Post-Graduate Degree Programme (CBCS)
in
ZOOLOGY
(M.Sc. Programme)

SEMESTER-IV

Parasitology and Immunology
ZDSE(MJ)T-404

Self-Learning Material

DIRECTORATE OF OPEN AND DISTANCE LEARNING
UNIVERSITY OF KALYANI
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Director's Message

Satisfying the varied needs of distance learners, overcoming the obstacle of distance and reaching the unreached students are the threefold functions catered by Open and Distance Learning (ODL) systems. The onus lies on writers, editors, production professionals and other personnel involved in the process to overcome the challenges inherent to curriculum design and production of relevant Self Learning Materials (SLMs). At the University of Kalyani, a dedicated team under the able guidance of the Hon'ble Vice-Chancellor has invested its best efforts, professionally and in keeping with the demands of Post Graduate CBCS Programmes in Distance Mode to devise a self-sufficient curriculum for each course offered by the Directorate of Open and Distance Learning (DODL), University of Kalyani.

Development of printed SLMs for students admitted to the DODL within a limited time to cater to the academic requirements of the Course as per standards set by Distance Education Bureau of the University Grants Commission, New Delhi, India under Open and Distance Mode UGC Regulations, 2020 had been our endeavour. We are happy to have achieved our goal.

Utmost care and precision have been ensured in the development of the SLMs, making them useful to the learners, besides avoiding errors as far as practicable. Further suggestions from the stakeholders in this would be welcome.

During the production-process of the SLMs, the team continuously received positive stimulations and feedback from Professor (Dr.) Kallol Paul, Hon'ble Vice- Chancellor, University of Kalyani, who kindly accorded directions, encouragements and suggestions, offered constructive criticism to develop it within proper requirements. We gracefully, acknowledge his inspiration and guidance.

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Their persistent and coordinated efforts have resulted in the compilation of comprehensive, learner-friendly, flexible texts that meet the curriculum requirements of the Post Graduate Programme through Distance Mode.

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Theory (Discipline Specific Elective – Major) - ZDSE(MJ)T-404-Parasitology & Immunology

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	VIII	Principles of immunity in relation to helminths.		
	IX	T-cell receptor organ and functions of immune response		
	X	Antigen-antibody reaction and its role in clinical parasitology.		
	XI	Basic immunological changes due to parasitic infection		
	XII	Basic immunological changes due to antigen vaccination		
	XIII	Basic immunological changes due to immunopathology.		
	XIV	Structure and function of antibody.		
	Total counseling session 12hrs.			

Unit I

Membrane transport mechanism in parasites

Objective: In this unit we will discuss about membrane transport mechanism in parasites.

Introduction

Membrane transport mechanisms in parasites are essential for their survival, growth, and ability to cause disease. These mechanisms enable parasites to acquire nutrients, excrete waste products, and evade the host's immune system. The transport processes are often highly specialized to meet the needs of the parasite in various environments, whether inside or outside a host.

Here are the primary types of membrane transport mechanisms found in parasites:

1. Passive transport

Diffusion: In the process of diffusion, a substance tends to move from an area of high concentration to an area of low concentration until its concentration becomes equal throughout a space. **Simple diffusion** allows small, non-polar molecules like oxygen, carbon dioxide, and some lipids to pass directly through the parasite's membrane without the use of energy. Molecules can move through the cell's cytosol, and some molecules also diffuse across the plasma membrane. Each individual substance in a solution or space has its own concentration gradient, independent of the concentration gradients of other materials, and will diffuse according to that gradient. Other factors being equal, a stronger concentration gradient (larger concentration difference between regions) results in faster diffusion. Thus, in a single cell, there can be different rates and directions of diffusion for different molecules. For example, oxygen might move into the cell by diffusion, while at the same time, carbon dioxide might move out in obedience to its own concentration gradient.

In **Facilitated Diffusion**, molecules diffuse across the plasma membrane with assistance from membrane proteins, such as channels and carriers. Larger or polar molecules, such as glucose and ions, cross the membrane through these protein channels or carriers. This process does not require energy and moves substances down their concentration gradient. The concentration gradient has the potential to diffuse into or out of the cell by moving it down. However, because they are charged or polar, they can't cross the phospholipid part of the membrane at its own. Facilitated transporter proteins shield these molecules from the hydrophobic core of the membrane, providing a route by which they can cross the membrane. Two major classes of facilitated transporter proteins are channel and carrier proteins.

Channel proteins span the membrane and make hydrophilic tunnels across it, allowing their target molecules to pass through it by diffusion. Channels are very selective and will accept only one type of molecule (or a few closely related molecules) for transport. Passage through a

channel protein allows polar and charged compounds to avoid the hydrophobic core of the plasma membrane, which would otherwise slow or block their entry into the cell.

Carrier proteins can change their shape to move a target molecule from one side of the membrane to the other. Like channel proteins, carrier proteins are typically selective for one or a few substances. The carrier proteins involved in facilitated diffusion, simply provide hydrophilic molecules with a way to move down an existing concentration gradient. Channel and carrier proteins transport material at different rates.

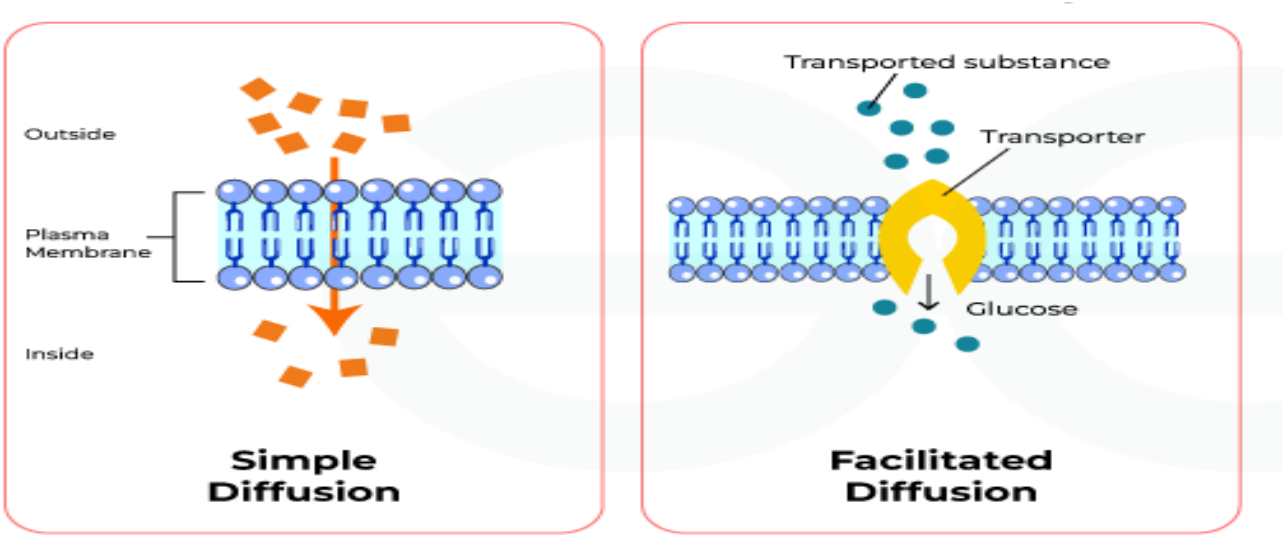
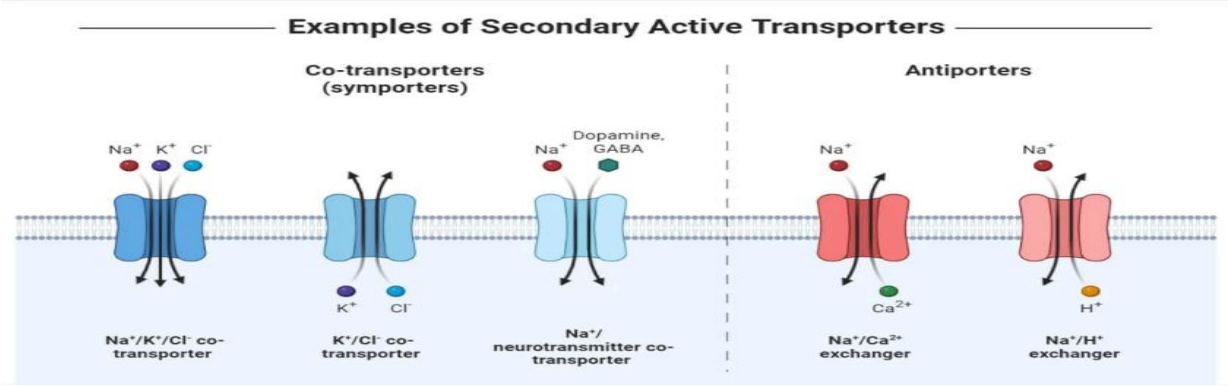


Fig- Simple diffusion and facilitated diffusion

2. Active transport

Primary active transport: This involves the direct use of ATP to move molecules against their concentration gradient. An example is the sodium-potassium pump, which is crucial for maintaining ion gradients across the membrane. Not only does the sodium-potassium pump maintain correct concentrations of Na^+ and K^+ in living cells, but also it plays a major role in generating the voltage across the cell membrane in animal cells. Pumps like this, which are involved in the establishment and maintenance of membrane voltages, are known as **electrogenic pumps**. The primary electrogenic pump in plants is one that pumps hydrogen ions (H^+) rather than sodium and potassium.

Secondary active transport: The electrochemical gradients set up by primary active transport store energy, which can be released as the ions move back down their gradients. Secondary active transport uses the energy stored in these gradients to move other substances against their own gradients. In secondary active transport, the two molecules being transported may move either in the same direction (i.e., both into the cell), or in opposite directions (i.e., one into and one out of the cell). When they move in the same direction, the protein that transports them is called a **symporter**, while if they move in opposite directions, the protein is called an **antiporter**.



Fig– Secondary active transporters

Membrane transport in parasites

- In respect to malarial parasite, these undergo a complex life cycle between their hosts and vectors. During this cycle the parasites invade different types of cells, migrate across barriers, and transfer from one host to another. Some 12–18 h after its invasion by the malaria parasite, the infected erythrocyte undergoes a profound increase in its permeability to low-molecular-weight solutes. The increased permeability of the infected cell has been attributed to the induction in the erythrocyte membrane of channels that allow the passage, both into and out of the cell, of a wide range of low-molecular-weight solutes including nutrients, inorganic ions and metabolic wastes. The number of different types of channels involved and as well as their origin (i.e. whether they are comprised of parasite- or host-encoded proteins, or both) is controversial and there is recent evidence both for the involvement of endogenous channels of the host cell and for the involvement parasite-encoded protein. These pathways are thought to play an important role in the delivery of a number of key nutrients, including the vitamin pantothenic acid and the essential amino acid isoleucine to the parasite, as well as being responsible for the dramatically increased Na+ concentration in the host cell cytosol. They are therefore of significant interest as potential drug targets, although the majority of inhibitors presently available are of low affinity and poor specificity.

  Within the infected cell, solutes are thought to pass freely between the erythrocyte cytosol and parasitophorous vacuole, via high-capacity, low-selectivity channels that render the vacuolar membrane freely permeable to low-molecular-weight solutes. By contrast, the parasite plasma membrane supports a significant membrane potential and substantial transmembrane ion gradients. Solutes move back and forth across this membrane, into and out of the parasite, via a range of channels, transporters and pumps.

- **Trypanosomes**

In **Trypanosomes**, African trypanosomes e.g. *T*. *brucei* and related subspecies, are uniflagellated parasites that cause African trypanosomiasis in humans and in wild and domestic animals. *T*. *brucei* is the causative agent of human African trypanosomiasis, a fatal disease that is commonly referred to as "African sleeping sickness." These parasites are digenetic organisms, completing part of their life cycle in a mammalian host and part in an insect vector, the tsetse fly. *T*. *brucei* is transmitted to the bloodstream of a mammalian host through the bite of an infected tsetse fly. Once in the bloodstream, the parasites multiply extracellularly for a period of weeks to months. They eventually penetrate the blood vessel endothelium, spread within the connective tissues, and infiltrate the host's central nervous system (CNS), where they initiate a cascade of events that result in fatal sleeping sickness. Clinical manifestations of sleeping sickness are divided into an early stage, in which parasites are found in the blood and lymph, and a late stage, when parasites have invaded the CNS. The early and late stages of the disease are characterized by distinct clinical symptoms and respond very differently to antiparasitic drugs. If untreated, sleeping sickness is always fatal, and the fatal course of the disease is directly linked to the presence of parasites in the CNS. Hence, the pathogenic features of sleeping sickness are directly related to migration of the parasite to specific host tissues. Since T. brucei is extracellular at all stages of its life cycle, it is dependent upon its own vigorous cell motility for extravasation and dissemination within the host.

