragments formed by activation of a component are denoted by small letters. In most cases, the smaller fragments resulting from cleuage of a component is designated “a” and layer fragments designated “b” note that C_2 is an exception : C_{2a} is the largest clevage. The layer

fragments bind to the target near the site of can initiate localized inflammatory respouses by binding to specific receptors. The complement fragments interact with one another to form functional complexes. Those complexes that have enzymatic activity are designated by a bar over the number or symbol.

Table 1.4.4.1

	Component Name	No. of Chains in Native Molecule	Mol Wt (dalton—approximate)	Plasma or Serum Concentration (μg/mL—approximate)	Cleavage Fragments	Chromosome
Early classical pathway	C1q	18	410,000	70–300		1
	C1r	1	83,000	34–100		12
	C1s	1	85,000	30–80		12
	C4	3	204,000	350–600	C4a, C4b, C4c, C4d	6
	C2	1	102,000	15–30	C2a, C2b	6
Third component	C3	2	190,000	1200–1500	C3a, C3b, C3c, C3d, C3f, C3g, C3dg, C3d-K, iC3b	19
Membrane attack complex (terminal complement complex)	C5	2	196,000	70–85	C5a, C5b	9
	C6	1	125,000	60–70		5
	C7	1	120,000	55–70		5
	C8	3	150,000	55–80		1,9
	C9	1	66,000	50–160		5
Alternative pathway	factor B	1	100,000	140–240	Ba, Bb	6
	P	4	224,000	20–30		X
	factor D	1	24,000	1–2		?
Mannan binding lectin pathway	Mannan binding lectin	18	540,000	1		10
	MASP-1	1	94,000			?
	MASP-2	1	76,000			?
Classical pathway control	C1INH	1	105,000	180–275		11
	C4BP	7	550,000	250		1
Alternative pathway control	factor H	1	150,000	300–560		1
	factor I	2	100,000	34–50		4
Miscellaneous control	AI		310,000	35		?
	S protein (vitronectin)		83,000	150–500		17
	factor J	1	20,000	2.6–8.2		?
	SP40,40		80,000	50		8

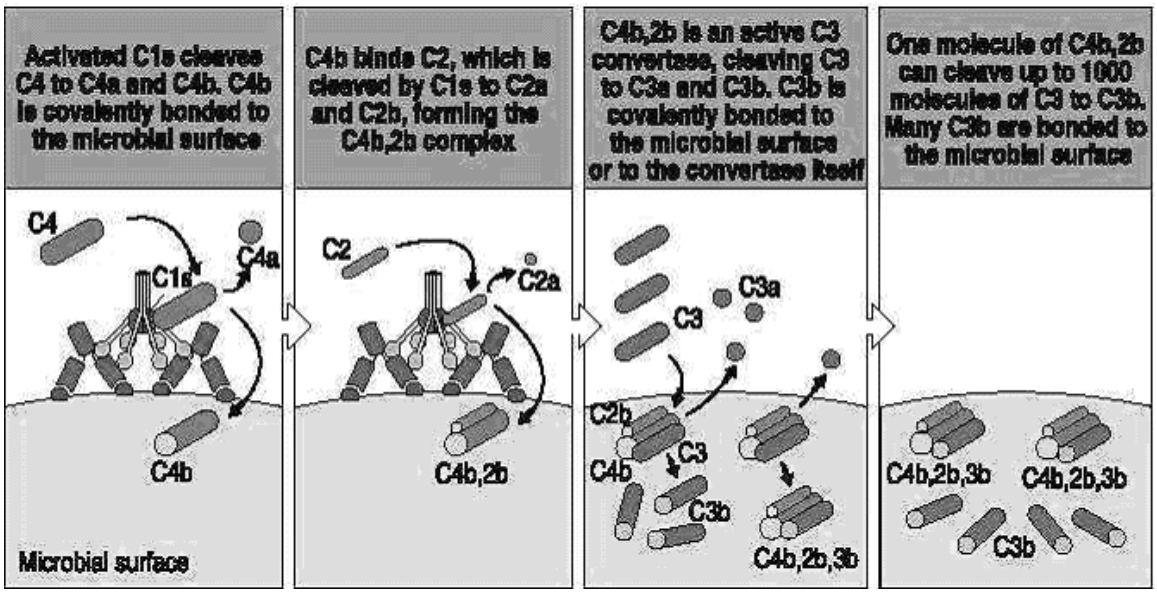
1.5.1.5 Complement activation

The early steps in complement activation culminating in formation of C_{5b}, can occur by the classical path way, the alternative pathway, or the lectin pathway. The final steps that lead to a membrane attack are the same in all pathways.

1.5.1.5.1 Classical pathway

Complement activation by the classical pathway commonly begins with the formation of soluble antigen antibody complexes or with the binding of antibody to antigen an a suitable target, such as a bacterial ceu-IgM and certain sub classes of IgG. IgG_2 and IgG_3 can activate the classical complement pathway, as can certain nonimmunologic activators. The initial stage of activation involves C_1, C_2, C_3 and C_4 which are present in plasma in functionally inactive forms. Because the components were named in order to their discovery and before their functional roles had been determined the numbers in their names do not always reflect the order in which they react.

Fig 1.5.1.1 The classical Path way



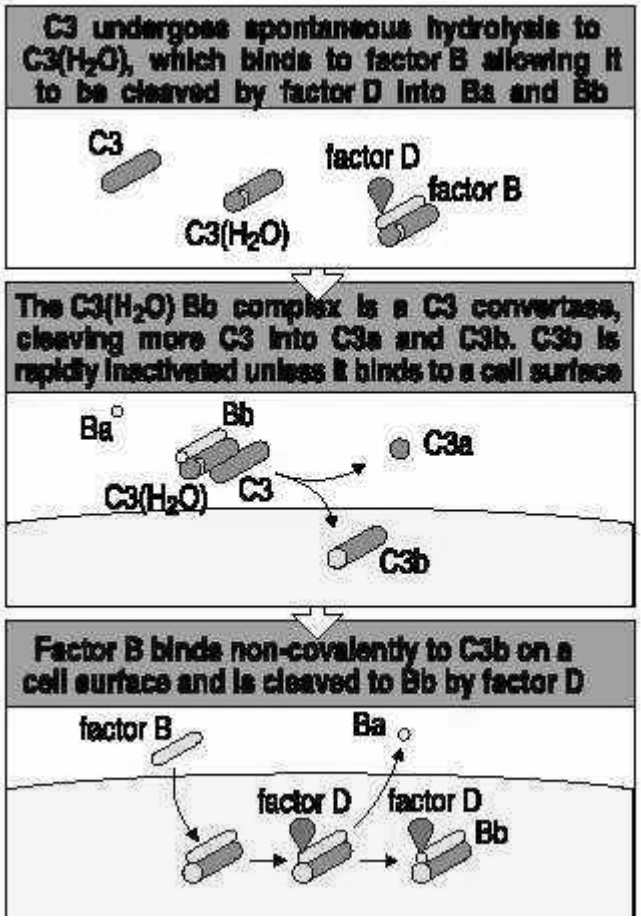
1.5.1.5.2 Alternative path way

The alternative path way is an antibody independent means to generate bound C_{5b} the same product as the classical path way generates in a sequence of events requiring antigen antibody complements for iniciation no antibody is required, the alternative pathway is a component of the innate immune system. This major path way of complement activation involves four serum proteins C_3 factor B, factor D, and properdin ulike the classical path way the alternative path way is initiated with out requiring antibody, in most cases by various cell-surface constitutes that are foreign to the host for example both gram-ve and gram+ve bacteria have cell-wall constitutes that can activate the alternative path way. The inter mediates in the alternative path way for generating C_{5b} (are depicted schematically in fig 13-7).

In classical pathway, C_3 is cleaved to C_{3a} and C_{3b} rapidly by the enzymatic activity of the C_3 convertase. In the alternative pathway. Serum C_3, which contains an unstable thioester bond is subject to slow spontaneous hydrolysis to yield C_{3a} and C_{3b}. The C_{3b} component can bind to foreign surface antigens or even to the host's own cells. The membrane of the most mammalian cells have high level of sialic acid, which contribute to the rapid inactivation of bound C_{3b} molecules on host cells; consequently this binding rarely leads to furthur reactions on the host cell membrane because many foreign antigen surfaces. Eg: bacterial cell wall, yeast cell wall, and certain viral envelope have only low levels of sialic acid, C_{3b} bound to these surface remains active for a longer time. The C_{3b} present on the surface of the foreign ceu can bind auother serum protein called factor B by way of a Mg^{2+} dependent bond binding to C_{3b} exposes a site on factor B that serves as `the substrate for an enzymatically active serum protein called factor D. factor D cleves the C_{3b} bound factor B, releasing a small fragment (B_a), which diffuses away, and generates $C_{3b}Bb$. The $C_{3b}Bb$ complex has C_3 convertase activity

and thus is analagus to the $C_{4b}2a$ complex in the classical pathway. The C_3 convertase activity of $C_{3b}Bb$ has a half life of only 5 minutes unless the serum protein properidin binds to it, stabilizing it and extending the half-life of this convertase activity to 30 minutes.

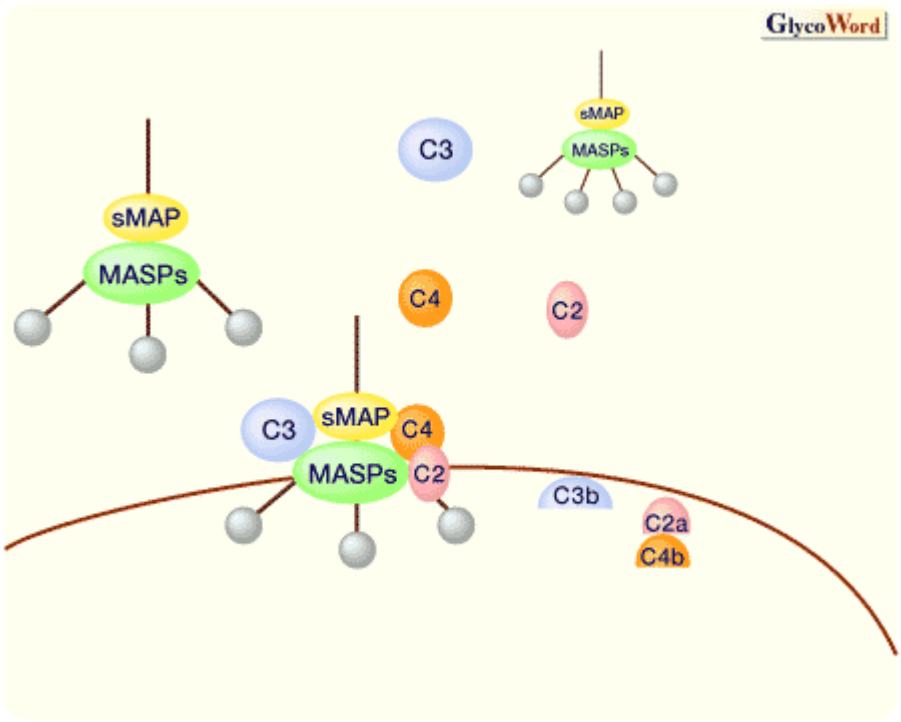
Fig.1.5.1.2. The Alternative Pathway



1.5.1.5.3 The lectin pathway

Recently, an additional means by which the complement cascade can be activated was described. This addition to the complement repertoire is called the lectin pathway. Lectins are proteins that bind to a carbohydrate. The lactin pathway like the alternative pathway does not depend on antibody for its activation. However, the mechanism is more like that of the classical pathway, because after initiation proceeds, through the action of C_4 and C_2 to produce C_5 convertase. The path way is activated by the binding of mannose binding lectin (MBL) to mannose residues on glycoprotein or carbohydrates on the surface of micro organisms. MBL is an acute phase protein produced in inflammatory responses its function in the complement path way is similar to that of Ciq, which resembles in structure. After MBL binds to the surface of a ceu or pathogen MBL-associated serine proteases, or MSP, binds to it. The active complex formed by this association causes clevage and activation of C_4 MASP has structural similarity to cir and cis and mimics their activities. Details of the lectin pathway remain obscure, but this means of activating the C_2-C_4 components to form a C_5 convertase with out need for specific antibody binding certainly represents an important innate defense mechanism comparable to the alternative pathway.

Fig 1.5.1.3 The lectin Pathway



1.5.1.5.4 Terminal sequence: Formation of membrane – attack complex

The terminal sequence of complement activation involves C_{3b}, C_6, C_7, C_8 and C_9, which interact sequentially to form an macro molecular structure called the membrane attack complex (MAC). This complex displaces the membrane phospho lipids, forming a large trans membrane channel that disrupt the membrane of the target cell and enables ions and small molecules of diffuse through it freely.