The requirement for trypanosome cell motility is especially acute during transmission through the tsetse fly, where the parasite must undergo an ordered series of developmental transformations and directed migrations in order to achieve its goal of being delivered to a new, mammalian host. Following a blood meal, ingested quiescent bloodstream-form, trypomastigotes first differentiate into actively dividing procyclic trypomastigotes and establish an infection in the tsetse fly midgut. The parasites then migrate from the midgut into the ectoperitrophic space and then through the proventriculus into the foregut, where they differentiate into elongated and asymmetrically dividing "postmesocyclic" epimastigotes. These elongated epimastigotes complete the journey through the proboscis and hypopharynx to reach the lumen of the salivary gland, where the final stage of development occurs. Parasites advancing to the foregut and proboscis exhibit dramatically increased motility compared to those found in the midgut. Once parasites are in the salivary gland, cell division is completed, generating short epimastigotes, which attach themselves to the gland epithelium through intricate membrane and cytoskeletal connections that are established between the parasite flagellum and the epithelial cell membrane. These attached epimastigotes differentiate into variant surface glycoprotein (VSG)-coated metacyclic trypomastigotes that detach from the epithelium and are now uniquely suited for survival in the mammalian bloodstream. Thus, migration of the parasite from the midgut to the salivary gland and the concomitant developmental changes that occur along the way are required for transmission to the mammalian host.

The importance of trypanosome motility for completion of the journey from the midgut to the salivary gland is obvious but remains to be tested experimentally. In addition, other important questions arise concerning development in the tsetse.

- **Tapeworm**

In **Tapeworms**, the "cuticle" is, in reality, a cellular syncytium, referred to in the recent literature as a tegument. Lacking a gut, tapeworms utilize the tegment alone for chemical interchange with the host. Attendant to this function is a brush border containing a number of transport mechanisms and hydrolytic enzymes. The external limiting membrane is coated with a layer of carbohydrate-rich polyelectrolytes, viz. a glycocalyx. This structure serves as a binding surface for inorganic ions and higher molecular weight organic compounds, including host enzymes. Certain of the latter retain activity, and may thereby contribute to contact-digestive functions at the surface of the worm. Others are bound in a catalytically-inactive configuration, which may serve to protect the parasite from digestion. Intrinsic enzymes of the tegument membrane surface include phosphohydrolases and a ribonuclease; the hydrolysis products of these activities interact with proximal transport loci and are thereby absorbed. Thus, the tapeworm tegument includes a digestive-absorptive-protective surface, whose functions involve the interaction of both parasite and host components within the structural framework of a membrane-defined interface.

- **Digenea**

In **Digenea**, the tegument is folded into concentrically arranged furrows and ridges bearing numerous tightly packed tubercules, and extends into the oral cavity. An area of specialized tegument is present on the ventral surface, anterior to the disc region. Mitochondria are absent from the tegumental syncytium and underlying tegumental cells, suggesting that the tegument may serve principally as a protective layer rather than in active uptake phenomena. However, extensions of the lymph and parenchyma systems are closely associated with the base of the tegumental syncytium and may provide ATP for active processes. Ciliated and non-ciliated sensory papillae are present, particularly around the oral opening. Numerous lymph channels are present in the sub-tegument and may be involved in osmoregulation. The Transport is complicated by presence of alimentary canal. Gut plays a major role in nutritional transport. *Schistosoma mansoni* & *Faciola hepatica* transports monosaccharide by carrier mediated transport. *Schistosoma mansoni* follows tegumental carrier to transport amino acid where else *F. hepatica* absorbs amino acid by simple diffusion only.

Probable Questions

1. Write a note on membrane transport mechanism in parasite.
2. What role does passive transport play in membrane transport mechanism in parasites?
3. How do parasites utilize active transport process to maintain homeostasis and acquire essential nutrients?
4. How do nutrient molecules transport through Digenean tegument?

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Unit II

Reproductive physiology in parasites

Objective: In this unit we will discuss about reproductive physiology in parasites.

Introduction:

➢ Most parasites reproduce asexually but they can switch to sexual reproduction to encourage diversity and to remain infectious.

➢ Certain species of parasites can even sexually reproduce with other species, via a process called Hybridization.

➢ Many parasites have complex life cycles which involve both sexual and asexual processes.

➢ Three processes are involved- 1. Asexual reproduction 2. Sexual reproduction 3. Reproductive synchrony

➢ Another parasite that reproduces both sexually and asexually is the *Podosphaera plantaginis*. They reproduce asexually by producing infective spores throughout their life cycle. When the host's growing season comes, they produce resting spores that can survive during the winter. Spores can either be produced by mating or by asexual process known as haploid selfing. Reproducing sexually comes at a much greater cost, bringing into question why pathogen continue to maintain this reproductive strategy.

How does parasite reproduce?

1. **Asexual reproduction:** All parasite reproduces asexually by four different methods-
    i. **Binary fission**- It is the division of the parent body into two equal daughter individuals. The nucleus divides first and is followed by the division of cytoplasm.

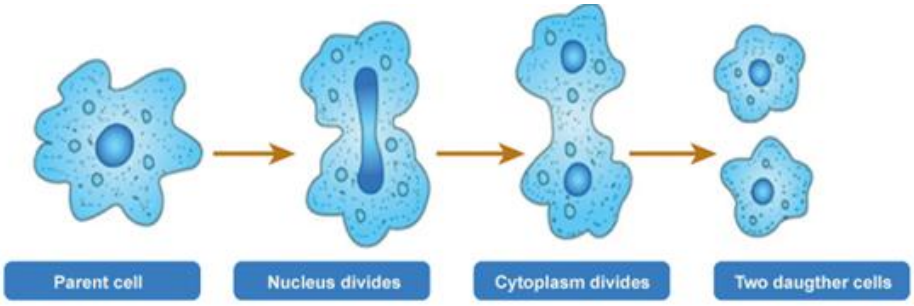

Fig- Binary fission

ii. **Multiple fission-** The nucleus divides many times either by mitosis or fragmentation. The daughter nuclei migrate towards the periphery and are surrounded by the fragments of cytoplasm forming daughter individuals.

Fig- Multiple Fission

iii. **Plasmotomy-** It is a very special type of asexual division in a multinucleate animal in which the cytoplasm divides but the nuclei do not divide.

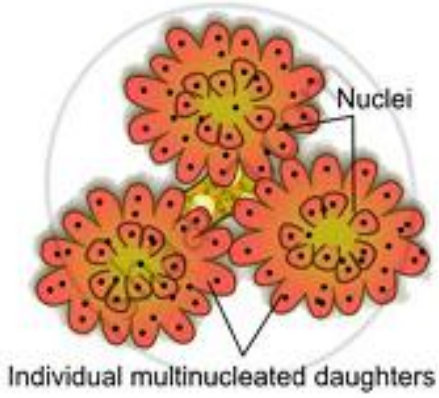

Fig- Plasmotomy

iv. **Budding-** It is a form of fission in which one or more smaller individuals separate from parent body and each undergoes differentiation either before or after separation.

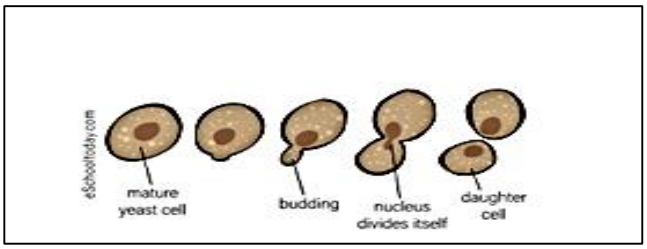

Fig- Budding

Mechanism of asexual reproduction:

Asexual reproduction in free-living species usually involves nuclear division and the division of the cell into two identical daughter cells of equal size by binary fission. In parasitic protozoans and some free-living species, multiple fission, resulting in the production of many offspring that may not resemble the parent cell. During the cycle of growth and division, the protozoan undergoes a series of identifiable phases: a division phase, a growth phase during which the cell increases substantially in size, a phase of DNA synthesis, and a phase of preparation for division, which extends from the end of DNA synthesis until the initiation of division. The division of the cytoplasm (cytokinesis) is preceded by the division of the nucleus or nuclei.

The plane of division in protozoan cells varies among the different groups and is of taxonomic significance. The ciliates normally divide in an equatorial, or transverse, plane, thereby maintaining the correct number of ciliary rows, or kineties. The cell mouth and any specialized cilia around it are replicated in different ways among the various ciliate groups, depending on the complexity of the cytostome. The replication of the cytostome precedes the division of the cytoplasm. Some ciliates (e.g., Colpoda) divide within thin-walled reproductive cysts into two daughter ciliates, each of which then divides so that the cyst contains four progeny, which are released when the cyst wall ruptures.

The sedentary suctorians do not reproduce by binary fission, because the production of an identical non swimming offspring would rapidly lead to overcrowding. They instead produce single ciliated offspring, a process called budding. Budding can occur endogenously, in which the bud forms within the parent and is ejected when mature, or exogenously, in which the swarmer is formed outside the parent. They swim away from the parent, settle on a substrate, lose their cilia, and develop feeding tentacles and an attaching stalk.

The foraminiferan and radiolarian *Amoebae* have evolved multiple fission. Both produce many flagellated swarmers, or zoospores. The common planktonic foraminiferan *Globigerinoides sacculifer*, for example, can produce 30,000 swarmers at one time. Each

swarmer is about 5 micrometres (0.005 mm) long. In planktonic species the parent usually loses buoyancy and sinks by shedding spines and withdrawing the complicated pseudopodial network into the shell. produced in deep water and migrate upward as they mature. Each secretes a shell around itself, which is added to as the organism grows.

- **Reproduction in *Plasmodium falciparum***

P. falciparum enters the host in the form of a sporozoite through a bite from female *Anopheles* mosquito and enter the bloodstream. They reproduce asexually by dividing into schizonts that consist of many merozoites.

i. Polyembryony:

In polyembryony, found in some parasitic *Hymenoptera* or helminth, one sexually produced embryo is split into up to several thousand others during development after the egg is oviposited. It thus shares some of the characteristics of both sexual reproduction and parthenogenesis.

Polyembryony is found in some parasitic *Hymenoptera*. One sexually produced embryo is split into up to several thousand others during development after the egg is oviposited.

It thus shares some of the characteristics of both sexual reproduction and parthenogenesis. Unlike parthenogenesis, which produces many copies of a single genotype, in polyembryony the genotypes of the mother and offspring differ. Unlike sexual reproduction, however, all the genotypes produced are the same as each other. As it occurs after the egg is oviposited, polyembryony enables the offspring, rather than the parent, to determine optimal brood size.

Twinning is an accidental form of polyembryony that has been observed in most animals in which an individual egg may split to form two or more embryos. The host completes embryogenesis and hatches into a first instar larva, and during the four host instars, the copidosoma embryo proliferates. At the fifth larval instar, up to 2000 larvae of copidosoma are formed and begin to consume the host, ultimately creating a mummy. The wasp larvae pupate and then emerge. The single wasp egg that is laid in the egg of a lepidopteran is surrounded by a thin chorion. Lacking yolk, the wasp egg exploits the nutritive environment of the host both for embryogenesis in the absence of the syncytium, there is also no opportunity for the distribution of patterning morphogens within the developing egg, and the specification of developmental axes occurs later in each embryo. The segmentation proteins commonly found during *Drosophila* embryogenesis are expressed later in development. Individual numbers are dramatically increase by internal budding.