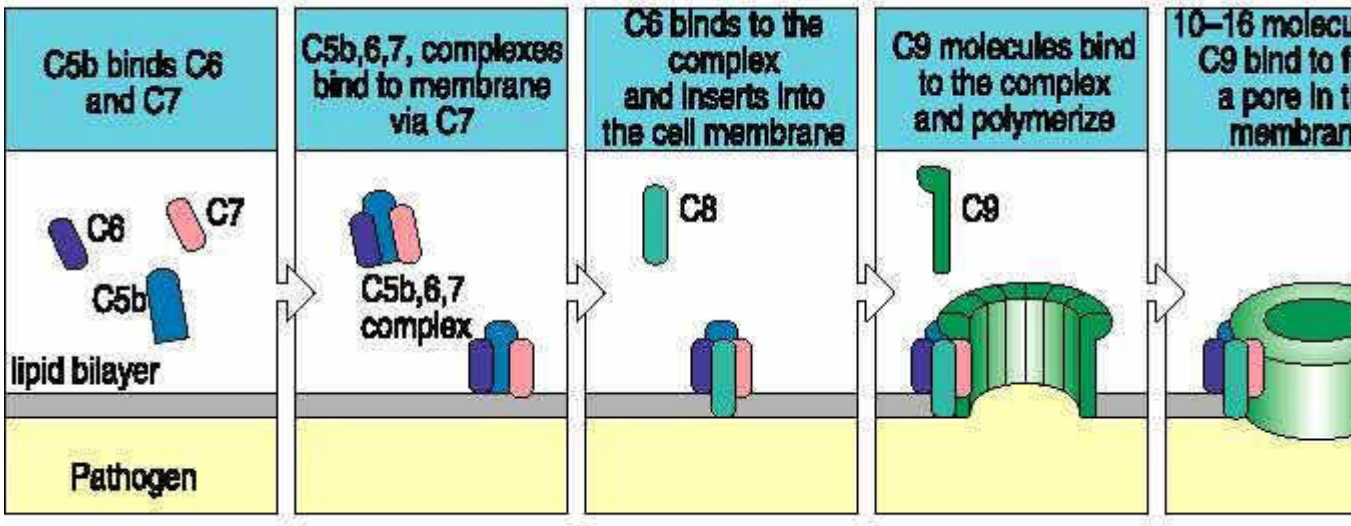
As noted previously in the classical, lectin and alternative pathways, a C_5 convertase cleaves C_5, which contains two protein chains (α and β) after binding of C_5 to the non enzymatic C_{3b} components of the convertase, the amino terminus of the α chain is cleaved. This generates the small C_{5a} fragment, which diffuses away, and the large C_{5b} fragment, which binds to the surface of the target ceu and provides a binding site for the subsequent components of the membrane attack complex. The C_{5b} component is extremely labile and is in activated with in 2 minutes unless C_6 binds to it and stabilizes its activity up to this

point, all the complement reactions takes place on the hydrophilic surface of membranes or on immune complexes in the fluid phase. As C_{5b6} binds to C_7, the resulting complex under goes a hydrophyllic – amphiphilic structural transition that exposes hydrophobic regions, which serve as binding sites for membrane phospho lipids of the reaction occurs on a target-cell membrane, the hydrophobic binding sites enable the C_{5b67} complex to insert into the phospholipid bilayer if, however the reaction occurs on an immune complex or other noncellular activating surface. Then the hydrophobic binding sites can not anchor the complex and it is released. Released C_{5b67} complexes can insert in to membrane of near by cells and mediate "innocent – by stander" lysis, although this rarely occurs. In a number of diseases in which immune complexes are produced, tissue damage may result from such innocent by stander lysis. (This autoimmune process will be discussed in chapter 20).

Binding of C_8 to membrane-bound C_{5b67} induces a conformational change in C_8, so that it too undergoes a hydrophylic – amphiphilic structural transition, exposing a hydrophobic region, which interacts with the plasma membrane. The C_{5b678} complex creates a small pore 10 A^o in diameter: formation of this pore can lead to lysis of red blood cells but not of nucleated cells. The final step in formation of the MAC is the binding and polymerization of C_9, a perforin-like molecule, to the C_{5b678} complex. As many as 10-17 molecules of C_9 can be bound and polymerized by a single C_{5b678} complex. During polymerization, the C_9 molecules under go a hydrophilic – amphophilic transition, so that they also insert into the membrane. The completed MAC, which has a tubular form and functional pore size of 70-100 A^o, consits of a C_{5b678} complex surrounded by a poly-C_9 complex. Since ions and small molecules can diffuse freely through the central channel of the MAC, the cell can not maintain its osmotic stability and is killed by an influx of water and loss of electrolytes. The components of the terminal sequence of the complement pathways.

Fig 1.5.1.4

Figure 7.39a



1.5.1.6 Biological consequences of complement activation

Complement serves as an important mediator of the humoral response by amplifying the response and converting it into an effective defense mechanism to destroy invading micro organisms and viruses the MAC mediates cell lysis while other complement components or split products participate in the inflammatory response, opsonization of antigen, viral neutralization, and clearance of immune complexes.

Many of the biological activities of the complement system depend on the binding of complement fragments to complement receptors, which are expressed by various cells. In additions, some complement receptors play an important role in regulating complement activity by binding biologically active complement components and degrading them in to inactive products. The complement receptors and their primary ligands, which include various complement.

1.5.1.6.1 Cell lysis

The membrane attack complex formed by complement activation is capable of lysing a broad spectrum of micro organisms, viruses, erythrocytes, and nucleated cells. Because the alternative and lectin path ways of activation generally occur with out an initial antigen- antibody interaction, these pathways serve as important innate systems of non specific defense against infections micro organisms. The requirement for an initial antigen-antibody reaction in the classical path way supplements these non-specific innate defense with a more specific defense mechanism.

The importance of ceu mediated immunity in host defense against viral infections has been emphasized nevertheless, antibody and complement do play a role in host defense against viruses and are often crucial in containing viral spread during acute infection and in protecting against reinjection. Most-perhaps-all enveloped viruses are susceptible to complement-mediated lysis. The viral envelope is largely derived from the plasma membrane of the infected host cell and is therefore susceptible to pore formation by the membrane-attack complex. Among the pathogenic viruses shown to be lysed by complement-mediated lysis are herpes virus, orthomyno viruses, paramyno viruses and retero viruses.

The complement system is generally quite effective in lysing gram –ve bacteria. However, some gram-ve bacteria and most gram +ve bacteria have mechanisms for evading complement mediated damage for example: a few gram –ve bacteria can develop resistance to complement mediated lysis that correlates with the virulence of the organism. In E.coli and solmonella, resistance to complement is associated with the smooth bacterial phenotypic, which is characterized by the presence of long polysaccharide side chains in the cell-wall lipopolysaccharide component. It has been proposed that the increased LPS in the wall of resistant strains may prevent insertion of the MAC in to the bacterial membrance, so that the complex is released from

the bacterial cell rather than forming a pore. Strains of neisseria gouorrheae resistant to complement mediated killing have been associated with disseminated gonococal infections in humans. Some evidences suggests that the membrane proteins of resistant Neisseria strains undergo non covalent interactions with the MAC that prevent its insertion in to the outer membrane of the bacterial cells. Most gram negative bacteria are susceptible to complement –rediated lysis. In contrast, gram +ve bacteria are generally resistant to complement – radiated lysis because the thick peptidoglycon layer in their cue wall prevents insertion of the MAC into the inner membrane. Although complement activation can occur on the cell membrane of encapsulated bacteria such as streptococcus preumonia, the capsule prevents interaction b/u C_{3b} deposited on the membrane and the CR_1 on phasocytic cells some bacteria possess an elastase that inactivates C_{3a} and C_{5a}, precenting these split products from inducing an inflammatory response. In addition to these mechanisms of evasion, various bacteria, viruses, fungi, and protozovan contain proteins that can interrupt the complement cascade on the surfaces, thus mimicking the effects of the normal complement regulatory proteins C_{4b}BP, CRI and DAF.

Nucleated cells tend to be more resistant to complement mediated lysis that red blood cells are lysis of nucleated cells requires formation of multiple membrane attack complexes, where as single MAC can lyse a red blood cell. Many nucleated cells, including the majority of cancer cells, can endocytose the MAC if the complex is removed soon enough, the cell can repair any membrane damage and restore its osmotic stability. This is the reason why complement mediated lysis by antibody specific for tumor-cell antigens is often not effective.

Fig 1.5.1.5

1.5.1.6.2 Inflammatory response

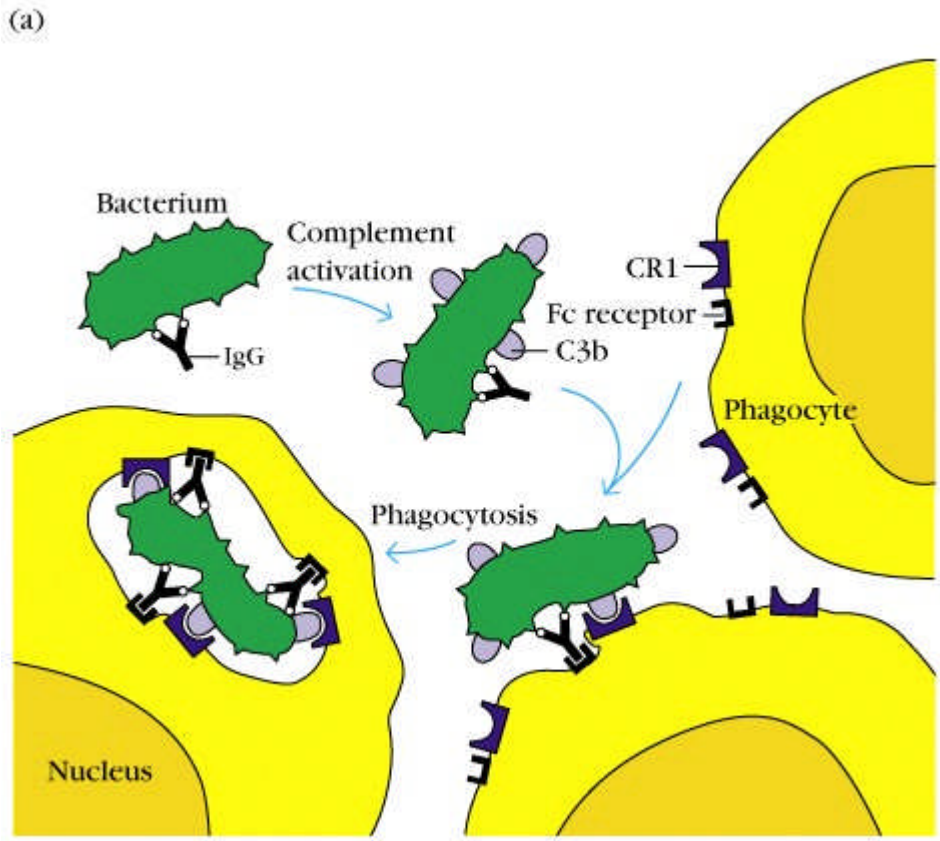
The complement cascade is often viewed in terms of the final out come of cell lysis, but various peptides generated during

formation of the MAC play a decisive role in the development of an effective inflammatory response. The smaller fragments resulting from complement clevage, C_{3a}, C_{4a} and C_{5a} called anaphylatoxins bind to receptors on most cells and blood basophils and induce degranulation with release of histamine and other pharmacologically active mediators. The an aphylatoxins induce smoot-ruscle contraction and increased vascular permeability activation of the complement system thus results in influxes of fluid that carries antibody and phagocytic cells to the site of antigen entry. The activities of these highly reactive anaphylatoxins are regulated by a serum protein called carboxypeptidase N_2 which cleaves an Arg residue from the `C' terminus of the molecules, resulting in what are termed the des-Arg forms. The des Arg forms of C_{3a} and C_{4a} are completely inactive while that of C_{5a} retains about 10% of its chemotactic activity and 1% of its ability to cause smooth muscle contraction.

Each of C_{3a} and C_{5b67} can induce moncytes and neutrophils to adhere to vascular endothelial ceus, entravasate through the endothelial living of the capillary, and migrate toward the site of complement activation in the tissues. C5a is most potent in mediating these processes, with picomolar quantities being effective the role of complement in leukocyte chemotaxis (is discussed in 15 chapter).

1.5.1.6.3 Opsonization of antigen

C_{3b} is the major opsonin of the complement system, although C_{4b} and ic3b also have opsonizing activity. The amplification that occurs with C_3 activation results in a coating of C_{3b} on immune complexes and particulate antigens. Phapocytic cells as well as some other cells, express complement receptors that bind C_{3b}, C_{4b}. When antigen has coated with C_{3b} during complement activation, the coated antigen binds to cells bearing CRI. If the cell is a phasocyte (Neutrophil, Monocyte), phasocytosis will be enhanced. Activation of phasocytic cells by various agents, including C_{5a} an apnylatoxin, has been shown to increase the No. of CRIS from 5000 on resting phasocytes to 50,000 an activated cells, greatly facilitating their phapocytosis of C_{3b} – coated antigen.



1.5.1.6.4 Viral neutralization

The complement system plays an imp role in host defense by neutralizing viral infectivity. Some viruses retero viruses, (Epstein – Barr Virus, New castle disease virus, and rubella virus) can activate the alternative, lectin, or even the classical pathway in the absence of antibody for most viruses, the binding of serum antibody to the repeating subunits of the viral structural proteins creates particulate immune complexes ideally suited for complement activation by classical pathway. The complement system mediates viral neutralization by a no. of mechanisms. Some degree of neutralization is achieved through the formation of large viral aggregates. Simply because these aggregates reduces the net no. of infections viral particles. Although antibody does play a role in formation of viral aggregates, in vitro studies. Show that the C_{3b} components

facilitates aggregate formation in the presence of a little as two molecules of antibody per viriou. Eg: Polyoma virus coated with antibody is neutralized when serum containing activated C_3 is added.

1.5.1.7 Summary

The complement system is over 20 proteins commonly found in plasma that are part of the so-called innate immune system. These can be traced back in evolution to the most primitive immune defense mechanisms and to the "first line of defense". The two main parts of this network include the activation route (classical pathway) and an amplification loop (the alternative pathway). According to P McGeer, complement is one of the significant factors in neurotoxicity in the AD brain and often found in markedly elevated levels in post-mortem AD brain samples.
The three main actions of the complement protein cascade include opsonization (marking of target invading cells, bacteria, or debris for elimination by phagocytotic cells)[C1q, C3b], anaphylacsis (the activation of other inflammatory signals, including cytokines) [C4a,C3a, C5a], and formation of the membrane attack complex which leads to the lysis of cells directly. Each of these aspects is regulated in the healthy individual, where checks and balances prevent runaway undesired reactions. For example, inhibitors of the activation of C1 in solution and on cell surfaces and substances that protect cells from complement injury are known. In states of chronic inflammation, including neuroinflammation, such checks and balances fail.

1.5.1.8 Model Questions

Essay Questions

1.What is a complement? explain the classical pathway?

2.Describe the different pathway of the complement?

Short Questions

1.Membrane attack complex

2.Lectin Pathway

1.5.1.9 Key words

1.complement

2.clssicial pathway

3.Lectin Pathway

4.components

1.5.1.10 Refrences

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1.5.2 HYPERSENSITIVE REACTIONS

1.5.2.1 Objective:

The objective of this chapter is to explain the different kind of the hyper sensitive reactions which will take place during heightened immune response.

1.5.2.2 Introduction:

An immune response evokes a battery of effecter molecules that act to remove antigen by various mechanisms described in previous chapters. Generally these effecter molecules induce a sub clinical, localized inflammatory response that eliminates antigen without extensively damaging the host's tissue. Under certain circumstances, however, this inflammatory response can have deleterious effects, resulting in significant tissue damage or even death. This inappropriate immune response is termed hypersensitivity or allergy. Although the word hypersensitivity implies an increased response, the response is not heightened always but may instead be an inappropriate immune response to an antigen. These may develop in the course of either humoral or cell-mediated responses.

Anaphylactic reactions within the humoral branch initiated by antibody or antigen-antibody complexes as immediate hypersensitivity, because the symptoms are manifest within minutes or hours after a sensitized recipient encounters antigen three types of such hypersensitive reactions are commonly recognized. IgE mediated (type-1), antibody mediated (type-II) and immune complex mediated (type-III) hypersensitivity. A fourth type of hypersensitivity depends on reactions within the cell mediated branch. These are initiated by T_{DTH} cells and are referred as delayed type hypersensitivity (DTH), in recognition of the delay of symptoms until days after exposure.

1.5.2.3 Gell and coombs classification

Several forms of hypersensitive reaction can be distinguished, reflecting differences in the effector molecules generated inn the course of the reaction. In immediate hypersensitive reactions, different antibody isotypes induce different immune effector molecules. IgE antibodies, for example, induce most cell degranulation with release of histamine and other biologically active molecules. IgG and IgM

abs, induce hypersensitive reactions by activating complement. The effector molecules in these reactions are the membrane attack complex and such complement split products as C_3a, C_4a and C_5a. In delayed type reactions the effector molecules are various cytokines secreted by T_{DTH} cells.

As it became clear that different immune mechanisms can give rise to hypersensitive reactions, P.G.H Gell and R.R.A. Coombs proposed a classification scheme in which these reactions are divided into 4 types, I, II, III and IV each involving distinct mechanisms, cells and mediator molecules. This classification scheme has served an important function identifying the mechanistic differences among various hypersensitive reactions. But it is important to point out that a great deal move complexity exists in a vast array of secondary effects that blur the boundaries between the four categories.

1.5.2.3.1 IgE mediated (Type-I) hypersensitivity

Type I hypersensitive reaction is induced by certain types of antigens, referred to as allergens and has all the hallmarks of a humoral response. That is an allergen induces a humoral antibody response by the same mechanisms. For other soluble antigens, resulting in the generation of antibody screening plasma cells and memory cells. Plasma cells secrete IgE. This class of antibody binds with high affinity to F_c receptors on the surface of tissue most cells and blood basophylls. Such IgE coated most cells and basophylls are said to be sensitized. A later exposure to the same allergen cross-links the membrane bound IgE on sensitized most cells and basophils, causing degranulation of these cells. The pharmacologically active mediators released from the granules act on the surrounding tissues. The principal effects – vasodilation and smooth muscle contraction may be either systemic or localized, depending on the extent of mediator release.

Components of Type I reaction

Several components are critical to development of type I hypersensitive reactions.

Allergens

The majority of humans mount significant IgE responses only as a defense against parasitic infections. After an individual has been exposed to a parasite, serum IgE levels increase and remain high until the parasite is successfully cleared from the body. Some persons, however may have an abnormality called atopy, a hereditary predisposition to the development of immediate hypersensitivity reactions against common environmental ags. The IgE regulatory, defects suffered by atopic individuals allow nonparasitic ag to stimulate inappropriate IgE production, leading to tissue damaging hypersensitive reactions type I.

Several common allergens associated with Type I hypersensitivity are proteins such as Foreign serum and vaccines (which are available in)foods such as Nuts, Seafoods, Eggs, Peas, Beans and Milk. Plant Pollens such as Rye grass, Ragweed, Timothy grass, Birch trees, Drugs such as Penicillin, Sulfonamides, local anesthetics, salicylates, Insect products such as Beevenom, Wasp Wenom, Anl venom, Cockroach calyx, Dust mites, Mold spores and Animal hair and dander.

It has been reported that Regweed yields 16 tons of pollen in a single season. The pollen particles are inhaled and their tough outer walls is dissolved by enzymes in the mucous secretions, releasing the allergenic substances.

Reaginic antibody IgE

The existence of a human serum factor that reacted with allergens was first demonstrated by K. Prausnitz and H. Kustner

in 1921. The local wheal and flave response that occurs when an allergen is injected into a sensitized individual is called the P.K reaction.

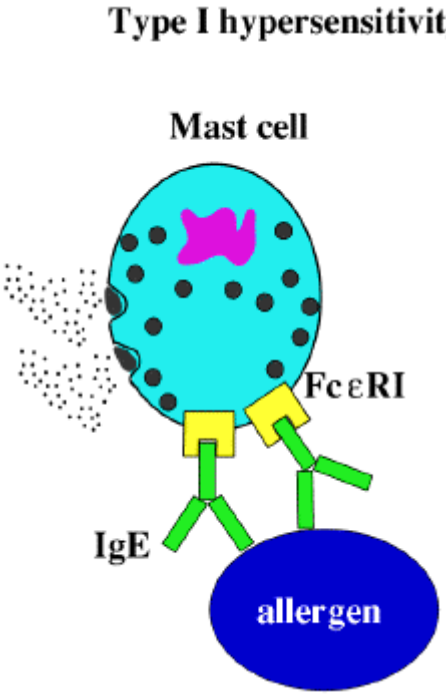
Experiments conducted by K and T. Ishizaka in the mid 1960s showed that biological activity of reaginic antibody in a P.K test could be neutralized by rabbit antiserum against whole atopic human seva but not by rabbit antisera specific for the human immunoglobulin classes known at that time (IgA, IgO, IgG and IgM). In addition when rabbits were immunized with sera from ragweed sensitive individuals, the rabbit antiserum could inhibit a positive ragweed P.K test even after precipitation of the rabbit antibodies specific for the human IgG, IgA, IgM and IgD isotypes. The ishizakas called this new isotype IgE in reference to the Eag of ragweed that they usually used to characterize it.

Serum IgE levels in normal individuals fall within the range of 0.1-0.4 μg/ml. IgE consists of two heavy and two light chains with a combined molecular weight of 190,000. the higher molecular weight compared with IgG (150,000) is due to the presence of an additional constant region domain. Halflife of IgE is 2-3 days only. These has been bound to its receptors on most cells and basophils.

Mast cells and basophils

The cells that bind IgE were identified by incubating human leukocytes and tissue cells with either ^{125}I labeled IgE myeloma protein. In both cases, autoradio graphy revealed that the labeled probe bound with high affinity to blood basophils and tissue most cells. Basophils are granulocytes that circulate in the blood of most verlegrates, in humans they account for 0.5% - 1.0% of the circulating white blood cells. Their granulated cytoplasm stains with basic dyes, hence the name basophil. Electron microscope revealed a multilobed nucleus, few mitochondria, numerous glycogen granules and electron dense membrane bound granules, which contain pharmacologically active mediators, scattered throughout the cytoplasm.

Most cell precursors are formed in the bone marrow during haomatopoiesis and are carried to all vascularized peripheral tissues. Most cells are found throughout connective tissue, particularly near blood and lymphatic vessels. Some tissues, including the skin and mucous membrane surfaces of the respiratory and gastro intestinal tracts contain high concentrations of most cells; skin for example contains 10,000 most cells / mm^3. Electron micrographs of most cells reveals numerous membrane bounded granules, which like basophils contain, pharmacologically active mediators, distributed throughout the cytoplasm most cells also secrete a variety of cytokines, including IL-1, IL-3, IL-4, IL-5, IL-6, GM-CSF, TGF-β and TNF-α. Active mediators released from the granules manifests the type I hypersensitive reactions.Fig 1.5.1.1

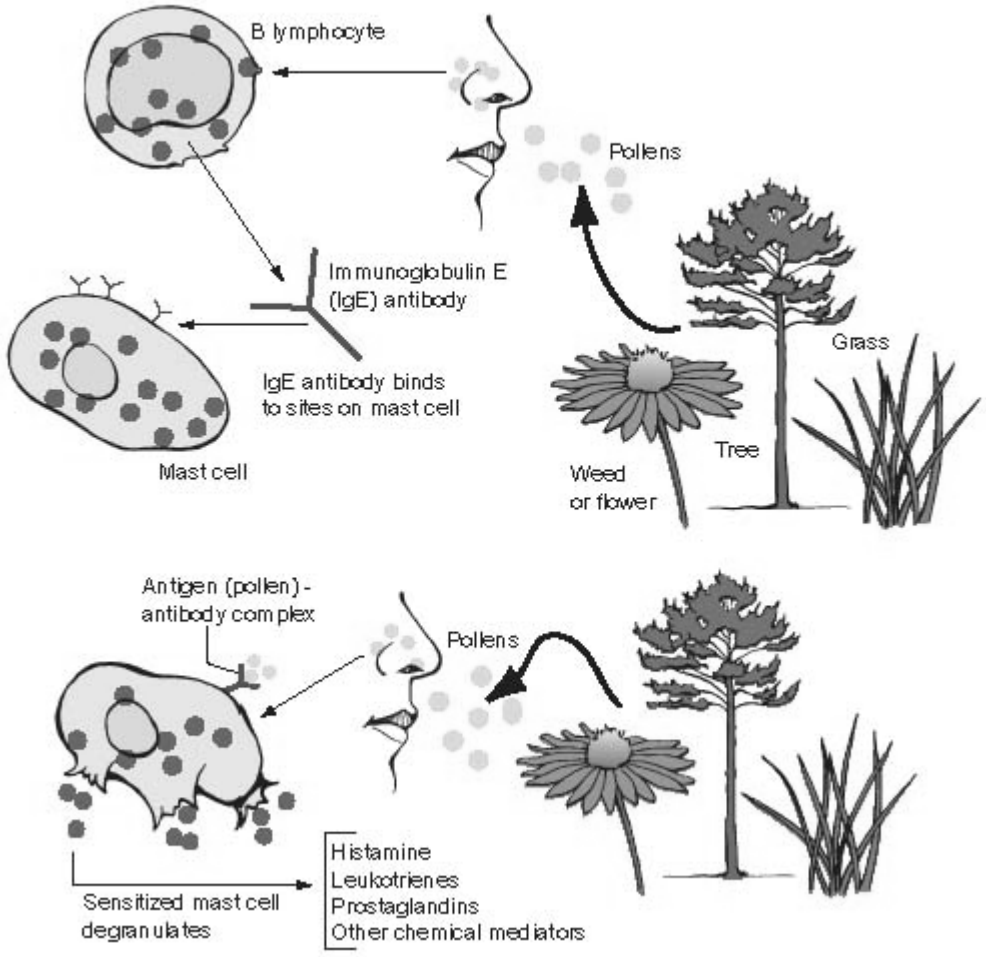


Mechanism of IgE-mediated degranulation

The biochemical events that mediate degranulation of most cells and blood basophils have many features in common. This section presents a general overview of most cell degranulation mechanisms without calling attention to the slight differences between most cells and basophils. Although most cell degranulation generally is initiated by allergen cross linkage of bound IgE, a number of other stimuli can also initiate the process, including anaphylatoeins (C_3a, C_{4a} and C_{5a}). Various drugs such as synthetic ACTH (adrenocorticotropic hormone) codeine and morphine; and compounds such as the calcium ionophore.

Fig1.5.2.2 Type I Hypersensetivity

Type I hypersensitivity reaction: allergic rhinitis. (A) The first exposure of mast cells in nasal mucosa to inhaled antigens (eg, pollens from weeds, grasses, trees) leads to the formation of immunoglobulin E (IgE) antibody molecules. These molecules then bind to the surface membranes of mast cells. This process sensitizes mast cells to the effects of inhaled antigens (allergens). (B) When sensitized mast cells are re-exposed to inhaled pollens or other antigens, they release histamine and other chemical mediators which then act on nasal mucosa to produce characteristic symptoms of allergic rhinitis.



Mediators of Type I reactions

These mediators are pharmacologically active agents that act on local tissues as well as populations of secondary effector cells, including eosinophils, neutrophils, T-lymphocytes, monocytes and platelets. The mediators thus serve as an amplifying terminal effector mechanism, much as the complement system serves as an amplifier and effector of an ag-ab interaction.

The mediators can be classified as either primary or secondary. The primary mediators are produced before degranulation and are stored in the granules. The most significant primary mediators are histamine, proteases, eosinophyll, chemotactic factor, neutrophil chemotactic factor, and heparin. The secondary mediators either are synthesized after target cell-activation or are released by the breakdown of membrane phospholipids during the degranulation process. The secondary mediators include platelet activating factor, leukotrienes, prostaglandins, bradykinin, and various cytokines. The differing manifestations of type I hypersensitivity in different species or different tissues partly reflect variations in the primary and secondary mediators present. The main biological effects of several of these mediators are described briefly.

Histamine

Histamine which is formed by decarboxylation of the aminoacid histidine, is a major component of most cell granules, accounting for about 10% of granule weight. Because it is stored-preformed-in the granules, its biological effects are observed within minutes of most –cell activation. Once released from most cells, histamine initially binds to specific receptors on various target cells. Three types of histamine receptors-designated H_1, H_2 and H_3 – have been identified. These receptors have different tissue distributions and mediate different effects when they bind histamine.

Most of the biological effects of histamine in allergic reactions are mediated by the binding of histamine to H_1 receptors. This binding induces contraction of intestinal and bronchial smooth muscles increased permeability of venules, and increased mucus secretion by goblet cells. Interaction of histamine with H_2 receptors increases vasopermeability and dilation and stimulated exocrine glands. Binding of histamine to H_2 receptors on most cells and basophils suppresses degranulation. Thus histamine exerts negative feedback on the release of mediators.

Leukotrienes and prostaglandins

As secondary mediators, the leukotrienes and prostaglandins are not formed until the most cell undergoes degranulation and the enzymatic breakdown of phospholipids in the plasma membrane. An ensuring enzymatic cascade generates the prostaglandins and the leukotrienes. It therefore lakes a longer time for the biological effects of these mediators to become apparent. This effects are move pronounced and longer lasting, however than those of histamine. The leukotrienes mediate bronchoconstriction, increased vascular permeability and mucus production. Prostaglandins D_2 causes bronchoconstriction.