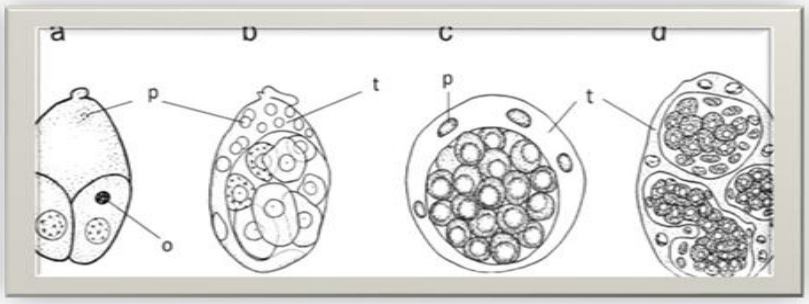

Fig- Polyembryony

ii. Sexual reproduction

Many protozoans reproduce by a form of sexual reproduction but it is not always easy to distinguish between the fusion of individual parasites and the fusion of gametes. The gametes are morphologically distinct:

✓ Male- Microgamete

✓ Female- Macrogamete

Asexual schizogony increases number of merozoites in the host blood but sexual gametocytes are also formed which are transmitted to mosquitoes in which host gamete fusion and sexual proliferation takes place

i. Gametocytogenesis:

Gametocytogenesis is the creation of gametocytes by mitotic division of gametogonia. During this process a series of morphological and biochemical changes occurs to facilitate the transmission from human host to mosquito vector. It is regarded as an escape mechanism for unfavourable conditions by means of the genomic variation conferred by random fusion of gametes. All monogeneans are hermaphrodites and asexual mechanism are unknown.

ii. Self fertilization offers reproductive assurance in circumstances where mates are encountered rarely Example- liver fluke, roundworms

A prerequisite for **cross fertilization** studies is the availability of parasite strains that exhibit intraspecific variation

iii. Cross fertilization in *Plasmodium falciparum*: Two clones of the human malaria parasite Plasmodium falciparum, denoted 3D7 and HB3, were grown in vitro under conditions permitting the development of gametocytes clones differ in their allelic forms of two antigen genes MSP1 and MSP2 gene. Mosquitoes (*Anopheles stephensi*) were fed on a mixture of these gametocytes. A total of 128 oocysts was isolated from the midguts of infected mosquitoes from 9 crossing experiments between the clones. Oocysts which contained both alleles of each gene (MSP1 and MSP2) had developed from heterozygotes produced by cross fertilization events

between 3D7 and HB3 gametes. The remaining oocysts contained single alleles of each gene, in parent clone combinations, and these had developed from homozygotes formed by self-fertilizations. The physiology of egg production in mosquitoes is well understood. Eggs are released from mature ovary and enter oviduct. Spermatozoa from the partner are stored after copulation in a seminal receptacle released along with a small number of vitelline cell. During development the egg shell becomes tanned and on release the egg is fully protected by the rigid shell.

The tapeworm egg is not tanned like digenea but is surrounded by a capsule comprising various constituent layers.

Acanthocephalans have separate sexes and they are sexually dimorphic. Eggs are fertilized and liberated from the ovarian balls to complete develop in the pseudocoelom. Females release large number of eggs that protected by covering of protein and chitin.

Nematodes are sexually dimorphic. In some groups such as the filarial nematodes egg hatching takes place in utero and egg shell reduced in size and chemical complexity

Reproductive synchrony:

Malaria parasites of the species *Plasmodium falciparum* classically progress synchronously through their 48-h lifecycle in the erythrocytes, from invasion to release of new merozoites, which gives rise to the periodic fevers. However, the extent to which natural infections display a synchronous pattern of growth is unclear; fever patterns, especially in falciparum malaria, are often irregular. Both oscillatory and non-oscillatory patterns in parasite densities were observed in non-immune patients deliberately infected during malaria therapy for neurosyphilis. The degree of synchrony is relevant clinically. Individuals with asynchronous infections have been reported to have higher parasite multiplication rates, associated with increased disease severity, and result in a more rapidly expanding parasite population, which may outstrip antiparasitic host responses, or interventions such as drug treatment.

A parasite infection is defined as synchronous when merogony (schizogony) occurs with a standard deviation i.e. 68% of circulating parasites are within a 4 h age window. Using a combination of morphological features to define the “age”, but the presence of two parasite broods shifted by 24 h has also been described in some patients, supported by the observation that parasite negative samples are rarely seen in infected. However, the morphological characteristics used can be influenced by the immune status of the patient and the distinction between young and old ring-stage parasites can be difficult to assess objectively. What drives synchrony in natural infections is unknown. The asynchronous growth of *P. falciparum* in vitro suggests a role for host factors. Parasite dynamics in malaria patients may also depend on how the blood-stage infection initiates. A single mosquito bite inoculates 30–50 sporozoites of which one or two successfully infect hepatocytes. Non-simultaneously rupture of the mature infected hepatocytes

releases broods of parasites with shifted temporal patterns. Furthermore, infections with multiple clones (genotypes) of the parasite are also very common in endemic areas and these may originate at different times. Reproductive events in a small number of parasite species are synchronized to host sexual cycles and breeding pattern. This relationship serves to liberate infection. Example- Flagellated protozoans of amphibians and arthropod.

The hypermastigina that inhibit the gut of arthropod can be stimulated to reproduce sexually.

Flagellates of termites are lost with each successive moult. Synchrony of sexual process in the parasites with moulting in the host ensures reinfection.

• Sexual reproduction in protozoan parasite

✓ Syngamy

✓ Conjugation

i. **Syngamy**- Fusion of two gametes resulting in the formation of zygote. Fused nucleus of zygote is called as synkaryon.

Types-

Hologamy

Isogamy

a) **Hologamy**- In which the two premature Protozoan do not form gametes and fuse together but behave as gamates and fused together to form a zygote.

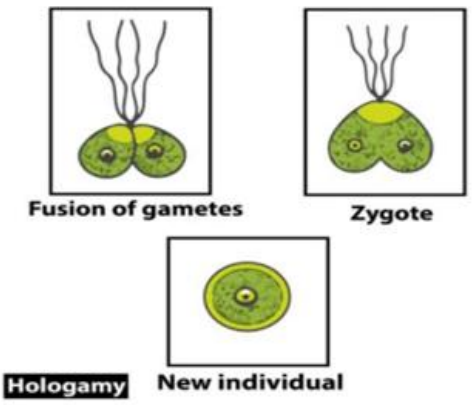

Fig- Hologamy

b) **Isogamy**- When two fusing gamates are morphologically similar but differ in behaviour they are called isogamete and their union is called isogamy.

c) **Anisogamy**- When two fusing gamates differ in morphology as well as in behaviour.

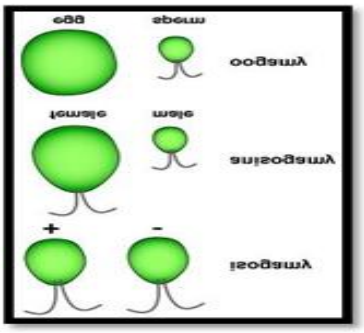

Fig- Isogamy and anisogamy

d) Autogamy- It involves the fusion of gamates derived from the same parent cell.

Conjugation-

Also called amphimixis. It is the temporary union of two individuals where exchange of genetic material takes place by direct cell to cell contact. Conjugation takes place only same group of organisms. Organisms take part in conjugation known as Conjugants.

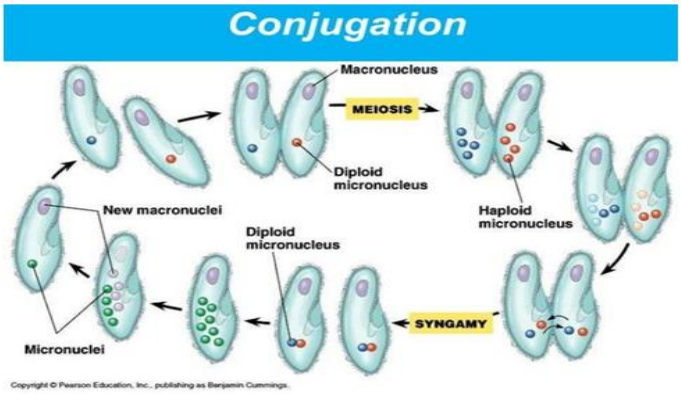

Fig- Conjugation process in protozoa

Probable questions:

1. What is plasmotomy?
2. What is polyembryony?
3. What is binary fission?
4. What is multiple fission?
5. What is conjugation?
6. What is syngamy?
7. Give an account on reproductive physiology in protozoan parasites.
8. How does parasite reproduce asexually?
9. Describe the mechanism of asexual reproduction.
10. Give an account of reproduction in *Plasmodium falciparum.*
11. Give an account of reproduction in protozoa by gametocytogenesis.
12. Describe the conjugation process in protozoa.

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Unit III

Energy metabolism in parasitic protozoa

Objective: In this unit we will discuss about energy metabolism in parasitic protozoa.

Energy metabolism plays a key role in all living organisms and is strictly related to their ecological niche and lifestyle. Anaerobic energy metabolism is broadly diffused among protozoa. It is typical in commensal and parasite species, such as digestive tract symbionts and pathogens in general, but it is also present in free-living species adapted to anoxic or hypoxic habitats. Energy metabolism pathways in extant obligate or facultative anaerobes are varied and anaerobically functioning mitochondria or mitochondrion-related organelles seem to be ubiquitous in such organisms. In fact, it has been recently suggested that the endosymbiosis which gave origin to mitochondria in the eukaryote ancestor, occurred as early as the nucleus acquisition and that organelles such as hydrogenosomes and mitosomes are degenerate forms of mitochondria which evolved independently in different eukaryotic lineages. While mitochondria and hydrogenosomes are directly involved in ATP generation, however, mitosomes are not, but arguably still retain such essential functions that no eukaryotic organism could do without them. This chapter critically reviews the present knowledge on the anaerobic energy metabolism in protozoa, in view of the current (although still debated) picture of the evolution of eukaryotes and of their mitochondrion related organelles.

Anaerobic energy metabolism in protozoa:

The universal quantum of biological energy is represented by the adenosine triphosphate (ATP) molecule. In heterotrophic organisms, ATP is generated by phosphorylation of adenosine-diphosphate (ADP) in two ways: substrate-level phosphorylation and oxidative phosphorylation. The opposite reaction, ATP hydrolysis, is highly exergonic, because end products are much more stable than reagents, and thus a considerable amount of energy is released. This reaction can be coupled with thermodynamically unfavorable reactions to give an overall negative change in Gibbs free energy (ΔG) for the entire sequence. While the initial breakdown of organic compounds is highly conserved in protozoa, the subsequent enzymatic pathways are diverse and can take place in different compartments of the eukaryotic cell, especially as regards anaerobic energy metabolism. As we will see, these dissimilarities are directly linked to species lifestyle and ecological niche, but the overall picture only makes sense in the light of earth early history and of biological evolution.

These organisms are *Giardia lamblia*, an inhabitant of the small intestine of mammals, *Entamoeba histolytica* that lives in the large intestine of humans, and two members of the family Trichomonadidae, *Trichomonas vaginalis* and *Trichomonas foetus* that are parasites of human and bovine genitourinary tracts, respectively. Several other mitochondrial organisms, and a few parasitic protozoa that possess mitochondria, are also mentioned. The chapter explains that the organisms do not contain mitochondria and rely on fermentative processes via an extended glycolytic pathway for adenosine triphosphate (ATP) generation. Fermentative metabolism persists in all these species, even when oxygen is present. Inorganic pyrophosphate rather than ATP is used in one or more of the glycolytic reactions. Glycolysis in trichomonads is also cytosolic, but pyruvate oxidation and hydrogen formation occur in a separate organelle. The enzymes of energy metabolism in these anaerobic parasites are more closely related to those found in eubacteria and eukaryotes than to those present in archaea bacteria. Thus, the chapter states that the energy metabolism of the anaerobes is well adapted to their luminal habitats, which contain little or no oxygen. Its evolutionary history remains to be elucidated, but there are clear indications that the strategy has appeared several times during eukaryotic evolution.