The contraction of human bronchial and tracheal smooth muscles appears at first to be mediated by histamine but within 30-60s further contraction is mediated by the leukotrienes and prostaglandins. In humans, the leukotrienes are thought to contribute to the prolonged bronchospasm and buildup of mucus seen in asthmatics.

Cytokines

Adding to the complexity of the type I reaction is the variety of cytokines released from most cells and eosinophils some of these may contribute to the clinical manifestations of type I hypersensitivity. Human most cells secrete IL-4, IL-5, IL-6 and TNF-α. These cytokines alter the local micro environment, eventually leading to the recrutement of inflammatory cells such as neutrophils and cosinophils. IL-4 increases IgE production by B cells. IL-5 is specially important in the recruitment and activation of eosinophils. The high concentrations of TNF-α secreted by most cells may contribute to shock in systemic anaphylaxis.

Consequences of type I reactions

The clinical manifestations of type I reactions can range from serious life threatening conditions such as systemic anaphylaxis and asthma, to hay fever, eczema, which are merely annoying.

Systemic anaphylaxis

Systemic anaphylaxis is a shock like and often fatal state whose onset occurs within minutes of a type I hypersensitive reaction. This was the response observed by Portier and Richet in dogs after antigenic challenge symptoms cohabiting are which reflects differences in the distribution of most cells and in the biologically active contents of their granules.

Active sensitization in guinea pigs is induced by a single injection of a foreign protein such as egg albumin. After an incubation of about 2 weeks, the animal is usually challenged with an intravenous injection of the same protein within 1 min, the animal becomes restless, its respiration becomes labored, and its blood pressure drops. As the smooth muscles of the gastrointestinal tract and bladder contract, the guinea pig defecates and urinates. Finally bronchiole constriction results in death by asphyxiation within 2-4 min of the injection. These events all stem from the systemic vasodilation and smooth muscle contraction brought by mediators released in the course of reaction. Postmortem examination reveals that massive edema, shock and broncliole constriction are the major causes of death.

Localised anaphylaxis (Atopy)

In localized anaphylaxis, the reaction is limited to a specific toget tissue or organ, often involving epithelial surfaces at the site of allergen entry. The tendency to manifest localized anaphylactic reactions is inherited and is called atophy. Atopic allergies which afflict at least 20% of the population in developed countries, include a wide range of IgE-mediated disorders, including allergic rhinitis (hey fever), asthama, atopic dermatitis and food allergies.

Allergic rhinitis

The most common disorder affecting 10% of the U.S population, is allergic rlinitis, commonly known as hey fever. This results from the reaction of air borne allergens with sensitized most cells in the conjunctivae and nasal mucosa to

induce the release of pharmacologically active mediators from most cells; these mediators then cause localized vasodilation and increased capillary permeability. The symptoms include watery exudation of the conjunctivae, nasal mucosa and upper respiratory tract as well as sneefing and coughing.

Asthma

Another common manifestation of localized anaphylaeis is asthma. In some cases, air borne or blood borne allergens, such as pollen, dust, fumes, insect products or viral antigens, trigger an asthmatic attack; in other cases, an asthmatic attack can be induced by exercise, or cold, apparently independently of allergen stimulation. It also triggers by degranulation of most cells with release of mediators; but instead of occurring in the nasal mucosa, the reaction develops in the lower respiratory tract. The resulting contraction of the bronchial smooth muscles leads to bronchoconstriction. Airway edema, mucus secretion, and inflammation contribute to the bronchial constriction and to airway obstruction.

Food allergies

Various foods also can induce localized anaphylaxis in allergic individuals. Allergen crosslinking of IgE on most cells along the upper and lower gastro intestinal tract can induce localized smooth muscle contraction and vasodilation and thus such symptoms as vomiting or diarrhea. Most cell degranulation along the gut can increase the permeability of mucus membranes, so that the allergen enters the blood stream various symptoms can ensure, depending on where the allergen is deposited. When a food allergen is carried to a most cell in the skin, causing swollen (edematous) red (erythematous) eruptions, this response is called a wheal and flase reaction.

Atopic dermatitis

Atopic dermatitis is an inflammatory disease of skin that is frequently associated with a family history of atopy. Serum IgE levels are often elevated. They develops skin eruptions that are erythematous and filled with pus. Unlike a delayed type

hypersensitive reactions which involves T_{H1} cells, the skin lesions in atopic dermatitis have T_H2 cells and an increased number of eosinophils.

Therapy for type I hypersensitivities

First step in controlling type I hypersensitives is to identify the offending allergen and avoid contact if possible. Immunotherapy with repeated injections of increasing doses of allergens to reduce the severity. Such repeated introduction of allergens by subcutaneous injections appears to cause a shift toward IgG production or to induce T-cell mediated suppression that turns off the IgE response. In this situation, IgG is referred to as blocking antibody because it competes for allergen, binds to it and forms a complex that can removed by phagocytosis.

Another approach for treating allergies stems from finding that soluble ag tend to induce a state of energy by activating T cells in the absence of necessary costimulatory signal. Soluble ag is internalized by endocytosis processed and presented with class II MHC molecules. Antihistamines have been the most useful drugs for symptoms of allergic rhinitis. These drugs act by binding to the histamine receptors on target cells and blocking the binding of histamine. The H_1 receptors are blocked by the classical antihistamines and the H_2 receptors by a newer class of antihistamines.

Several drugs block release of allergic mediators by interfering with various biochemical steps in most cell activation and degranulation. Disodium cromoglycate prevents Ca^{2+} influx into most cells. Theophylline which is commonly administered to asthmatics orally or through inhalers, blocks phosphodiesterase, which catalyzes conversion of CAMP levels to 5^1-AMP. Cortisone and various other anti-inflammatory drugs also have been used to reduce type I reactions.

1.5.2.3.2 Type II Antibody mediated cytotoxic hypersensitivity

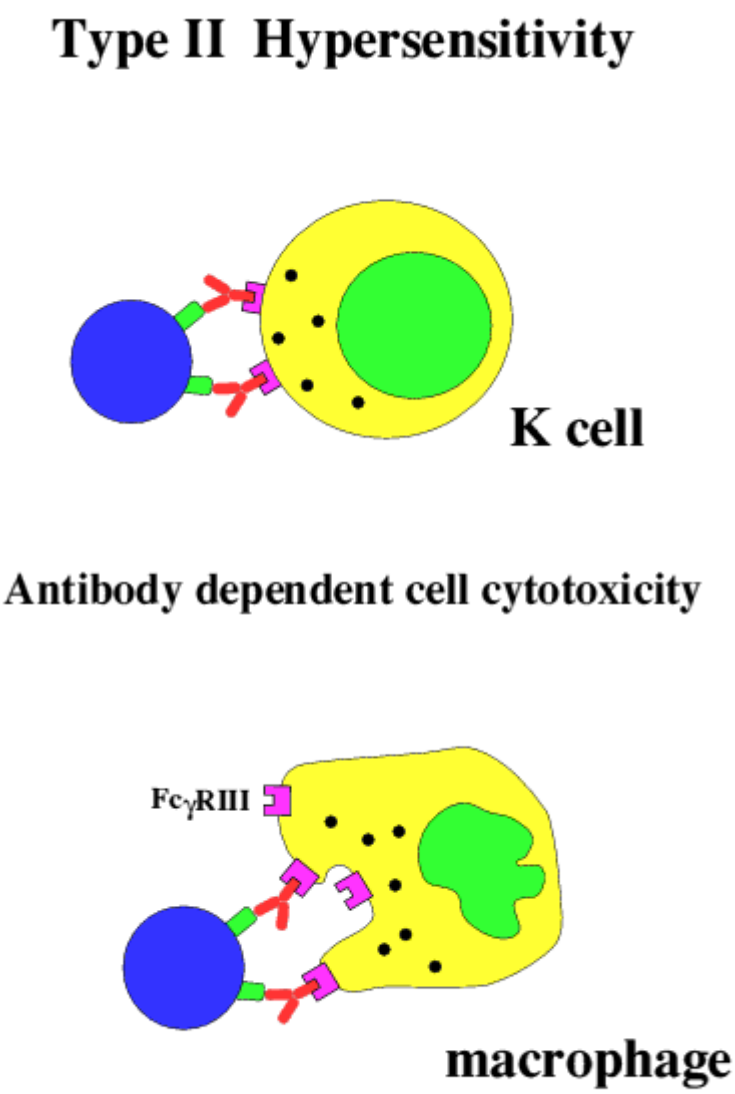
Type II hypersensitive reactions involve antibody mediated destruction of cells. This type of reaction is best exemplified by

blood transfusion reactions, in which host antibodies react with foreign ag on the incompatible transfused blood cells and mediate distruction of these cells. Ab mediate cell destruction by activating the complement system to create pores in the membrane of the foreign cell. Ab can also mediate cell destruction by ab dependent cell mediated cytotoxicity (ADCC). In this process cytotoxic cells with F_c receptors bind to the F_c region of antibodies on target cells and promote killing of the cells. Ab bound to a foreign cell also can serve as opsonin, enabling phagocytic cells with F_c or C_{3b} receptors to bind and phagocytose the ab-coated cells.

Transfusion reactions

A large number of proteins and glycoproteins on the membrane of RBC are encoded by different genes, each of which has an alternative alleles. An individual possessing one allelic form of a blood group ag can recognize other allelic forms on transfused blood-group antigen as foreign and mount an ab response.

Antibodies to the A, B and O ags called isohemagglutinins are usually of IgM class. Transfusion of blood into a recepient possessing ab's to one of the blood group antigens can result in a transfusion reaction. The clinical manifestation of transfusion reaction results from massive intrarascular hemolysis of transfused RBC by ab plus complement these manifestations may be either immediate or delayed. Reactions that begin immediately are most commonly associated with ABO blood group incompatibilities which lead to complement mediated lysis triggered can be seen in plasma; it is filtered through the kidneys, resulting in hemoglobinuria. Some of the hemoglobin gets converted to bilirubin which at high levels is toxic. Typical symptoms include fever, clills, nausea, clotting within blood vessels, pain in the lower back, and hemoglobin in the urine. Treatment involves prompt termination of the transfusion and maintenance of urine flow with a diuretic because the accumulation of hemoglobin in the kidney can cause acute tubular necrosis. Fig 1.5.2.3



Hemolytic diseases of the newborn

Hemolytic diseases of the new born develops when maternal IgG antibodies specific for fetal blood group antigens cross the placenta and destroys fetal RBC. The consequences of such transfer can be minor, serious or lethal. Severe hemolytic disease of the newborne, called erythroblastosis fetalis, most

commonly develops when an Rh^+ fetus expresses an Rh antigen on its blood cells that the Rh^- mother does not express.

During pregnancy, fetal RBC are separated from the mother's circulation by a layer of cells in the placenta called the trophoblast. During her first pregnancy with an Rh^+ fehes and Rh^- women in usually not exposed to enough fetal RBC to activate her Rh-specific B cells. At the time of delivery, however, seperation of the pacenta from the uterine wall allows larger amounts of fetal umbilical cord blood to enter the mother's circulation. These fetal RBC activate Rh-specific B cells, resulting in production of Rh-specific plasma cells and memory B cells in the mother. The secreted IgM ab clears the Rh^+ fetal RBC from the mothers circulation but the memory cells remain a threat to any subsequent pregnancy with Rh^+ fetes. Activation of these memory cells in a subsequent pregnancy results in the formation of IgG anti-Rh as which cross the placenta and damage the fetal RBC. Mild to severe anemia can develop in the fetes, some times with fetal consequences. In addition, conversion of hemoglobin to bilirubin can present an additional threat to the new borne because the lipid soluble bilirubin may accumulate in the brain and cause brain damage.

Hemolytic disease of the newborne caused by Rh incompatibility in a subsequent pregnancy can be almost entirely prevented by administering abs against the Rh ag to the mother. Within 24-48 hours after the first delivery. These antibodies called Rhogam bind to any fetal RBC that enter the mothers circulation at the time of delivery and facilitate their clearence before B cell activation and ensuing memory cell production can take place. In a subsequent pregnancy with an Rh^+ fetes, a mother who has been treated with Rhogam is unlikely to produce Igg anti Rh abs thus the fetes is protected from the damage that occurs when these abs cross the placenta.

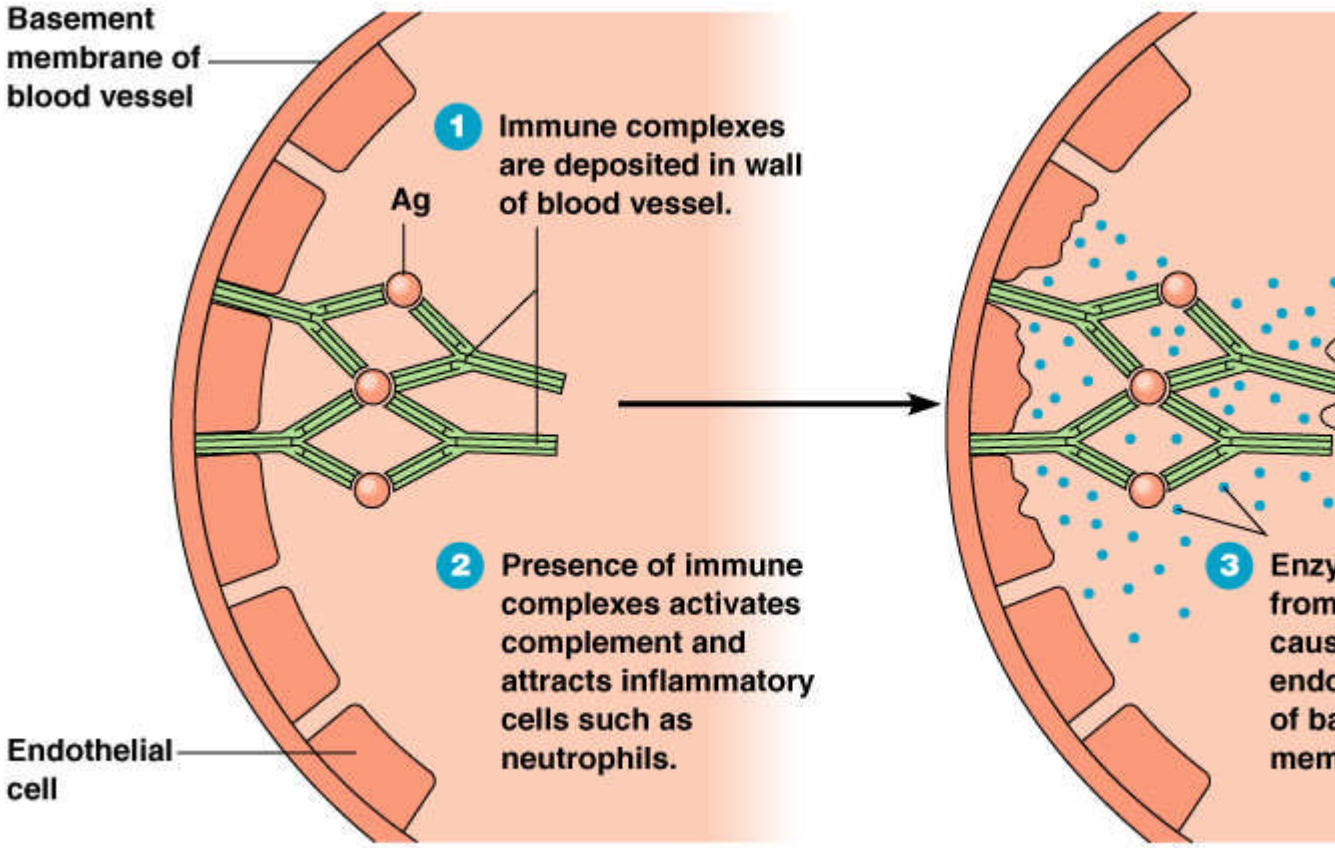
Hemolytic disease is caused by Rh incompatibility is detected during pregnancy. The treatment depends on severity of (disease) reaction. The fetes can be given an intrauterine blood exchange transfusion to replace fetal Rh^+ RBC with Rh –ve

cells. The mother can also be treated during the pregnancy by plasmapheresis.

1.5.2.5.3 Immune complex-mediated (Type III) Hypersensitivity

The reaction of antibody with antigen generates immune complexes. Generally this complexing of ag with ab facilitates the clearence of ag by phagocytic cells. In some cases however large amounts of immune complexes can lead to tissue damaging type III hypersensitive reactions. The magnitude of reaction depends on quantity of immune complex as well as distribution in their body. When complexes are deposited in tissues very near the site of ag entry-a localized reaction develops. The depositions of these complexes initiates a reaction that results in the recruitment of neutrophils to the site. In particular complex deposition was frequently observed in synovial membrane joints, on the glomerular basement membrane of the kidney, blood vessel walls and on the choroids plexus of the brain. The tissues there is injured as a consequence of granular release from the neutrophil.

Type III hypersensitive reaction develops when immune complexes activate the complement systems array of immune effector molecule. Localized and generalized type III reactions were observed. Fig 1.5.1.4



Localized type III reactions

Injection of an ag intradesmally or subcutaneously into an animal that has high levels of circulating ab specific for that ag leads to formation of localized immune complexes which mediate an acute arthous reaction within 4-8 hours. Neutrophils adhering to the vascular endothelium and then migrating in to the tissues at the site of immune complex deposition. As the reaction develops, localized tissue and vascular damage results in an accumulation of fluid and RBC at that site. The severity of the reaction may vary from mild swelling and redness of tissue necrosis.

After an insect bite a sensitive individual may have a rapid, localized type I reaction at the site. Often some 4-8 hours a typical Arthers reaction also develops at the site with pronounced erythema and edma. Intrapulmonary Arthus type

reactions induced by bacterial spores, fungi or dried fecal proteins can also cause pneumonitis or alveolitis. These reactions are known by a variety of common names reflecting the source of ag. For example `farmers lung' develops after inhalation of thermophilic actinomycetes inhalation of a serum protein in dust derived from dried pegion feces.

Generalised type III reactions

When large amounts of ag enter the blood stream and bind to ab, circulating immune complexes can form. If ag is in excess, small complexes form because these are not easily cleared by phagocytic cells, they can cause tissue damaging type III reactions at various sites. These generalized type III reactions were often observed after the administration of antitoein containing foreign serum, such as horse antitetanus or antidiphtheria serum. In such cases the recepient develops ab specific for foreign serum proteins. These abs then form circulating immune complexes. With the foreign serum ag's. After days or weeks. He individual manifests symptoms that are called serum sickness. Which the symptoms are weakness, generalized vasculitis with edema and erythema lymphadenopathy, arthritis and sometimes glomerulonephritis.

Formation of circulating immune complexes contribute to the pathogenesis of a number of conditions otherthan serum sickness. They include

→ Autoimmune Diseases :

systemic lupus erythematosus

Rheumatoid arthritis

Good Pasteur's syndrome

→ Drug Reactions :

Allergies to penicillin and sulpnamides

→ Infections diseases :

Post streptococeal glomerulonephritis

Mening it is

Hepatitis

Mononucleosis

Malaria

Trypanosomiasis

Complexes of ab with various microbes have been shown to induce type III reactions, including skin rashes, arthritic symptoms and glomerulonephritis.

1.5.2.3.4 T_{DTH} – mediated (Type IV) hypersensitivity

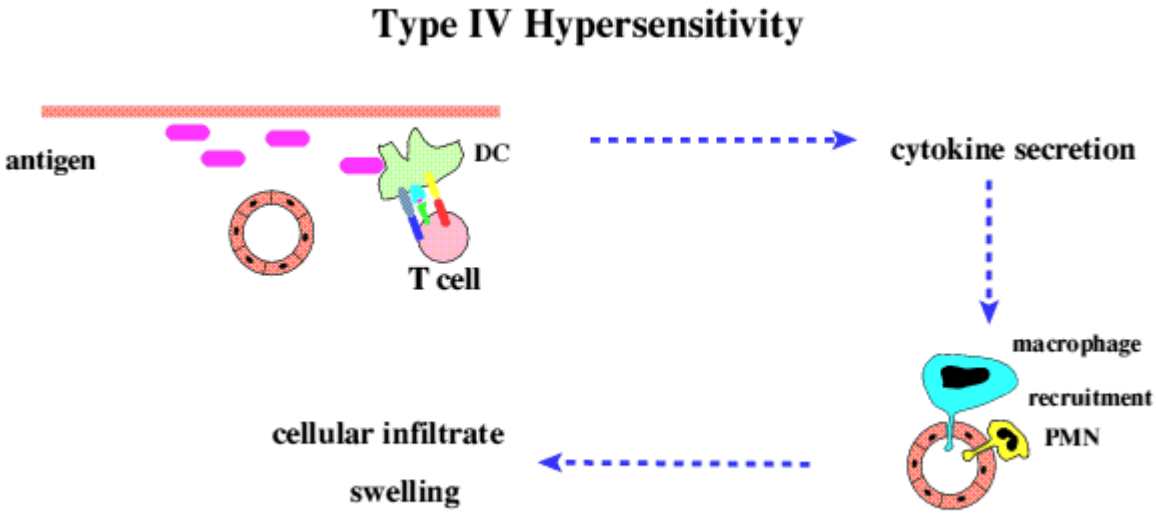
Type IV hypersensitivity reactions develop when ag activates sensitized T_{DTH} cells; these cells generally appear to be a T_H1 subpopulation, although sometimes T_C cells are involved. Activation of T_{DTH} cells by ag on appropriate ag presenting cells results in secretion of various cytokines, including IL-2, interferon gamma, MIF, and TNF-β. The over all effect of the cytokines is to draw the macrophages into the area and activate them promoting increased phagocytic activity and increased concentrations of lytic enzymes for more effective killing. As lytic enzymes leakout of the activated macrophages into the surrounding tissues localized tissue distruction can ensue. These reactions typically take 48-72 hours to develop the time required for initial T_{DTH} cell activation and cytokine secretion to mediate accumulation of macrophages and the subsequent release of their lytic enzymes. The hall mark of type IV reaction are delay in time required for the reaction to develop and the recruitment of macrophages as opposed to neutrophils as found

in type III reaction. Macrophages are the major components of the infiltrate that surrounds the site of inflammation.

Type IV reaction is important in host defence against parasite and bacteria that can live within cells. Once these organisms are inside the host cells, circulating abs can not reach them. However, the heightened phagocytic activity and buildup of lytic enzymes from macrophages into the area of infection lead to nonspecific destruction of cells and thus of the intracellular pathogen. The granulomatous skin lesions seen with mycobacterium leprae and lung cavitation seen with M. tuberculosis are examples of tissue damage that can result when chronic delayed-type hypersensitive reactions develop the reaction to an intradermal injection of an ag can serve as a test for presence of T_{DTH} cells previously sensitized by ag. The use of PPD ag to detect previous exposure to M.tuberculosis. A depletion in CD_4+ T cells assosciated with AIDS can be monitered by repeated skin testing with any of various ags, such as haptens or ags that induce type IV DTH response in most normal individuals. As AIDS progresses the decline in T_{DTH} cells is reflected in decreased skin reactivity to such ag's.

Many contact dermatitis including the respones to formaldehyde, trinitrophenol, nickel, turpentine, various cosmetics and hair dyes, poison oak and poison ivy, are mediated by T_{DTH} cells. Most of these substances are small molecules that can complex with skin proteins. This complex is internalized by ag presenting cell in the skin then processed and presented together with class II MHC molecules causing activation of sensitized T_{DTH} cells.

Fig.1.5.1. 5



1.5.2.6 summary

. Hypersensitivity is the name given to a state in which an immune response damages the body's own tissues. There are four or five types of hypersensitivity that are often described, each is a different way the immune system can damage the host. The four group classification was expounded by P H G Gell and R A A Coombs in 1968. Type I, anaphylactic or immediate-type hypersensitivity is an allergic reaction provoked by re exposure to a specific antigen called allergen Exposure may be by injestion , inhalationor injection or direct contact. The reaction is mediated by IgE antibodies and produced by the immediate release of histamine arachidonate and derivatives by basophils and mast cells. This causes an inflammatory response leading to an immediate (seconds to minutes) reaction (anaphylaxis) The reaction may be either local or systemic and symptoms vary from mild irritation to sudden death from anaphylactic shock In type II hypersensitivity, the antibodies produced by the immune response bind to antigens on the patient's own cell surfaces. The antigens

recognised in this way may either be intrinsic ("self" antigen, innately part of the patient's cells) or extrinsic (absorbed onto the cells during exposure to some foreign antigen, possibly as part of infection with a pathogen). IgG and IgM antibodies bind to these antigens to form complexes that activate the classical pathway of complement activation for eliminating cells presenting foreign antigens (which are usually, but not in this case, pathogens). That is, mediators of accute inflammation are generated at the site and membrane attack complexes cause cell lysis and death. The reaction takes hours to a day

In type III hypersensitivity, soluble immune complexes (aggregations of antigens and IgG and IgM antibodies) form in the blood and are deposited in various tissues (typically the skin ,kidney and joints) where they may trigger an immune response according to the classical pathway of complement activation (see above). The reaction takes hours to days to develop. Type IV hypersensitivity is often called delayed type as the reaction takes two to three days to develop. Unlike the other types, it is not antibody mediated but rather is a type of cell mediated response.

1.5.2.7 Model Questions

Essay questions

1.What is hypersensitivity and add a note on the immediate hypersensitivity?

2.What is delayed type of hypersensitivity and explain the how it is induced?

3.explain the immune complex mediated immunity?

Short Questions

1.Atopy

2. IgE reaginic antibody

3. Erthroblastis Fetalis

1.5.3.6 References

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1.5.3 AUTO IMMUNITY

1.5.4.1 Objective:

The objective of this chapter is to describe the different types of autoimmunity, different mechanisms of auto immunity and the treatment

1.5.4.2 Introduction

Early in this century, Paul Ehrlich realized that the immune system could go awry and instead of reacting against foreign antigens, could focces its attack on self antigens. He termed this condition "horror autotoeicus". We now understand that while mechanisms of self-tolerance normally protect an individual from potentially self-reactive lymphocytes, there are failures. They result in an inappropriate response of the immune system against self-components, and that is termed as Autoimmunity. In the 1960's, it was believed that all self-reactive lymphocytes were eliminated during their development and that a failure to eliminate these lymphocytes led to autoimmune consequences.

Sometimes the damage to self-cells or organs is caused by antibodies; in other cases, T cells are the culprit. For example, a common form of autoimmunity is tissue injury by mechanisms similar to type II hypersensitivity reactions. Autoimmune hemolytic anemic is an excellent example of such an tutoimmune disease. In this disease, antigens on RBC are recognized by auto-antibodies, which results in the destruction of blood cells, which in turn results in anemia. Auto-antibodies also are the major offender in Hashimoto's thyroiditis, in which abs reactive with tissue specific ags such as thyroid peroeidase and thyroglobulin cause severe tissue destruction.

Many automimmune diseases are characterized by tissue destruction mediated directly by T cells. A well known example of this is rheumatoid arthritis, in which self-reactive T-cells attack the tissue in joints, causing an inflammatory response that results in swelling and tissue destruction. Additionally diseases such as insulin – dependant diabetes mellihes and

multiple sclerosis are primarly due to the action of self-reactive T-cells.

1.5.4.3 Organ specific Auto Immune disease

In this, the immune response is directed to a target antigen unique to a single organ or gland, so that the manifestations are largely limited to that organ. The cells of the target organs may be damaged directly by humoral or cell-mediated mechanisms.

1.5.4.3.1 Disease mediated by direct cellular damage

Autoimmune diseases involving direct cellular damage occur when lymphocytes or antibodies bind to cell membrane antigens, causing cellular lysis and/or an inflammatory response in the affected organs. Gradually, the damaged cellular structure is replaced by connective tissue and the function of the organs decline.