Energy metabolism in *Trypanosoma*:

African trypanosomes are parasitic protozoa of the order of Kinetoplastida, which cause sleeping sickness and nagana. Trypanosomes are not only of scientific interest because of their clinical importance, but also because these protozoa contain several very unusual biological features, such as their special energy metabolism. The energy metabolism of *Trypanosoma brucei* differs significantly from that of its host, not only because it comprises distinct enzymes and metabolic pathways, but also because some of the glycolytic enzymes are localized in organelles called glycosomes. Furthermore, the energy metabolism changes drastically during the complex life cycle of this parasite. Anaerobic glycolysis produces a large amount of lactate, which is utilized as an energy source and is associated with the Warburg effect. Tumor cells opt for relatively inefficient anaerobic glycolysis to generate abundant lactate even under oxygen-rich conditions. In *T. brucei*, BSFs rely heavily on glycolysis for ATP biosynthesis even under aerobic conditions, rather than tricarboxylic acid cycle and oxidative phosphorylation, whereas PCFs rely primarily on oxidative phosphorylation. During glycolysis, glucose consumed by cells is broken down to generate energy. Nevertheless, the glycolytic pathway in early branched trypanosomatid parasites substantially differs from those in other organisms. In contrast to other eukaryotes, trypanosomes compartmentalize their glycolytic enzymes in peroxisome-derived organelles known as glycosomes. Glycolysis generates several metabolic end-products that must be eliminated. Lactate is abundantly produced during glycolysis in conventional eukaryotes. However, as BSF *T. brucei* lacks lactate dehydrogenase (LDH), it converts glucose into pyruvate under aerobic conditions but forms pyruvate plus glycerol under

anaerobic conditions. High rates of glycolysis yield methylglyoxal, which is a toxic by-product of the triosephosphate isomerase (TPI) reaction. Dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (GA-3P) generate methylglyoxal through an enediol intermediate As *T. brucei* expresses glyoxalase II (GLO2) but lacks glyoxalase I (GLO1), the end-product of methylglyoxal metabolism is mainly *L*-lactate, while other eukaryotic cells produce *D*-lactate vances made in understanding the process of ATP production in *T. brucei* during its life cycle and the consequences of the special subcellular compartmentation.

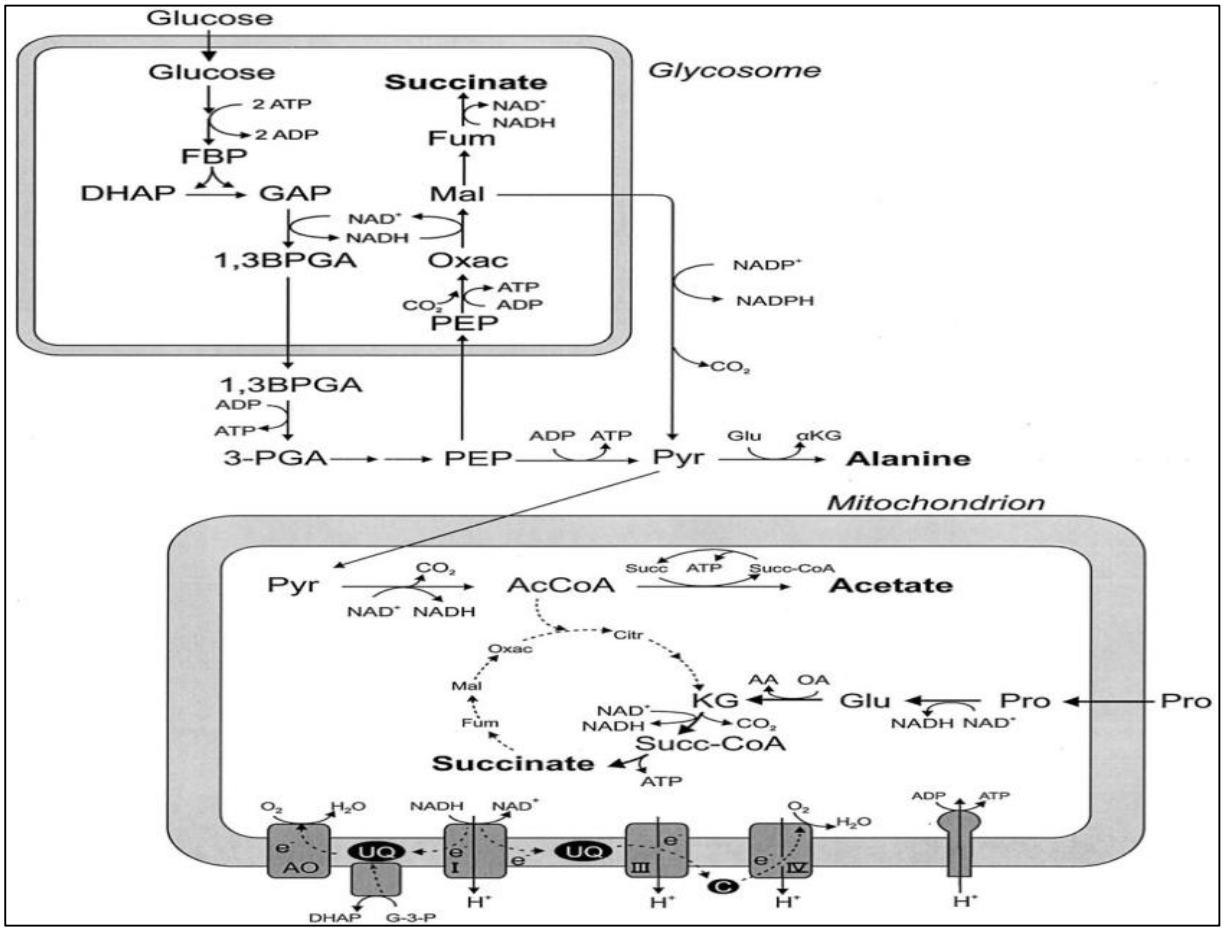

Fig: Energy metabolism in *Trypanosoma*

Energy metabolism in *Trichomonas*:

Glucose is the major energy source that is converted to pyruvate for ATP generation in the trichomonad hydrogenosome. Under glucose restriction (GR), the regulation of amino acids metabolism is crucial for trichomonad growth and survival. RNA-sequencing (RNA-seq) analysis has been used to identify differentially expressed genes in *Trichomonas* vaginalis under GR, leading to significant advances in understanding adaptive responses of amino acid metabolism to GR. However, the levels of amino acid metabolites modulated by GR are unknown in *T. vaginalis*.

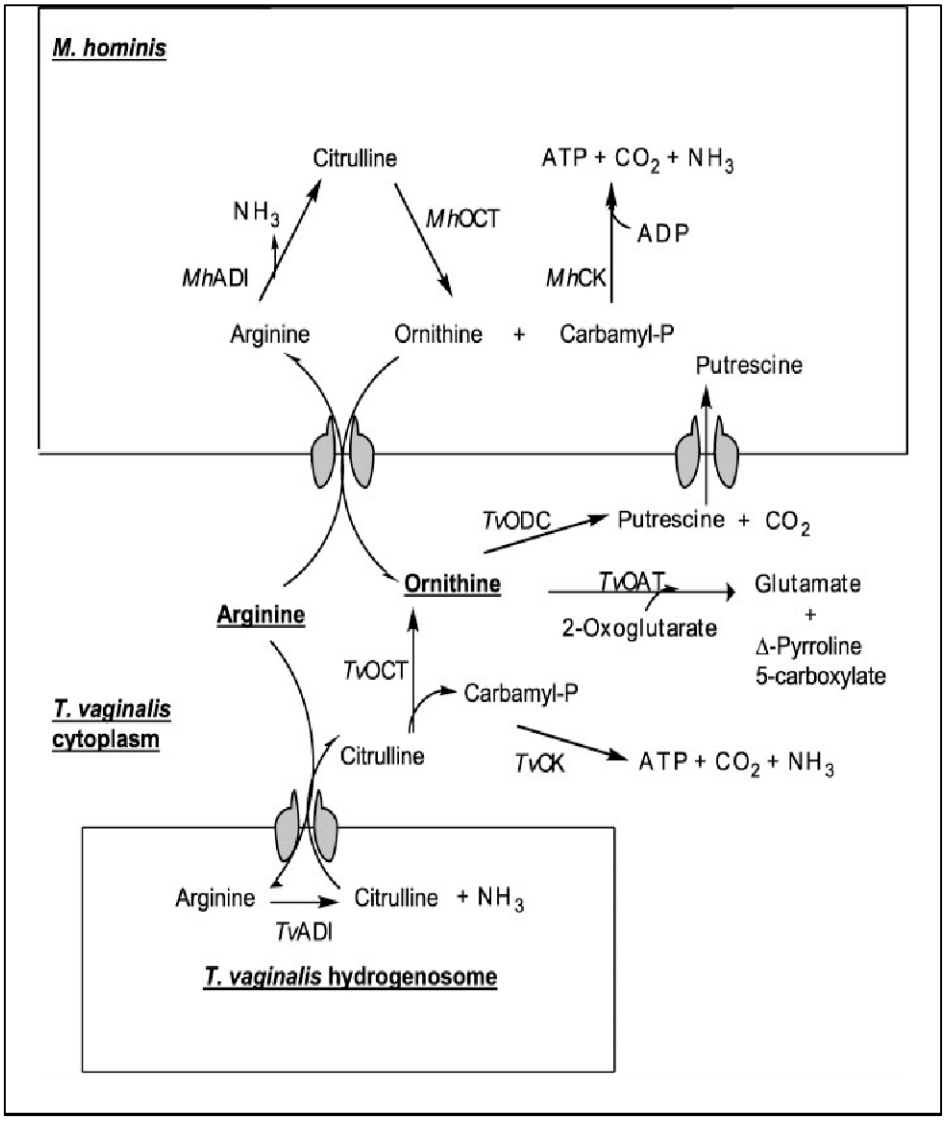

Fig: Energy metabolism in *Trichomonas*

Energy metabolism in *Entamoeba hystolytica*:

The amebicidal action of metronidazole is activated when the enzyme pyruvate: ferredoxin oxidoreductase transfers reducing equivalents to the nitro group of the drug. The enzyme is present in *Entamoeba histolytica* and other anaerobic parasites like *Giardia* and *Trichomonas* that lack mitochondria. The selectivity of the drug can be ascribed to the absence of the reductase in the human host. *E. histolytica* possesses other enzymes involved in glucose catabolism that are interesting for the rational design of new drugs. It has glycolytic enzymes that are important for the production of energy like phosphofructokinase, pyruvate phosphate dikinase, phosphoenolpyruvate carboxytransphosphorylase and acetate thiokinase, which use pyrophosphate as a phosphate donor and have no human counterparts. The first part of this article describes the reactions by which *E. histolytica* obtains energy from glucose degradation,

and includes recent advances in the cloning of genes for the various participating enzymes. The second part shows an alternative view for the study of target enzymes that are unique to the parasite, and indicates their importance in therapeutic research.