1.5.4.3.2 Hashimoto's Thyroiditis

In Hashimoto's thyroiditis, which is most frequently seen in middle aged women, an individual autoantibodies are produced and sensitized TDTH cells for thyroid ag's also. The DTH response is characterized by an intense infliltration of thyroid gland by lymphocytes, macrophages and plasma cells, which form lymphocytic follicles and germinal centers. It causes a goiter or visible enlargement of the thyroid gland. Ab's are formed to a no. of thyroid proteins, including thyroglobulin and thyroid peroxidase. Which involves iodine uptake.

1.5.4.3.3 Auto Immune Anemia's

These include pernicious anemia, autoimmune hemolytic anemia and durg – induced hemolytic anemia. Pernicious anemia is caused by auto – abs to a membrane bound intestinal protein on gastric parietal cells, called intrinsic factor, that

facilitates uptake of vit B_{12} from the small intstine. Pennicious anemia is treated with injections of vitamin B_{12}.

An individual with autoimmune hemolytic anemia makes auto – ab to RBC ags, triggering complement mediated lysis or ab mediated opsonization and phagocytosis of RBC. One form of autoimmune anemia is drug induced.

1.5.4.3.4 Good Pasture's syndrome

In Good Pastures Syndrome, auto ab's specific for certain basement membrane ags binds to the basement membranes of the kidney glomeruli and the alveoli of the lungs. Subsequent complement activation leads to direct cellular damage which leads to progressive kidney damage and pulmonary hemorrhage, death ensues often within several months of the onset of symptoms. Biopsies from patients with Good Pasture's Syndrome stained with fluorescent labeled anti IgG and anti C_3b reveal linear deposits of IgG and C_3b along the basement membrane.

1.5.4.3.5 Insulin Dépendent Diabètes Millitus

A disease effecting 0.2% of the population, IDDM is caused by an autoimmune attack of pancreas. The attack is directed against beta cells that are located in spherical clusters called the islets of Langerhans, scattered throughout the pancreas. This attack destroys beta cells, resulting in decreased production of insulin and consequently increase blood glucose levels.

Several factors are important in destruction of Beta cells. First activated CTLs migrate into an islet and begin to attack the insulin producing cells. Local cytokine production released during this response include IFN-γ, TNF-α and IL-1. Autoantibody production also can be a contributing factor in IDDM. The first CTL infiltration and activation of macrophoages, frequently referred to as insulitis is fallowed by cytokine release

and the presence of auto antibodies, which leads to a cell mediated DTH response. The sub sequent beta cell destruction is thought to be mediated by cytokines released from the activated macrophages. Autoantibodies to beta cells may contribute to cell destructionby facilitating either antibody – plus – complement lysis or antibody – dependant cell mediated cytotoxicity (ADCC).

The abnormalities in glucose metabolism that are caused by the destruction of islet beta cells result in serious metabolic problems that include ketoacidosis and increased urine production. The late stages of the disease are often characterized by atherosclerotic vascular lesions which inturn cause gangrene of the extremities due to impeded vascular flow – renal failure and blindness. Clearly, diabetes is a disease that, if not properly treated can result in death. The most common therapy for diabetes is daily administration of insulin. This is quite helpful in managing the disease but because sporadic doses are not the controlled release of the harmone, periodically injected doses of insulin do not totally alleviate the problems caused by the disease. Another complicating feature of diabetes is that people who suffer from it can go undetected for several years and thus suffer irreparable loss of pancreatic tissue.

1.5.4.3.6 Disease Mediated by stimulating or block auto antibodies

In some autoimmune diseases, antibodies act as agonists binding to hormone receptors in lieu of the normal ligand and stimulating inappropriate activity. This usually leads to an overproduction of mediators or an increase in cell growth. In other auto immune conditions, auto antibodies binds to hormone receptors but act as antagonists, blocking receptor function. This generally causes impaired secretion of mediators and gradual atrophy of the affected organs.

1.5.4.3.7 Graves Disease

The production of thyroid hormones is carefully regulated by thyroid stimulating hormone (TSH), which is produced by the pituitary gland. Binding of TSH to a receptor on thyroid cells activates adenylate cyclase and stimulates the synthesis of two thyroid hormones, thyroeine and triiodothyronine. A patient with Graves' disease produces auto antibodies to the receptor for TSH. Binding of these auto – antibodies to the receptor mimics the normal action of TSH, activating adenylate cyclase and resulting in production of the thyroid hormones. Unlike TSH, however the autoantibodies are not regulated and consequently they overstimulate the thyroid. For this reason these auto – intibodies are called long acting thyroid stimulating (LATS) antibodies.

1.5.4.3.8 Myasthenia Gravis

Myasthenia gravis is the prototype autoimmune disease mediated by blocking antibodies. A patient with this disease produces auto – antibodies to the acetylcholine receptors on the motor end plates of muscles. Binding of these auto – autobodies to the receptors blocks the normal binding of acetyl choline and also induces complement mediated degradation of the receptors resulting in progressive weakening of the skeletal mussles. Ultimately, the antibodies destroy the receptors. The early signs of this disease include drooping eyelids and inability to retract the corners of the mouth; which gives the appearance of snarling.

1.5.4.4 Systemic AutoImmune Disease

In systemic autoimmune diseases, the response is directed towards a broad range of target antigens and involves a number of organs and tissues. These diseases reflect a general defect in immune regulation that results in hyperactive T-cells and B-cells. Tissue damage is widespread, both from cell-mediated

immune response and from direct cellular damage caused by auto – antibodies or by accumulation of immune complexes.

1.5.4.4.1 Systemic Lupes Erythematosus

One of the best examples of a systemic autoimmune disease is systemic lupus erythematosus (SLE) which typically appears in women between 20 and 40 years of age; the ratio of female to male Patients is 10:1. SLE is characterized by fever, weakness, arthritis, skin rashes, pleurisy and kidney dysfunction. Lupus is more frequent in some places. Affective individuals may produce auto – antibodies to a vast array of tissue antigens such as DNA, histons, RBC's, Platelets, leucocytes and clotting factors; interaction of these auto – antibodies with their specific antigens produces various symptoms. Auto antibodies specific for RBCs andplatelets for example can lead to complement mediated lysis, resulting in hemolytic anemia and thrombocytopenia, respectively. When immune complexes of auto – antibodies with various nuclear antigens are deposited along the walls of small blood vessels, a type III hypersensitive reaction develops. The complexes activate the complement system and generate membrane attack complexes and complement split products that damage the wall of the blood vessel, resulting in vassulitis and glomerulorephritis.

Excessive complement activation in patients with severe SLE produces elevated serum levels of the complement split products C3a and C5a, which may be three to four times higher than normal. C5a induces increased expression of the type 3 complement receptor (CR3) on neutrophils, facilitating neutrophil aggregation and attachment to the vascular endothelium. As neutrophils attach to small blood vessels, the number of circulating neutrophils declines and various occlusions can lead to widespread tissue damage.

Laboratory diagnosis of SLE focuses on the characteristic antinuclear antibodies, which are directed against double

stranded or single stranded DNA, nucleoprotein, histones, and nuclear RNA. Indirect immunofluorescent staining with serum from SLE patients produces various characteristic nucleus – staining patterns.

1.5.4.4.2 Multiple Sclerosis

Multiple Sclerosis (MS) an autoimmune disease that affects the central nervous system is the most common cause of neurologic disability associated with disease in western countries. The symptoms may be mild, such as numbness in the limbs or severe, such as paralysis or loss of vision. Most people with MS are diagnosed between the ages of 20 and 40. Individuals with this disease produce autoreactive T cells that participate in the formation of inflammatory lesions along the myelin sheath of nerve fibres. The cerebrospinal fluid of patients with active MS contains activated T lymphocytes which infiltrate the brain tissue and cause characteristic inflammatory lesions destroying the myelin. Since myelin functions to insulate the nerve fibres a breakdown in the myelin sheath leads to numerous neurologic dysfunctions.

The identical twin of a person with MS has a one in 3 chance of developing the disease. These data points strongly to the genetic component of the disease. MS affects women two to three times more frequently than men.

1.5.4.4.3 Rheumatoid Arthritis

Rheumatoid arthritis is a common autoimmune disorder most often affecting women from 40 to 60 years old. The major symptom is chronic inflammation of the joints though he hematologic, cardiovascular and respiratory systems frequently are also affected. Many individuals with rheumatoid arthritis produce a group of auto – antibodies called rheumatoid factors, that are reactive with determinants in the Fc region of IgG. The classic rheumatoid factor is an IgM antibody with that reactivity. Such autoantibodis bind to normal circulating IgG, forming IgM

– IgG complexes that are deposited in the joints. These immune complexes can activate the complement cassade, resulting in a type III hypersensitive reaction, which leads to chronic inflammation of the joints.

1.5.4.4.4 Scleroderma

Scleroderma is an antoimmune disease that can have local effectson the skin or more wides pread systemic effects on internal organs and the vasculature. The most commonly identified symptom of scleroderma is a gradual tightening of the skin, usually in the extremities such as hands, feet, and face. The term "Scleroderma" literally means "hard skin" it is a chromic degenesative disease that leads to the over production of collagen in connective tissue. The localized form of the disease results in thickening of the skin and tightness in the face and hands. Many individuals affected with the localized form develop CREST syndrome. The term "crest" is an acronym for five symptoms that commonly occur together: calcinosis, a painful calcium deposit in the skin, Reynaud's phenomenon, abnormal blood flow in response to cold or stress, esophageal dysfunction problem with swallowing, sclerodactyly, tightening of the skin, and telangietasia the formation of red spots on the hands, palms forearms, face and lips. These five symptoms frequently appear after the first localized skin effects. Most patients who develop CREST do not develop the systemic form of the disease. The more severe systemic form attacks internal organs such as kidneys, lungs, heart, gastrointestinal tract and joints as well as the skin. This form of the disease can be quite painful and sometimes leads to premature death.

1.5.4.7 Proposed Mechanisms for Induction of Auto Immunity

A variety of mechanisms have been proposed to account for T-cell-mediated generated of autoimmune diseases. Evidence exists for each of these mechanisms and likely that autoimmunity does not develop from a single event but rather from a number of different events.

In addition, susceptibility to many autoimmune diseases differs between the two sells. As noted earlier, Hastimotos thyroiditis, systemic lupus erythematosus, multiple sclerosis, rheumatoid arthritis and scleroderma preferentially affect women. Factors that have been proposed to account for this preferential susceptibility, such as hormonal differences between the screes and the potential effects of fetal cells in the maternal circulation during pregnancy, are discussed in clinical focus.

1.5.4.5.1 Release of Sequestred antigens

The induction of self tolerence in T-cells is though to result from exposure of immature thymocytes to self antigens and the subsequent clonal deletion of those that are self reactive. Any tissue antigens that are sequestered from the circulation and therefore are not seen by the developing T-cells in the thymus, will not induce self-tolerance. Exposure to mature T-cells to such normally sequestered antigens at a later time might result in their activation.

Myelin basic protein (MBP) is an example of an antigen normally sequestered from the immune system, in this case by the blood – brain barrier. In the EAE model, animals are injected directly with MBP, together with adjuvant, under conditions that maximize immune exposure. In this type of animal model, the immune system is exposed to sequestered self-antigens under nonphysiologic conditions; however trauma to tissues following either an accident or a viral or bacterial infection might also release sequestered antigens into the circulation. A few ags are known to fall into this category. For example, sperm arise late in development and are requestered from the circulation. However after a vasectomy, some sperm

antigens are released into the circulation and can induce auto – antibody formation in some men. Similarly, the release of lens protein after eye damage or of heart – muscle ags after myocardial infarction has been shown to lead on occasion to the formation of auto – antibodies.

1.5.4.5.2 Molecular Mimicry

For several reasons, the notion that microbial or viral agents might play a role in autoimmunity is very attractive. It is well accepted that migrant human populations acquire the diseases of the area to which they move and that the incidence of autoimmunity has increased dramatically as populations have become more mobile. This, coupled with the fact that a number of viruses and bacteria have been shown to possess antigenic determinants that are identical or similar to normal most cell components led Michael oldstone to propose that a pathogen may express a region of protein that resembles, a particular self-component in conformation or primary sequence. Such molecular mimicry appears in a wide variety of organisms. In one study, 600 different monoclonal abs specific for 11 different viruses were tested to evaluate their reactivity with normal tissue antigens. More than 3% of the virus specific antibodies tested also bound to normal tissue, suggesting that molecular mimicry is fairly common phenomenon.

Molecular mimicry has been suggested as one mechanism that lads to autoimmunity. One of the best examples of this type of autoimmune reaction is post – vabies encephalitis which used to develop in some individuals who had received the rabies vaccine. In the past, the vabies virus was grown in rabbit – brain – cell cultures, and preparations of the vaccine included antigens derived from the rabbit brain cells. In a vaccinated person, these rabbit brain cells antigens could induce formation of antibodies and activated T cells, which could cross react with the recepient's own brain cells, leading to encephalitis. Cross – reacting antibodies are also thought to be the cause of heart

damage in rheumatic fever, which can some times develop after a streptococcus profection. In that case, the abs are to streptococcal ags, but they cross react with the heat muscles.

1.5.4.5.3 Mimicry between MBP and viral peptides

Since the encephalitogenic MBP peptides are known, the extent to which they are mimicked by proteins from other organisms can be accessed. For example, one MBP peptide is highly homologous with a peptide in the P_3 proteins of the measles virus. In one study, the sequence of another encephalitogenci MBP peptide was compared with the known sequences of large number of viral proteins. This computer analysis revealed sequence homologies between this MBP peptide and a number of peptides from animal viruses, including influenza, polyoma, adenovirus and hepatitis B virus.

Another compelling example of molecular mimicry comes from studies of herpes stromal keratinitis (HSK).

One peptide from the polymerase enzyme of the hepatitis B virus was particularly stricking, exhibiting 60% homology with a sequence in the encephalitogenic MBP peptide. Molecular mimicry can generate autoimmunity, rabbits were immunized with this hepatitis B virus peptide. The peptide was shown to induce both the formation of ab and the proliferationof T cells that cross reacted with MBP; in addition central nervous system tissue from the immunized rabbits showed cellular infiltration charactristic of EAE.