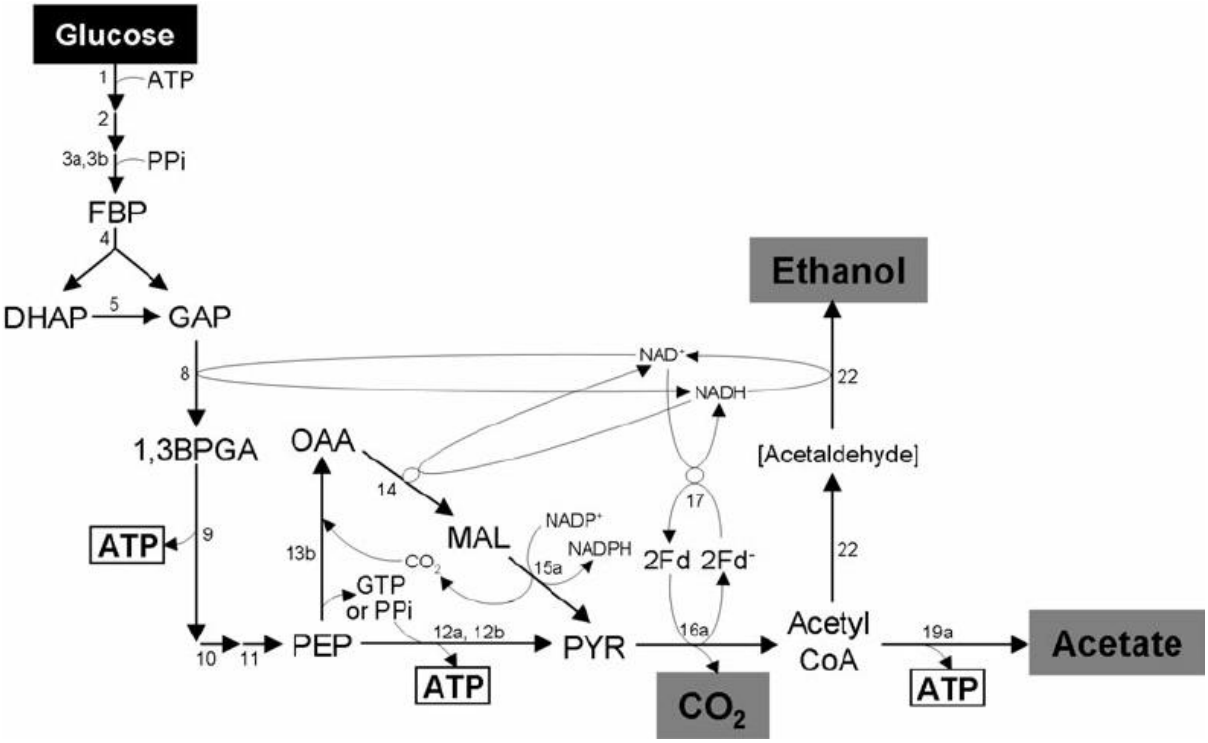

Fig: carbohydrate metabolism of trophozoite stage of *Entamoeba*

Energy metabolism in *Giardia*:

It is well known that this organism has minimal biosynthetic capacity. It lacks de novo lipid, de novo purine, and de novo pyrimidine syntheses (relying solely on salvage pathways). *Giardia* also lacks mitochondria and cytochrome-mediated oxidative phosphorylation and thus trophozoites use glycolysis (from glucose only) and the arginine dihydrolase pathways relying on substrate level phosphorylation for energy production; glucose is also shunted through a pentose phosphate pathway. The enzymes responsible for end product and energy production in *Giardia* are soluble—not found in subcellular organelles. The end products of glucose fermentation are acetate, ethanol, alanine, carbon dioxide, and hydrogen. Thus, it is clear that *Giardia* is well adapted to its environment; however, this comes at a cost because this organism must scavenge nearly all of its biosynthetic precursors from an environment containing a thriving microbial flora. Additionally, *Giardia*'s metabolism seems to be exquisitely balanced — and this would mean that processes such as the formation of the cyst wall carbohydrate have to be tightly controlled and regulated. Encysted *Giardia* slows their catabolism of glucose for energy, and begin converting glucose to the synthesis of a cyst wall specific sugar, N- acetylgalactosamine, for the synthesis of giardan. A complete

pathway of enzymes is induced during encystment for this synthesis including a novel enzyme, cyst wall synthase, that synthesized the giardanhomopolymer [β-1,3-N-acetylgalactosamine]. At the same time, trophozoites increase their catabolism of arginine ostensibly to offset the energy lost from slowing glycolysis.

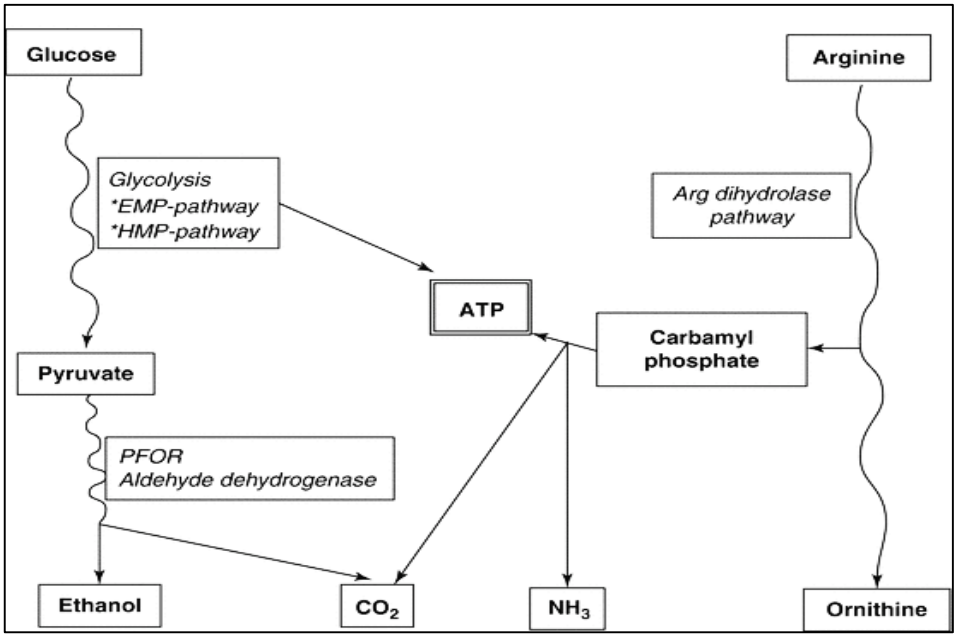

Fig- Energy metabolism in *Giardia*

Electron transport chain in protozoa

Among the parasitic protozoa only a few species have been studied in the detail necessary for characterizing the various components of the electron transport chain.

- ***Plasmodium*:**

Our biochemical information of the *Plasmodium* is limited to the erythrocytic stage in the life cycle of the organism. Several species have been studied, but since no significant differences have been discovered, so to treat them as a group seems best. No attempts to prepare pure enzymes has yet been made. Early work utilized infected blood of monkeys with uninfected blood as control. Further, washed parasitized avian erythrocytes were used. Saponin and specific hemolysins have been employed to prepare free parasites, and finally homogenates of free parasites have been studied for individual enzyme activities. With these preparations, several reactions of phosphorylative glycolysis, including the oxidation of 3-phosphoglyceraldehyde by NAD has been demonstrated. Lactic acid accumulates anaerobically, and to a lesser extent aerobically. NADH produced in the first reaction is reoxidized by pyruvate in the second and another coupling was demonstrated by showing lactate oxidation by indophenols requiring flavoprotein, which may be part of the electron transport chain. The utilization of glycerol by *P. knowlesi* probably indicates the presence of another system

for the reduction of NAD and oxidation of NADH, by glycerophosphate, although no evidence for phosphorylation was found. Recent studies have found adequate evidence for phosphorylation as well as for a functional citric cycle and free parasites utilized most of the cycle components. Pyruvate oxidation was stimulated by dicarboxylic acids and completely inhibited by malonate. Fumarate or malate relieved the inhibition on succinic dehydrogenase. So, the electron transport chain in *Plasmodium* contains proteins for reduction of NAD and perhaps NADP. Malonate inhibited oxygen uptake with succinate as substrate and is reversed by fumarate and malate. This indicates flavoprotein activity. The isolation of FAD from parasites, as well as reduction of indophenol and cresyl blue, support this interpretation. The oxidases being cyanide sensitive, among which one is azide sensitive, and one is inhibited by carbon monoxide. Though no supporting evidence has been obtained, but it indicates that these oxidases may be related to cytochrome oxidase. The only evidence for intermediate carriers so far found is the inhibition of oxygen uptake by naphthoquinones which in other organisms act upon cytochrome reductase.

- **Trypanosomes:**

Trypanosoma sp., a veterinary protozoan serves as a model for *Trypanosoma rhodesiense* and *Trypanosoma gambiense*, which cause human diseases known as African sleeping sickness. The bloodstream form of this parasite has neither oxidative phosphorylation, nor cytochrome-mediated electron transport system, as well as no tricarboxylic acid cycle. It has been seen that the high rate of oxygen consumption by these protozoa is not inhibited by cyanide, antimycin A, rotenone or azide and these parasites are totally dependent on glycolysis for their energy production. The respiratory system has been observed to be composed of glycerol-3-phosphate dehydrogenase and an oxidase. The respiratory system is linked to glycerol 3-phosphate shuttle. The shuttle and the respiratory system reoxidize NADH to NAD+ thus allowing continuous glycolysis. In the trypanosome most steps of glycolysis take place in a specialized microbody called a glycosome. Though, the electron transport system has not been well understood, but the respiratory system re-oxidizes the sn-glycerol3-phosphate produced by NADH-mediated reduction of dihydroxyacetone phosphate in the glycosome. The blood stream forms of vivax, congolense and brucei groups are characterized by high rates of glycolysis, low activity of the citric cycle enzymes, and active oxidases which are not inhibited by cyanide, azide, or carbon monoxide. Glycerophosphate plays a unique role as substrate for two enzymes, one linked to NAD and the other probably linked to FAD. The absence of hydrogen peroxide as end product has not been explained. It cannot be referred to the reaction with pyruvate as it would result in elimination of large amounts of carbon dioxide and acetate, none of them are found in cultures of the organisms. The absence of peroxide can neither be attributed to the action of catalase nor peroxidase, since these enzymes are not present in the organisms.

Probable questions:

1. Write a note on energy metabolism in *Trypanosoma*.
2. Discuss anaerobic energy metabolism in different protozoa.
3. Differentiate between aerobic and anaerobic energy metabolism.
4. Write a note on energy metabolism in *Trichomonas*.
5. How do protozoans adapt their energy metabolism to survive in anaerobic environments?
6. Write a note on energy metabolism in *Entamoeba*.
7. Describe the energy metabolism in organism which is a causative agent of giardiasis.
8. Describe the electron transport chain in different protozoa.
9. Describe the electron transport chain in *Plasmodium*/phylum apicomplexa.
10. Describe the electron transport chain in different trypanosomes.

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Unit IV

Energy metabolism in parasitic helminth

Objective: In this unit we will learn about energy metabolism in parasitic helminth.

Introduction

- All helminths utilise glucose as a respiratory substrate. Tape worms, incubated in vitro, are able to absorb almost all glucose provided incubation media.
- They have very active transport mechanism which binds glucose at very low concentration.
- Once glucose is absorbed, it is either converted into glycogen to act as an energy store or is metabolised directly via the glycolytic reactions as per as PEP (phosphoenolpyruvate).
- Then there are different options for the further metabolism.
- Although parasitic helminths are a very heterogeneous group of organisms, they share many interesting properties in their energy metabolism.
- In certain stages of their life cycle, they all have a large capacity for anaerobic functioning.
- In other stages, an aerobic energy metabolism prevails. Parasites have to adapt to different environments in which the availability of oxygen and food varies widely.
- These variations in their external conditions strongly influence their energy metabolism.

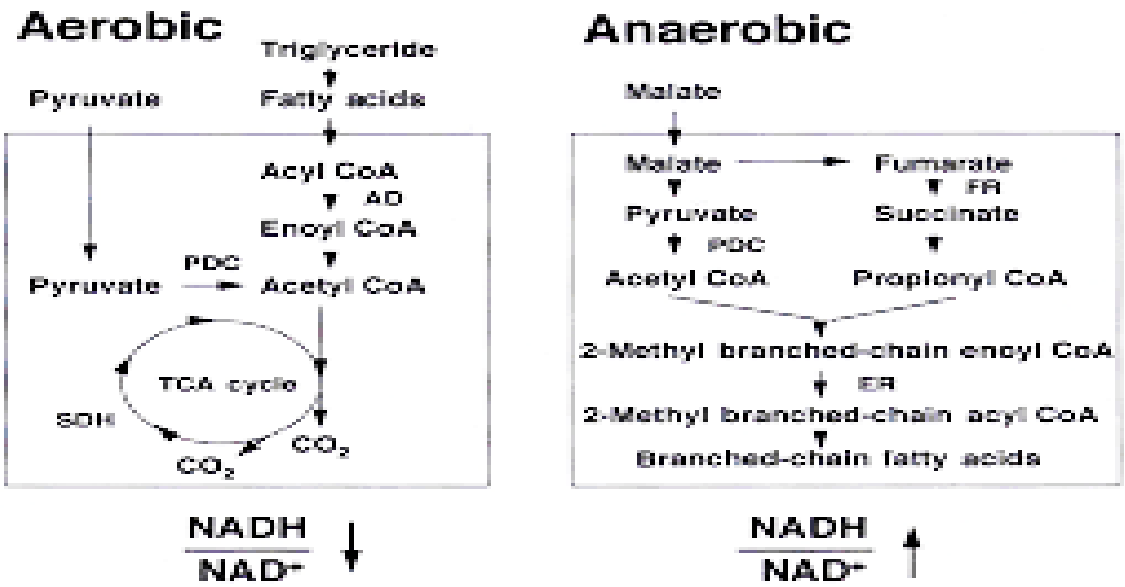

Fig- Aerobic and anaerobic energy metabolism

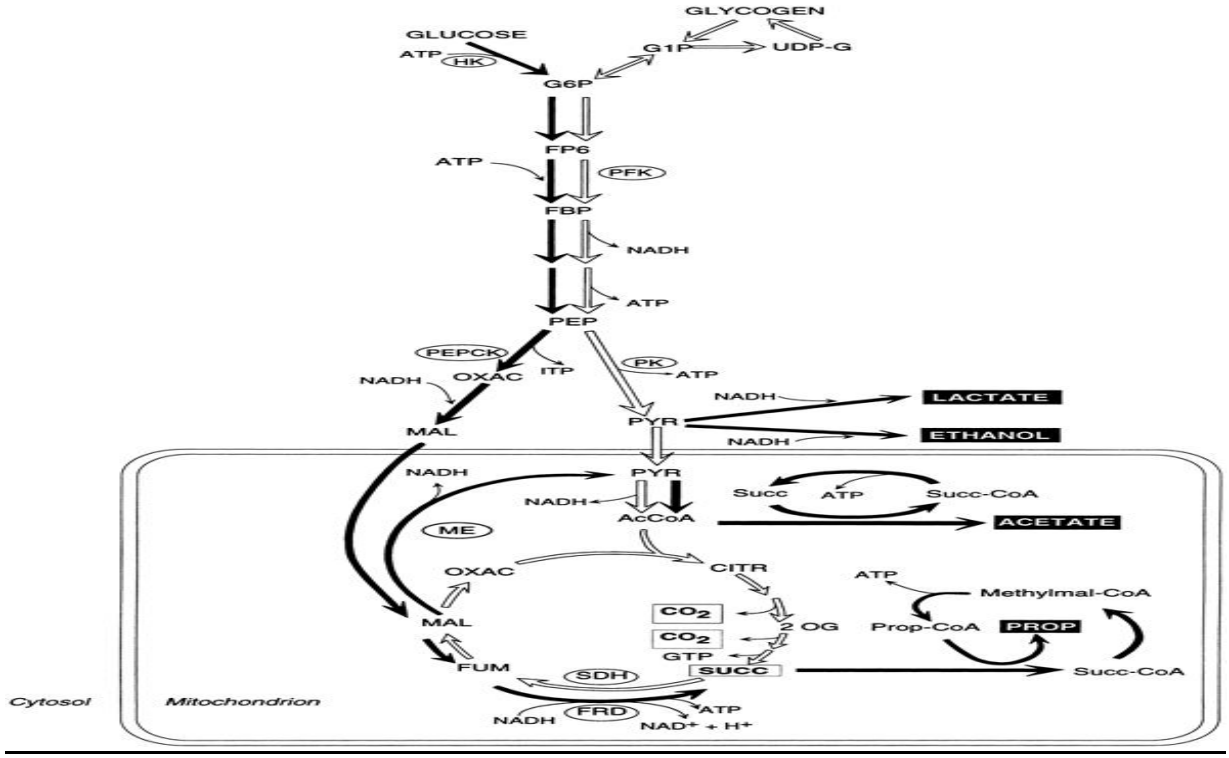

Fig- Energy metabolism

The strategies of energy conservation in helminths

- All adult helminths appear to be able to consume oxygen when it is available but none of them can use it to drive the pathways of complete substrate degradation, like typical aerobic organisms, as a major strategy for energy generation.
- These properties hold also true for those worms residing in a highly aerobic environment, such as the blood stream or the muscle and lung tissues.
- Although in a number of recent studies oxygen was found to play apparently a greater role in the bioenergetics of adult helminths than originally thought. Energy-generating mechanisms in adult worms seem to place greater emphasis on fermentations and anaerobic electron transport processes.
- These exhibit relatively low energy conservation efficiencies and result in the formation of a variety of organic end products.
- The obvious correlation between the type of bioenergetic strategy operative in a particular helminth species and its environmental conditions is not well understood.
- The increased capacity to generate chemical energy and key metabolites of helminths possessing multiple fermentations and anaerobic respirations may give the organism greater versatility and metabolic flexibility to respond to the environmental changes observed in its corresponding habitat.
- Other helminths, such as schistosomes and filariids, which have continuous access to a fairly constant nutrient supply, were found to depend primarily on the more inefficiently functioning and primitive strategy of glycolysis for energy production.
- The reason for the occurrence of limited oxidative capacities in helminths, is not completely clear.
- It may be assumed that the varieties of alternative anaerobic pathways have evolved in response to peculiar environmental conditions prevailing in most parasitic habitats and to the lack of a circulatory system.
- The fact that free-living and other larval or juvenile stages of helminths often have a typical aerobic bioenergetic pattern, is a clear indication that the DNA of these organisms carries the genetic message for all the enzymes involved in complete substrate degradation.

- **Fermentation**

Fermentation is an enzyme catalysed, metabolic process where organisms convert starch or sugar to alcohol or an acid anaerobically releasing energy.

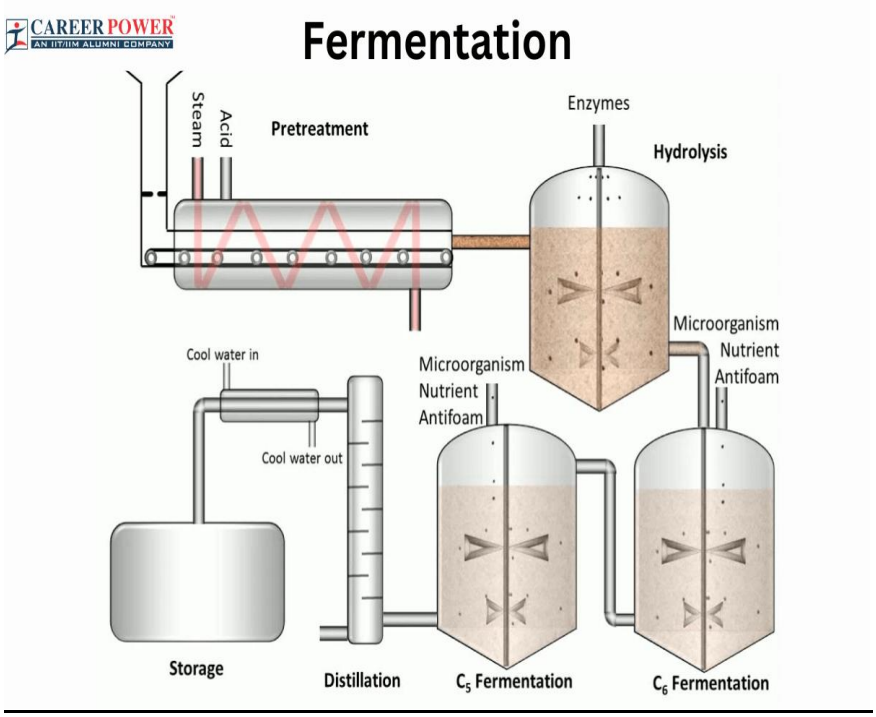

Fig- Fermentation

- Fermentation uses an organic molecule as a final electron acceptor to regenerate NAD^+ from NADH, so that glycolysis can continue.
- Fermentation does not involve an electron transport system, and no ATP is made by the fermentation process directly. Fermenters make very little ATP—only two ATP molecules per glucose molecule during glycolysis.
- Microbial fermentation processes have been used for the production of foods and pharmaceuticals, and for the identification of microbes.
- Microbes which perform **homolactic fermentation** produce only lactic acid as the fermentation product; microbes which perform **heterolactic fermentation** produce a mixture of lactic acid, ethanol and/or acetic acid, and CO_2.
- Lactic acid production by the normal microbiota prevents growth of pathogens in certain body regions and is important for the health of the gastrointestinal tract.

- During ethanol fermentation, pyruvate is first decarboxylated (releasing CO_2) to acetaldehyde, which then accepts electrons from NADH, reducing acetaldehyde to ethanol.
- Fermentation products of pathways (e.g., propionic acid fermentation) provide distinctive flavours to food products. Fermentation is used to produce chemical solvents (acetone-butanol-ethanol fermentation) and pharmaceuticals (mixed acid fermentation).
- Specific types of microbes may be distinguished by their fermentation pathways and products. Microbes may also be differentiated according to the substrates they are able to ferment.

Types of fermentation

- **Homo fermentation:** only one type of product formation
- **Hetero fermentation:** more than one product formed

On the basis of the end product formed, fermentation can be categorized as follows:

1. Lactic Acid Fermentation

- Lactic acid is formed from pyruvate produced in glycolysis. NAD+ is generated from NADH. Enzyme lactate dehydrogenase catalyses this reaction. Lactobacillus bacteria prepare curd from milk via this type of fermentation.
- During intense exercise when oxygen supply is inadequate, muscles derive energy by producing lactic acid, which gets accumulated in the cells causing fatigue.

$$C_6H_{12}O_6(Glucose) \xrightarrow[Lactate.dehydrogenase]{Lactobacillus} 2CH_3CHOHCOOH(LacticAcid)$$

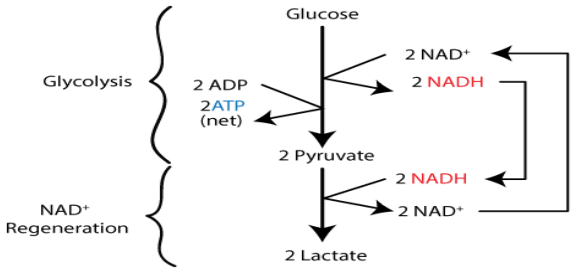

Fig- Lactic acid fermentation

2. Alcohol fermentation

- This is used in the industrial production of wine, beer, biofuel, etc. The end product is alcohol and CO_2.
- Pyruvic acid breaks down into acetaldehyde and CO_2 is released.
- In the next step, ethanol is formed from acetaldehyde. NAD+ is also formed from NADH, utilized in glycolysis. Yeast and some bacteria carry out this type of fermentation.
- Enzyme pyruvic acid decarboxylase and alcohol dehydrogenase catalyse these reactions.

$$C_6H_{12}O_6(Glucose) \xrightarrow[Decarboxylase]{Dehydrogenase} 2CH_3CHO(Acetaldehyde) + 2CO_2 \rightarrow 2C_2H_5OH(Ethanol)$$

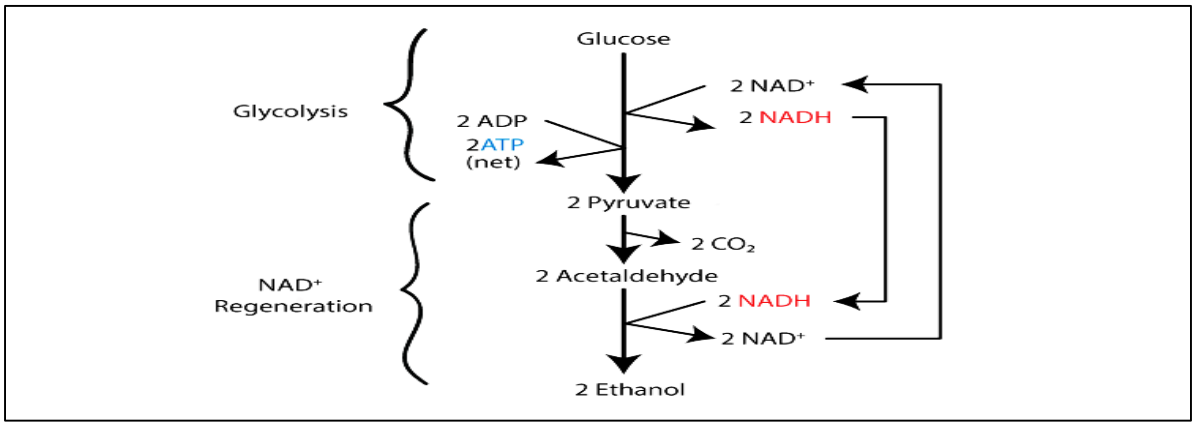

Fig- Alcohol fermentation

3. Acetic acid fermentation

- Vinegar is produced by this process. This is a two-step process.
- The first step is the formation of ethyl alcohol from sugar anaerobically using yeast. In the second step, ethyl alcohol is further oxidized to form acetic acid using acetobacter bacteria. Microbial oxidation of alcohol to acid is an aerobic process.

$$C_2H_5OH(ethanol) + O_2 \xrightarrow{acetobacter} CH_3COOH(acetic\ acid) + H_2O$$

4. Butyric acid fermentation

- This type of fermentation is characteristic of obligate anaerobic bacteria of genus clostridium. This occurs in retting of jute fibre, rancid butter, tobacco processing and tanning of leather.