1.5.4.5.3 Inappropriate expression of class II MHC molecules

The pancreated beta cells of individuals with insulin dependent diabetes millites (IDDM) express high levels of both

class I and class III MHC molecules, where as healthy beta cells express lower levels of class I and do not express class I at all. Similarly thyroid acinar cells from those with Grave's disease have been shown to express class II MHC molecules on their membranes. This inappropriate expression of class II MHC molecules, which are normally expressed only on ag – presenting cells may serve to sensitize TH cells to peptides derived from the beta cells or thyroid cells allowing activation of B cells or Tc cells or sensitization of TDTH cells against self – antigens.

For example, the T-cell mitogen phytohemagglutinin (PHA) has been shown to induce thyroid cells to express class II Molecular . In vitro studied reveal that IFN-γ also induces increases in class II MHC molecules including Beta cells, intestinal epithelial cells, melanoma cells and thyroid acenar cells. If IFN-γ induces class II MHC expression on non –ag presenting cells, inappropriate TH cell activation might fallow with autoimmune consequences.

An intrestign transgenic mouse system implies IFN-γ and inappropriate class II MHC expression in autoimmunity. Although an in appropriate class II MHC expression on pancreatic beta cells may be involved in the auto immune reaction in transgenic mice, other factors also may play a role. For example, IFN-γ is known to induce production of several cytokines, including IL-1 and TNF. ∴the development of autoimmunity involve ag presentation by class II MHC molecules on pancreatic beta cells together with co-stimulatory signal, such as IL-1 that may activate self reactive T cells.

1.5.4.5.5 Polyclonal B-cell activation

A number of viruses and bacteria can induce non specific polyclonal B-cell activation Gram –Ve bacteria, cytomegalovvirus and Epstein Barr Virus (EBV) are all known to be such polyclonal activators, inducing the proliferation of numerous clones of B cells that express IgM in the absence of TH cells. If B cells reactive to self – ags are activated by this mechanism, auto – antibodies can appear. For instance, during infectious mononucleros is which is caused by EBV a variety of auto –

antibodies are produced, including auto – antibodies reactive to T and B cells, rheumatoid factors and antinuclear antibodies. Similarly lymphocytes from patients with SLE produce large quantities of IgM in culture, suggesting that they have been polyclonally activated. Many AIDs patients also show high levels of non specific ab and auto antibodies to RBCs and platelets. These patients are often infected which may induce the polyclonal B-cell activation that results in auto antibody production.

1.5.4.8 Treatment of Auto Immune Diseases

Ideally, treatment for autoimmune diseases should be aimed at reducing only the autoimmune response while leaving the rest of immune system intact.

Current therapies for autoimmune diseases are not curse but merely palliatives, aimed at reducing symptoms to provide the patient with an acceptable quality of life. For the most part, these treatments provide non specific suppression of the immune system. Immuno suppressive drugs (Eg:- Corticosteroids, a zathioprine and cyclophosphamide) are often given with the intent of slowing proliferation of lymphocytes. By depressing the immune response in general, such drugs can reduce the severity of autoimmune symptoms. The general reduction in immune responsiveness, however puts the patient at greater risk for infection or the development of cancer. A somewhat more selective approach employs cyclosporin A or FK506 to treat autoimmunity.

Another theraputic approach that has produced positive result in some cases of myasthenia gravis is removal of the thymus because patients with this disease often have thymic absormalities (Eg:- Thymomas).

Studies with experimental autoimmune animal models have provided evidence that it is indeed possible to induce specific immunity to the development of autoimmunity.

1.5.4.6.1 T-cell Vaccination

T-Cell vaccination has been explored as a therapy for some autoimmune diseases. When rats were injected with low doses of cloned T-cells specific for MBP, they did not develop symptoms of EAE. Instead they became resistant to the development of EAE. When later challenged with a lethal dose of activated MBP specific T-cells or MBP in adjuvents. T-cell clones enhanced by cross – linking the cell membrane components with formaldehyde or glutoral dehyde when such cross linked T-cells were injectd into the animals with active EAE, permanent vemission of symptoms observed.

1.5.4.6.2 Peptide Blockade of MHC Molecules

Identification and sequencing of various auto antigens has led to the development of new approaches to modulate atoimmune T-cell activity. In EAE, for example the encephalihogenic peptide of MBP have been well characterized. Synthetic peptides differing by only one amino acid from their MBP counterparts have been shown to bind to the appropriate MHC molecule.

In other studies, blocking peptides complex to soluble class II MHC molecules reversed the clinical progression of EAE in mice, presumably by inducing a state of clonal energy in the auto immune T-cells.

1.5.4.6.3 Monoclonal Antibody Treatment

Monoclonal antibodies have been used successfully to treat autoimmune disease in several animal models. For example, a high percentage of F, mice given weekly injections of high doses of monoclonal antibody specific for the CD_4 membrane molecule

removed from their autoimmune lupus like symptoms. Similar positive results were obtained in NOD mice in which treatment with an anti $^{-}CD_4$ monoclonal antibody led to disappearence of the lymphocytic infiltration and diabetic symptoms.

Because Anti CD_4 (monoclonal antibodies block or regardless of their specificity, they) can deplete all TH cells, regardless of their specificity, they can threaten the overall immune responsiveness of the recipient one remedy for this disadvantage is to try to block ag activated TH cells only, since these cells are involved in the autoimmune state. To do this, researchers have used monoclonal antibody directed against the α subunit of the high affirity IL-2 receptor, which is expressed only by ag activated TH cells. Because the IL-2Rα subunit is expressed at higher levels on autoimmune T cells, monoclonal ab to the α subunit might preferentially block autoreactive T-cells. This approach was tested in adult vats injected with activated MBP – specific T-cells in the presence or absence of anti – TAC. All the control rats died of EAE where as six of the nine treated with Anti TAC had no symptoms and the symptoms in the other three were mild. The association of autoimmune disease with restricted TCR expression in a number of animal models has prompted researchers to see if blockage of the preferred receptors with monoclonal antibody might be theraputic. Injection of PL/J mice with monoclonal antibody specific for the VB 8.2 T-cell receptor prevented induction of EAE by MBP in adjuvent. The anti VB 8.2 monoclonal antibody could also reverse the symptoms of autoimmunity in mice manifested long term remission clearly, the use of monoclonal antibodies as a treatment for human autoimmune diseases presents exiting possibilities.

1.5.4.6.4 Tolerance Induction by oral antigens

When antigens are administered orally they tend to induce the state of immnologic unresponsivenesscalled tolerance. For example, mice fed MBP do not develop EAE after subsequent injection of MBP. This finding led to a double – blind pilot trial

in which 30 individuals with multiple selerosis were fed either a placebo or 300 mg of bovine myelin every day for a year. The results of this study reveal that T-cells specific for MBP were reduced in the myelin fed group. While the results of oral tolerance in mice were promising, the data from humans do not appear to be as beneficial. However the human clinical trials are in the early stages, and it may be that the peptides used so for were not the most effective. Because of the promise of this approach as shown in animal studies, it is likely that more clinical trials will be conducted.

1.5.4.7 Summary

Human auto immune diseases can be devided into organ specific and systemic disease. The organ sys specific disease involves an auto immune response detected primarly against a single orgon or gland. These include disease in which auto reactive lymphyocytes or auto antibodies bind to self antigens leading to direct cellular damage. Some organ specific auto immune disease are mediated by stimulating antibodies (Graues diseases) or blocking antibodies (Myas theria gravis). This systems disease are directed against broad spectrum of tissue and have manifestation in a variety of organs resulting from ell mediated responses and cellular damage. Caused byauto antibodies or immune complexes.

Variety of mechanisms have been proposed for induction of auto immunity, including release of sequestered antigens. Molecular mimicry in approapriate class II MHC expression on cell and poly clonal B-cell activation.

Current therapies for auto immune diseases include treatment with immuno suppressive drage. Theymictmy and plasmapheresis. These therapes are non specific and have significant side effect.

1.5.4.8 Key Words

1. Auto immunity
2. Good pasture's syndrome
3. Granes disease
4. Rebounatoid factors
5. Sequestered antigens.

1.5.4.9 Model Questions

1What is auto immunity and what kinds of effect it causes?

2. What are different molecular mechanisms of auto immunity?
3. What kind of therapies have been employed for auto immunity.
4. What is organ specific autoimmunity and add a note on different types of organ specific autoimmunity

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1.5.4 Vaccines

1.5.4.1 Objective

The main objective of this lesson is to explain the what the vaccine is and what are the different kinds of vaccines and in what way they are useful to man kind

1..5.4.2 Introduction

Vaccines have been the most effective tool for preventing and eliminating devastating infectious diseases and thus improving the quality of life. Their role will be increasingly important in the medicine of the future because biotechnology has now broadened the targets of vaccination to include new infectious diseases, immunotherapy of tumours, chronic infections, autoimmunity and allergies

The concept that people who survive an infectious disease do not develop the same disease again is as old as humankind. In written history, it was reported for the first time by the historian Thucydides, in the description of the Peloponnesian War in 430 Bc. He reports that, during the plague of Athens, it was a common practice to use those who had recovered from the disease, and therefore could not acquire it again, to take care of the sick. Today we know that we do not get the same diseases twice thanks to our immune system, which recognizes as 'nonself' the components (antigens) of infecting agents and engineers the genetic repertoire of the cells devoted to the defense of our organism (B and T lymphocytes), to code for receptor molecules that specifically recognize the antigens of the incoming pathogen. This generates specific B- and T-cell clones, which, respectively, combat the intruding agent by neutralizing it with antibodies or by killing the cells that have been infected by it. After infection, the specific B and T cells are stored away as memory cells capable of recognizing the agent and proliferating, should they meet it again.

The principle of vaccination is quite simple. We expose the body to biological material that mimics the infectious agent, so that the immune system mounts the resistance to a given pathogen and acquires memory to it, without experiencing the infection and disease. To do so, whole microorganisms (killed or attenuated) or parts of them are presented to the immune system (usually by injection), so that the immune system produces specific B and T cells able to combat the infectious agent in the absence of a dangerous infection. The first

report of the induction of immunity by deliberate infection of healthy people comes from Asia in ad 590; however, it is likely that it had been practiced long before then. In those early practices, infected material from a mild smallpox lesion, sometimes dried with a little cotton, was transferred to healthy people to make them resistant to subsequent exposures to the same disease (variolation). Historically, vaccination was born in 1796 when Edward Jenner, an English physician who, during his practice in the countryside, noticed that farmers exposed to infected materials from cows did not develop smallpox but acquired immunity to the disease. Jenner decided to use the material derived from the bovine (vaccinus) lesions to vaccinate a boy (James Phipps), and showed that the patient was immune to a subsequent challenge with smallpox. The scientific approach to vaccination came only a century later, when Louis Pasteur introduced the concept that infectious diseases were caused by microorganisms and discovered that they could be attenuated by growing them under adverse conditions. Using this empirical approach he developed the first live-attenuated bacterial and viral vaccines (chicken cholera bacillus and rabies, respectively). Large-scale vaccination came only following the discovery by Glenny and Hopkins (1923) and Ramon (1924) of safe and reproducible ways to inactivate toxins and pathogens by the use of formaldehyde, and of the stable attenuation of pathogens by serial passage *in vitro*. These simple, basic technologies were the only means used for vaccine development for most of the twentieth century, until the very recent biotechnological revolution that allowed the development of vaccines also against those infectious agents that are difficult or impossible to grow in the laboratory

1.5.4.3 The Existing Vaccines

Successful vaccines developed during the twentieth century as shown, many infectious diseases that at the beginning of the century caused millions of deaths are completely absent today, as a consequence of a simple and cheap practice such as vaccination. They can be divided into three broad categories, depending on whether they contain live-attenuated microorganisms, inactivated whole microorganisms or purified components of microorganisms (subunit vaccines). The last group can be either purified from the infectious agent or made by recombinant deoxyribonucleic acid (DNA). A successful category of subunit vaccines is made by semi synthetic vaccines in which a carbohydrate antigen is covalently linked to a protein (conjugate vaccines).

1.5.4.4 Vaccines containing live-attenuated organisms

The prototype of a live-attenuated vaccine is vaccinia virus vaccine, the one used against smallpox. It was so successful that it is no longer in use because by 1977 it had eradicated the infectious agent and the disease from Earth. Examples of widely used live-attenuated vaccines are the bacillus Calmette–Guérin (BCG) vaccine against tuberculosis, the Sabin type of polio vaccine, the vaccines against measles, mumps, rubella and varicella, and the Ty21A vaccine against typhoid fever. The recently developed vaccines against rotavirus and influenza that were licensed in 1998 are also expected to enter into wide use. Most of these vaccines were developed before the era of molecular biology and biotechnology, by *in vitro* passage of human or animal pathogens; therefore, the molecular mechanisms of their inactivation is usually unknown. Today it would be difficult to introduce some of these noncharacterized vaccines. However, very recently there was a new wave of success with live-attenuated vaccines against influenza and rotavirus that have been generated by the old Jennerian, empirical approach. Well-characterized, live-attenuated bacterial and viral strains can be built by rational modification of the genome of the pathogen. Until now, most of the work has been dedicated to obtaining attenuated strains of *Salmonella*, by deleting or inactivating the genes coding for the synthesis of aromatic amino acids, or regulatory pathways; of *Vibrio cholerae*, by deleting the gene coding for the A subunit of cholera toxin; and of *Vaccinia virus* by deleting genes involved in the nucleotide metabolism. In theory, however, any microorganism can be attenuated by deleting or modifying genes that are essential for the *in vivo* growth of the pathogen. Today, the most attractive reason for developing live-attenuated microorganisms resides in the possibility of engineering their genome to allow them to produce *in vivo* cloned antigens deriving from other microorganisms. A great deal of literature is available describing *Salmonella* spp., poxviruses and many other attenuated microorganisms expressing recombinant antigens. This approach can be very useful in inducing mucosal immunity and cytotoxic responses to target antigens

1.5.4.5 Vaccines containing killed microorganisms

Heat or chemical inactivation of bacteria and viruses has been the first, easy approach to the development of any vaccine. Generally the virulent microorganism is killed by exposure to a chemical such as

formaldehyde and used as such in the vaccine. The advantage is that all antigens present in the pathogen are included in the vaccine, so it is not necessary to know which are the protective antigens. The disadvantage is that some of the vaccine components may be toxic and responsible for undesirable side effects. Some vaccines widely used in the past, such as those containing killed bacteria against salmonella and cholera, are no longer in use because of the unacceptable level of side effects. Today this method of vaccine development is no longer widely employed, although several vaccines of this type are still in use. These include the whole-cell vaccine against pertussis, the Salk vaccine against polio, the influenza vaccine, the vaccines against rabies and tick-borne encephalitis, and the vaccine against hepatitis A.