- Butyric acid is produced in the human colon as a product of dietary fibre fermentation. It is an important source of energy for colorectal epithelium.
- Sugar is first oxidized to pyruvate by the process of glycolysis and then pyruvate is further oxidized to form acetyl-CoA by the oxidoreductase enzyme system with the production of H_2 and CO_2.
- Acetyl-CoA is further reduced to form butyric acid. This type of fermentation leads to a relatively higher yield of energy. 3 molecules of ATP are formed.

$$C_6H_{12}O_2 \xrightarrow[butyricum]{clostridium} C_4H_8O_2 + 2CO_2 + 2H_2$$

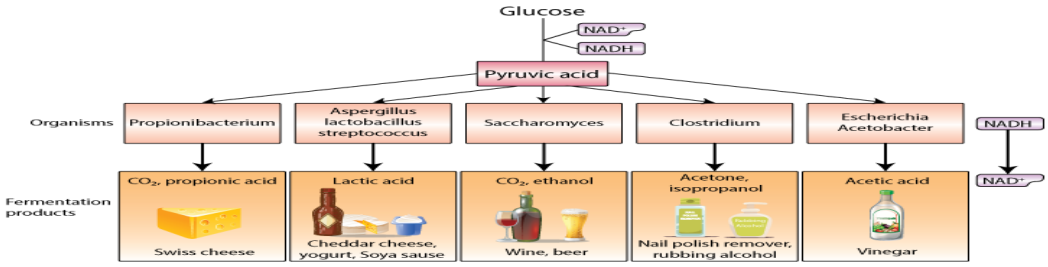

Figure- Types of fermentation

Homolactate fermentation

- True homolactate fermentation probably does not occur in helminths. In homolactate fermentation glucose is converted exclusively into **lactic acid** by glycolysis.
- The end product, lactic acid is excreted and energy generation is thus wholly independent of oxygen. ATP is synthesized at the **Phosphoglycerate kinase** and **pyruvate kinase** steps and NADH is re-oxidised by lactate dehydrogenase so that the pathway remains in redox balance.
- However, there is evidence that other energy yielding processes also occur in the so called Helminths homolactate fermenters. For example, Schistosomes display low level of TCA cycle activity that contribute significantly to energy metabolism. This is because of the aerobic oxidation of glucose generates 18 times more ATP per mole of glucose than Homo lactate fermentation.
- The same is true for filarial worm *L. Carinii.* Overall studies suggest that 2% of utilised carbohydrates in normal respiration invitro, undergo complete oxidation to carbon dioxide and water.

- A simple calculation shows that 98 moles of glucose converted to lactic acid, yield 196 moles of ATP while 2 moles of glucose converted to carbon dioxide and water by TCA cycle, yield 72. From this it is clear that 27% of energy generations in *L. Carinii* could be aerobic.

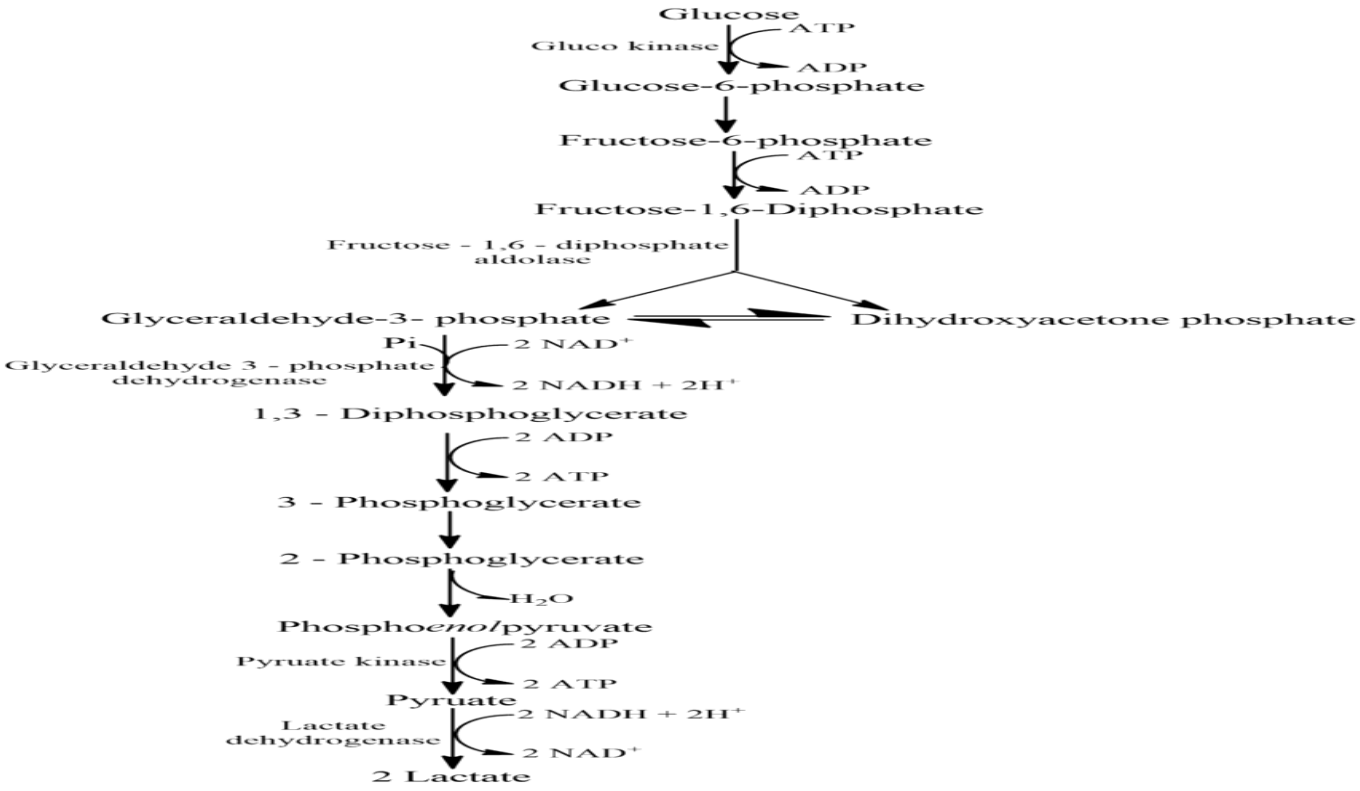

Fig -Homolactate fermentation

Malate dismutation

- The malate dismutation has been most extensively studied in *Ascaris* species ***Hymenolepis diminuta*** *&* ***Fasciola hepatica*****.** Glucose is oxidised to the level of **PEP**. A CO_2 fixation state then occurs catalysed by **PEPCK,** leading to the formation of oxaloacetate which is subsequently reduced to **malate**.
- This can be contrasted with what occurs in mammals. In Mammals PEP is converted to converted to pyruvate which in turn is converted to **acetyl coA** by the pyruvate dehydrogenase system and the subsequent Metabolism of **acetyl coA** is by the TCA cycle in Mitochondria.

- The role of **PEPCK** in Mammals is the reverse of that encounters in helminths. The decarboxylation of oxaloacetate during gluconeogenesis serves as a bypass of the pyruvate dehydrogenase state.
- Citrate synthase agonitate hydratase and isocitrate dehydrogenase are often detected in trematodes and cestodes but there are 3 enzymes of TCA cycle which are almost present in the mitochondria of parasitic helminths. They are **MDH, fumarate hydratase** and **fumarate reductase**. In the mitochondria there are also two important oxidative enzymes-**Malic enzyme, pyruvate dehydrogenase complex**. Malate then enters the mitochondrion where it undergoes dismutation. In a dismutation reaction one molecule of a given compound is oxidised while the second one is reduced. The oxidation step is coupled to the step which provides the reducing power to drive the reaction.
- In the malate dismutation there is an additional need of maintaining redox balance which demands the oxidation of one molecule to malate while two molecules are reduced.
- The products of dismutation are usually acetate and succinate or propionate, are then excreted as the free acid. The net result is the production of more ATP than Homolactate fermentation.
- The malate dismutation involves two subcellular compartments, named cytosol and mitochondria
- Further series of reaction that produce either lactate or malate are equivalent energetically because **PK** and **PEPCK** each brings about synthesis of ATP.

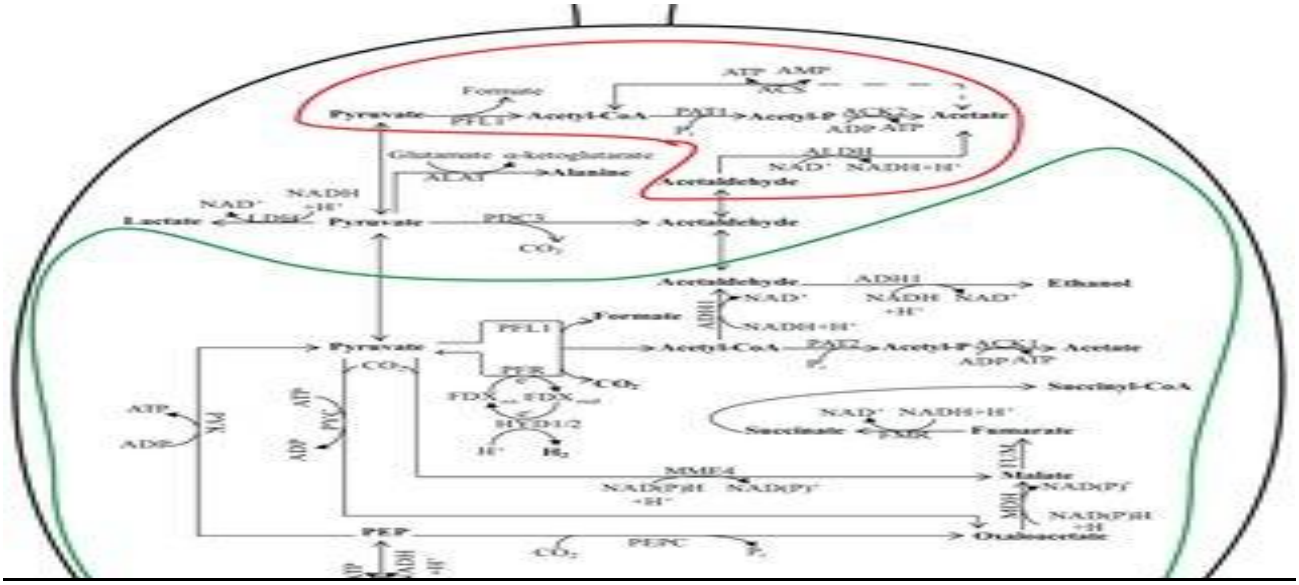

Fig- Malate dismutation and energy metabolism in anaerobic mitochondria.