1.5.4.6 Subunit vaccines

Subunit vaccines consist of one or more antigens purified from the microorganism or produced by recombinant DNA technology. Development of subunit vaccines requires knowledge of the protective antigen(s) and the ability to produce and purify them on a large scale. It is also desirable to know the type of immune response that will induce protection, in order to be able to construct and deliver the antigen in the appropriate way.

The first subunit vaccines to be developed were diphtheria and tetanus toxoids. The observation that both diseases were caused by a toxin produced by the bacterium suggested that serum antibodies able to neutralize the toxin were sufficient to protect against disease. The semipurified toxins were therefore inactivated by chemical (formaldehyde) treatment and used as vaccines.

A second example of subunit vaccines are polysaccharide vaccines against encapsulated bacteria. The observation that serum bactericidal antibodies against the capsular polysaccharide were enough to protect from invasive bacterial infection suggested the development of purified capsular polysaccharides as vaccines. Polysaccharide vaccines have been developed against meningococcus A, C, Y and W135, against 23 types of pneumococcus, against *Haemophilus influenzae* type b, and against *Salmonella typhi*. However, polysaccharide vaccines bring about T cell-independent immune responses, only inducing primary immunoglobulin M (IgM)-type antibody in adults, and no immunity at all in infants. These

vaccines have therefore had only a limited use, and only in adults. To overcome these problems, capsular polysaccharides were covalently linked to carrier proteins, thus obtaining semisynthetic conjugated vaccines, described in more detail below.

A third example of subunit vaccines is the recombinant vaccine against hepatitis B. In this case, it had been observed that serum antibodies elicited by the envelope protein of the *Hepatitis B virus* were able to neutralize the virus and to protect from infection; however, the vaccine could not initially be produced in large quantities because the virus did not grow *in vitro* and could only be purified from the plasma of infected patients. The solution to this problem was found by using recombinant DNA to engineer a yeast strain to produce the envelope protein. This turned out to be one of the unique cases where the yeast produced and assembled the protein in the correct conformation, so that the purified recombinant protein could be directly used as vaccine.

1.5.4.7 Conjugate vaccines

In 1996 the prestigious Laskey prize was awarded for the development of the first semisynthetic vaccine created by the artificial coupling of a polysaccharide and a protein molecule, the so-called conjugate vaccine. Experience had shown that many bacteria causing systemic infection are surrounded by a capsule composed of polysaccharides and that vaccination using purified polysaccharides could protect people from disease. Polysaccharide vaccines had been developed against meningococcus A, C, Y and W135, against 23 types of pneumococcus, against *H. influenzae* type b and against *S. typhi*; however, they were found unsuitable for mass prevention of disease and for vaccination of infants, who were often the major targets of diseases, because polysaccharides are recognized very poorly, if at all, by helper T cells. As a consequence, they only induce a T cell-independent, short-term, partial immune response in adults and do not prime memory. To overcome this, the polysaccharide was chemically linked to a carrier protein (diphtheria or tetanus toxoid) known to be recognized by T cells.
Within a few years of its use, the first conjugate vaccine (the one against type b *H. influenzae*), eradicated the disease and the bacterium from all countries where it has been introduced. A number of vaccines based on the same principle have been developed against meningococcus A and C, pneumococcus, *S. typhi* and shigellae. They

have all shown great performance in clinical trials and are expected to be ready for use in the control of these diseases in the near future.

1.5.4.8 Combination Vaccines and the Most Common Vaccines Used Today

Most vaccines in use today are for infants. To avoid too many injections, often several vaccines are combined and delivered together. The most common basic and combinations of vaccines available today are . Tetravalent and hexavalent vaccine combinations that will further reduce the number of injections are expected to be the ones mostly used in the future The vaccines recommended by the Advisory Committee on Immunization Practices (ACIP), the American Academy of Pediatrics (AAP) and the American Academy of Family Physicians (AAFP), and the World Health Organization that are mainly used in the USA and Europe and developing countries. See also: Vaccination

1.5.4.9 Vaccines: The Future

There is no doubt that vaccines have been the most effective means of combating deadly infectious diseases during the twentieth century. At the start of the third millennium, we are observing a new revolution in the field. Undoubtedly, there will be more vaccines both against those infectious agents for which a vaccine is not yet available and also for emerging infectious agents. . Vaccines against infectious agents causing cancer (*Hepatitis B virus* (HBV), *Hepatitis C virus* (HCV), *Papillomavirus* (HPV), *Helicobacter pylori*) will be developed and this will dramatically decrease the incidence of tumors in future generations. Finally, vaccines will not only be used to prevent infections, but also to cure, once and for all, chronic infectious diseases, tumors, autoimmune diseases and allergies. The way in which vaccines will be delivered and the target population will also change. Vaccines will no longer be just a painful childhood experience. Rather, they will be perceived as friendly tools both preventing and curing diseases throughout our whole lives. They will be safer, more efficacious, often delivered at mucosal sites rather than injected and given to infants, adolescents, adults, the elderly and to special groups at risk, such as travellers.

The technical revolutions giving the impetus to vaccine development have been: (1) the recombinant DNA that made possible the

production of vaccines previously impossible, such as against HBV, and the identification of new infectious agents such as HCV; (2) genomic sequencing; and (3) a better understanding of the immune system.

The genomic era will provide targets for new vaccines at a speed that is increasing exponentially. In the 1970s, sequencing a gene was impossible. In the 1980s, sequencing a gene became possible but for a research team to sequence a gene of approximately 2000 bp took about 1 year. Long and careful preliminary work was therefore necessary to ensure that the gene being sequenced was indeed a good vaccine candidate. Today, the situation is totally reversed: a whole bacterial genome containing three million base pairs can be sequenced in less than 6 months and all the protein vaccine candidates are available for computer search. Using appropriate algorithms, it is possible to select the most promising vaccine candidate molecules from the whole genome within a few hours. Three major areas appear very promising for the future of vaccinology: mucosal delivery of vaccines; DNA vaccines; and therapeutic vaccines

1.5.4.10 Mucosal delivery of vaccines

With few exceptions, most vaccines are presently delivered by intramuscular injection, which is a primitive way of delivering drugs. It is anticipated that within the next decades we will have more convenient methods of vaccine delivery that will be mostly based on mucosal delivery.

Development of mucosally delivered vaccines will not only provide a more convenient way of delivery but will stimulate an immune response at mucosal sites, which are usually not involved by systemic vaccination. Mucosal surfaces are exposed to the environment and are usually the first barrier encountered by pathogens during infection. The mucosal surface is enormous: the human body has a total mucosal surface of 400 m^2, an area equivalent to that of a tennis court. In order to fight pathogens at their portal of entry, in addition to fighting the pathogens once they are within the body (systemic immunity), the immune system has developed a special arm localized in mucosal tissues. This system utilizes different types of antibodies (IgAs instead of IgG) and different T cells that home specifically to the mucosal tissues. Most antigens are ignored when they are exposed to

a mucosal surface; however, there are some molecules that have the peculiar property of stimulating the mucosal immune system, which recognizes them as potentially dangerous. These are toxic molecules such as cholera toxin (CT) and the heat-labile toxins (LTs) of *Escherichia coli*. When they are delivered orally or intranasally they provoke a powerful mucosal immune response against themselves, and also against molecules that are codelivered. Unfortunately, they cannot be used in humans because they are too toxic. Recently, the genes coding for these molecules were engineered to eliminate the enzymatic activity that is responsible for toxicity. Single amino acid substitutions were found to eliminate completely the toxicity without affecting the ability of the immune system to recognize them as dangerous and mount a powerful immune response against them. Therefore, today we have available genetically detoxified CT and LT mutants that are no longer toxic, but maintain the property to stimulate the mucosal immune system. These molecules are currently the best candidates available as mucosal adjuvants and are beginning to undergo clinical testing.

1.5.4.11 Nucleic acid vaccines

The use of naked DNA as an approach to induce immune responses is a new, very popular field in vaccinology, potentially of great importance. In this approach, the gene(s) coding for the antigens against which we want to raise an immune response are cloned in appropriate plasmid vectors under strong promoters and directly injected into the host. A small fraction of the injected plasmid DNA is then taken up and expressed by antigen-presenting cells; this results in the elicitation of an immune response against the newly expressed foreign antigen. Although fairly new, this technology has been applied experimentally to many vaccines, often with very promising results. Influenza, tuberculosis, human immunodeficiency virus (HIV) infection, hepatitis B, human papillomavirus infection and malaria are just a few examples of the infections which are now being tackled by this fastly growing technology. The naked DNA approach has two great advantages over the conventional technologies. Firstly, it is very simple. The same, easy manufacturing technology (plasmid DNA preparation) is used for all antigens and all vaccines, so that once the protective antigen(s) have been identified and cloned, the vaccine development path is identical for all vaccines. Secondly, it is so far the

most efficient method known of inducing a cytotoxic immune response against an antigen. Some outstanding issues, however, remain.

(1) The safety issues of injecting DNA are still unresolved.

(2) Antigens are folded and posttranslationally modified by eukaryotic cells; for instance, bacterial antigens are often glycosylated, and may not have the appropriate conformation when they are given as naked DNA molecules.

(3) Polysaccharide vaccines cannot be approached by this method.

(4) The delivery of the DNA is very inefficient and large quantities of plasmid are still needed to elicit strong immune responses.

DNA immunization studies on a large number of infectious agents are currently in progress and the feasibility of this technology has been clearly demonstrated in small animal models. However, clinical application of this form of technology is still problematic. The main issue to be solved is the efficiency of DNA delivery, especially in primates. Large quantities of DNA are in fact required to stimulate appreciable immune responses in monkeys, stressing the point that more basic information needs to be obtained on the mechanisms of *in vivo* DNA uptake and on the understanding of the role of the different types of transfected cells in inducing the proper immune response.

Clinical trials will confirm whether or not the promising results so far obtained in mice and primates will result in the development of a class of new vaccines that will provide protection against many of the diseases in which the classic technologies have failed or were not adequate. For instance, the possibility of immunizing with many genes from the same pathogen may be used in the approach to complex parasitic diseases such as malaria.

1.5.4.12 Therapeutic vaccines

Beside preventing a disease, can a vaccine also cure a disease? Can our immune system be educated by vaccination to eradicate diseases for which conventional medicine has only temporary remedies? This could be the case for chronic infections, tumours and diseases due to

abnormal immune responses such as autoimmune diseases or allergies.

The assumption behind any vaccine therapy is that the immune system of the host has the potential to mount an immune response capable of controlling or eradicating a given disease which *per se* does not prime a protective immune response. Typical examples are some tumours.

Although most tumours are antigenic, i.e. express molecules that can be potentially recognized by the host immune system, few tumours are also immunogenic, i.e. an immune response against them is detectable in the patients. That is why the immune system is generally incapable of controlling tumour growth. Two major strategies have been followed in the design of tumour vaccines. One is based on the identification of tumour-associated antigens, with the aim of eliciting an immune response against them. Another strategy is to render tumour cells more immunogenic for the host immune system, for instance by creating a strong inflammatory milieu at tumour sites or providing the missing costimulatory molecules for T cells in soluble form. The most relevant tumour for which vaccine therapy is in advanced clinical trials is the melanoma.

A different situation is found in some chronic infections, such as hepatitis B. In these cases, the immune system responds to the infection but in a way that is not sufficient to eradicate the infection itself. Even worse, as is the case for heptatitis B, the immune response primed by the infection is the cause of the disease, which is due to damage of the infected liver by T cells in an attempt to clear the virus. See also: *Hepatitis B virus*

In these cases, the strategy for developing a therapeutic vaccine is to instruct the immune system to respond to the attack by the infectious agent in a way which is beneficial, rather than deleterious, for the host. In the case of hepatitis B, for example, the strategy is to generate a neutralizing antibody response and to avoid cytotoxic reactions.

Examples of chronic infections for which vaccines are being developed are those caused by *Herpes genitalis* (HSV-2), HBV, HCV, HPV, *H. pylori* and HIV. It is noteworthy that most of these infections have been implicated in tumorigenesis of the infected organs, e.g. HCV and liver, *H. pylori* and stomach, HPV and genital tract, HSV and uterus. Apart from curing chronic infections, it is clear that in most of these

cases the control of the infectious disease would result in a decreased risk of tumours of the infected organs

1.5.4.13 summary

Recent advances in immunology, biotechnology, and other sciences now give the prospect of a wide variety of new vaccines that can bring further improvements in health but that pose some theoretical issues relating to safety and efficacy, as well as practical issues relating to logistics, number of injections, and other factors. Combination vaccines are essential if society is to take full advantage of new vaccines that can further reduce the burden of infectious diseases in this country and around the world. The major issues relating to combination vaccines are much the same today as those discussed at a 1993 meeting. However, considerable progress has been made in developing solutions to the problems, and prospects are good that many of these issues will be resolved in the next 2-3 years

1.5.4.14 Model Questions

Essay Questions

1. What is a vaccine and explain the different kinds of vaccine ?

2. What are the Therapeutic Vaccine?

3. How are they useful to man kind?

Short questions

1. DNA vaccine

2. Live attenuated Vaccines

3. Subunit Vaccine

1.5.4.15 Key words

1. vaccine
2. Attenuated microorganism
3. Immunity
4. Killed microorganism
5. Antibody
6. protein antigen
7. Virus
8. Bactrium
9. killed microorganism
10. DNA vaccine

1.5.4.16 References

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