Mitochondrial malate dehydrogenase, decarboxylating and transhydrogenase activities of adult *Hymenolepis microstoma* (Cestoda)

- Adult *H. microstoma* mitochondria catalysed a malate dehydrogenase, decarboxylating ("malic" enzyme) activity. This "malic" enzyme was found as a soluble component of the mitochondrion, was specific for NADP, and required a divalent cation with Mn++ ion yielding the greatest activity.
- The *H. microstoma* "malic" enzyme could fulfil the need for generating intramitochondrial reducing equivalents required for electron transport. The *H. microstoma* mitochondria also exhibits an NADPH: NAD transhydrogenation reaction.
- The electron transport system of this cestode was apparently specific for NADH both in terms of the rotenone-sensitive oxidase and fumarate reductase systems.
- Electron transport-associated NADPH oxidation was increased markedly with the addition of NAD to the system.
- Coupling of NADPH utilization to fumarate reduction, in the presence of NAD, is apparent under conditions of reduced oxygen tension. This is consistent with the presence of the NADPH: NAD transhydrogenase which catalysed a transfer of

reducing equivalents from NADPH to NAD, producing NADH for electron transport function. The data presented suggest that *H*. *microstoma* mitochondria can engage in an anaerobic, electron transport-associated production of succinate, and presumably concomitant phosphorylation.

- Malate may serve as the mitochondrial substrate supplying reducing equivalents for electron transport via the activity of the "malic" enzyme coupled to the NADPH: NAD transhydrogenase.
- In addition to the NADPH: NAD transhydrogenase activity, *H. microstoma* mitochondria catalysed an NADH: NAD transhydrogenation.

Energy-linked mitochondrial pyridine nucleotide transhydrogenase of adult *Hymenolepis diminuta*

- Employing "phosphorylating" submitochondrial particles as the source of pyridine nucleotide transhydrogenase, the occurrence of an energy-linked NADH----NADP+ transhydrogenation in the adult cestode *Hymenolepis diminuta* was demonstrated.
- The isolated particles displayed rotenone-sensitive NADH utilization and the reversible transhydrogenase, with the NADPH----NAD+ transhydrogenation being more prominent. Although not inhibiting the NADPH----NAD+ reaction, rotenone, but not oligomycin, inhibited the catalysis of NADH----NADP+ transhydrogenation.
- In the presence of rotenone, Mg2+ and ATP are stimulated by more than 3-fold NADH----NADP+ transhydrogenation. This stimulation is ATP specific and abolished by EDTA or oligomycin.
- Succinate was essentially without effect on the NADH----NADP+ reaction. These data demonstrate the occurrence of an energy-linked transhydrogenation between NADH and NADP+ with energization resulting from either electron transport-dependent NADH oxidation or ATP utilization via the phosphorylating mechanism in accordance with the preparation of "phosphorylating" particles.
- This is the first demonstration of an energy-linked transhydrogenation in the parasitic helminths and apparently in the invertebrates generally.

Electron transport

- The mechanism of electron transport in mammal involves the reoxidation of reduce co-factors by system of enzymes and electron carrier. The later includes **Flavoprotein dehydrogenase, ubiquinone**, **cytochrome B, C, A** and finally **cytochrome oxidase** which transport the electron to oxygen with the formation of water. During electron transfer ATP formation occur at the sites.
- Electrons are transported along the chain of electrons either to cytochrome oxidase to alternative oxidase or to fumarate reductase system.
- The production of respiration is either water the potentially dangerous hydrogen peroxide (renewed either by catalase or peroxide both of which are found in helminth in high activities) or succinate and its derivatives by anaerobic with the mammalian system. It is presumed that there are three proton translocating sites for ATP synthesis.
- All parasitic helminths show a measurable uptake of oxygen while some of the oxygen is no doubt used for synthetic reaction. A part of it is used in respiration.
- It is widely accepted that while many adult helminth possesses low activities of classical electron transport system. Their specialised electron transport system is anaerobic system.
- In addition, they may also be branched and therefore possessed several terminal oxidases. In some helminths for example there maybe 3 branches -one with **cytochrome oxidase**, second with a **B type cytochrome** and third which generate **hydrogen peroxide**.

Probable question:

1. Write a note on energy metabolism in helminths.
2. Differentiate aerobic and anaerobic energy metabolism.
3. What are the key strategies employed by helminths for energy conservation, and how do these strategies contribute to their survival and adaptation in various host environments?
4. Comment on fermentation. State the utility of this.
5. Comment on homofermentation.
6. What is heterofermentation?
7. Describe lactic acid fermentation.
8. Discuss about alcohol fermentation.
9. Describe the steps of acetic acid fermentation. What is the utility of acetic acid fermentation?
10. Describe the steps of butyric acid fermentation.
11. Comment on homolactate fermentation.
12. Role of Malate dismutation in fermentation.
13. Discuss the role of mitochondrial malate dehydrogenase, decarboxylating and transhydrogenase activities of adult *Hymenolepis microstoma.*
14. Describe electron transport in parasitic helminths.

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Unit- V

Principles of immunity in relation to virus

Objectives:

In this section we will discuss on principles of immunity in relation to virus.

Introduction:

Viruses as obligate intracellular parasites require their host to replicate them and to facilitate their spread to others. In humans, viral infections are rarely lethal, even if they are highly cytolytic to individual cells. Mortality commonly occurs when viruses jump species (such as Ebola or human immunodeficiency virus (HIV)), when the virus undergoes a major antigenic change (i.e., influenza viruses), or when host immunity is compromised. HIV represents one of the most dramatic human examples of an exotic virus that kills its host. However, HIV kills slowly, providing ample time to spread to new hosts and an effective strategy for persistence in the species. Death or dire consequences following virus infection in mammals with inadequate immunity are well illustrated by observations that foetuses or neonates, especially if deprived of passive immunity, succumb to many agents well tolerated by normal adults. The increasing wealth of immunological tools, such as transgenic animal models and major histocompatibility complex (MHC) tetramers, have provided sensitive methods for defining the relevance of immune mechanisms for antiviral defence. In most situations, defence against viruses involves multiple immune components, and the impact of a single mechanism varies greatly according to the method by which individual viruses enter, replicate, and spread within the host. In this chapter, we highlight the principal means by which the host achieves immunity following infection by viruses.

Viral entry and infection:

Access to target tissues presents numerous obstacles for entry and infection by most human viruses. Most effective of these are the mechanical barriers provided by the skin and mucosal surfaces, as well as the chemically hostile environment of the gut (Fig 1). A number of common human viral pathogens enter through the gastrointestinal tract, including rotavirus, enteric adenoviruses, and hepatitis A virus (HAV). These are usually spread via person-to-person contact or contaminated food and water. Respiratory infections caused by influenza viruses, rhinoviruses, coronaviruses, measles virus, varicella-zoster virus (VZV), and respiratory syncytial virus (RSV) are often spread by aerosol transmission, as well as person-to-person contact. Many of the herpes viruses target the skin or the mucosae, such as herpes simplex virus (HSV) and VZV. HSV in particular can infect oral and genital mucosa, the eye, and the skin through small cuts and abrasions. Other herpes viruses, such as Epstein–Barr virus (EBV) and cytomegalovirus (CMV), target the mucosa. CMV can also spread vertically from mother to baby or rarely

via blood transfusions. Human papillomavirus (HPV) targets skin and mucosa and causes warts and may transform cells, inducing cancers such as cervical cancer. Viruses such as West Nile virus and Semliki Forest virus may also enter through the skin via insect vectors. HIV and hepatitis B virus (HBV) are commonly spread via sexual contact. HIV, HBV, and hepatitis C virus (HCV) can also infect humans via direct entry into the bloodstream via transfusions or contaminated needles.

Most human viruses replicate only in certain target tissues, this being mainly the consequence of viral receptor distribution. Many viruses use two receptors, such as the use of the CD4 co-receptor and CCR5 by HIV. After attachment to a cellular receptor, viruses may fuse with the cell membrane or be endocytosed and then gain entry into the cytoplasm or nucleus by fusing with the vesicular membrane (enveloped viruses such as HSV and HIV), or translocate across the cell membrane or induce lysis of the endocytic vesicle once in the cytoplasm (nonenveloped viruses such as Norwalk virus and poliovirus). Viruses then utilize host cell machinery and specialized virally encoded proteins to replicate rapidly within the cell. Once they have multiplied within the cell, many viruses induce cytolysis in order to facilitate release of new infectious virions (the poxviruses, poliovirus, and herpes viruses, for example). Other viruses are released from infected cells by budding through the cell membrane in the absence of cell death (i.e., HIV and influenza virus). Having entered the body, however, viruses encounter numerous innate defences and activate the components of adaptive immunity. The latter usually assures that clinical disease, if not infection, will not become evident. Successful exploitation of these defences through the use of vaccines remains a central challenge for many human viruses, particularly those that cause chronic infections such as HIV and HCV.

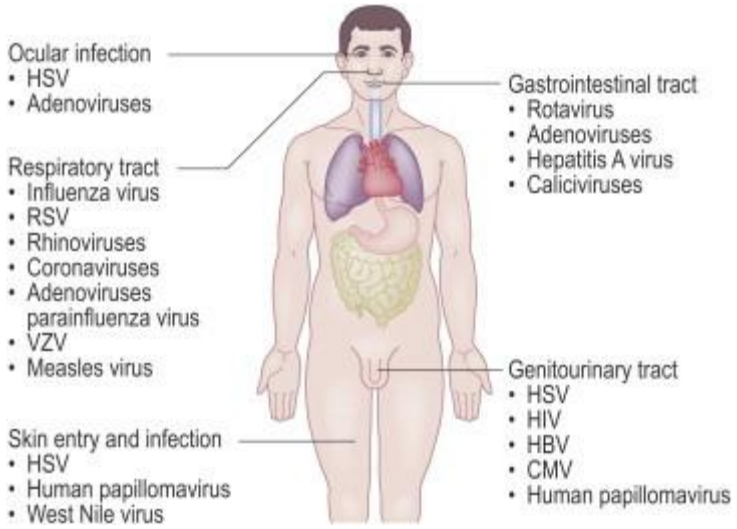

Figure1: Common routes of entry and infection for human viral pathogens. CMV, cytomegalovirus; HBV, hepatitis B virus; HIV, human immunodeficiency virus; HSV, herpes simplex virus; RSV, respiratory syncytial virus; VZV, varicella-zoster virus.

Innate immunity to viruses:

Viral infection induces an extensive array of defence mechanisms in the host. Innate defences come into play to block or inhibit initial infection, to protect cells from infection, or to eliminate virus-infected cells, and occur well before the onset of adaptive immunity. The innate immune defences are initiated via pathogen recognition receptors of the Toll-like receptor (TLR) family or a family of DExD/H box RNA helicases. These cellular sensors promote the expression of type I (α/β) interferons (IFN) and a variety of IFN-stimulated genes and inflammatory cytokines. TLRs are cell surface or endosomal membrane-bound proteins expressed by numerous cells including dendritic cells (DC), macrophages, lymphocytes, and parenchymal cells. Expression of TLRs is largely inducible in most cell types, though some (TLR7/8/9) are constitutively expressed at high levels by specialized plasmacytoid DC for rapid IFN production. Different TLR molecules recognize specific viral products such as single- and double-stranded RNA (TLR 3 and TLR7/8, respectively) or double-stranded DNA (TLR9). The more recently described non-TLR RNA helicases, retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated gene (MDA-5), mediate cytoplasmic recognition of viruses.[6] It is thought that other cytoplasmic sensors of viruses are also likely to exist such as the recently discovered cytosolic dsDNA sensor DAI (DNA-dependent activator of IFN).

The innate defence system consists of multiple cellular components and many specialized proteins. The longest-known and best-studied antiviral proteins are the α/β IFNs, which act by binding to the type I IFN receptor and result in the transcription of more than 100 IFN-stimulated genes. One consequence of this 'antiviral state' is the inhibition of cell protein synthesis and the prevention of viral replication. Type I IFNs also activate natural killer (NK) cells and induce other cytokines such as interleukin (IL)-12 that promote NK responses. NK cells produce proinflammatory cytokines, they can kill infected cells and interact with DC, and are an important component of innate defence against viruses. NK cells are regulated by an array of activating and inhibitory receptors whose expression and function are just beginning to be understood. Uninfected cells are usually protected from NK cell cytolysis as they deliver negative signals such as high expression of MHC molecules. In contrast, virus-infected cells are killed either because they deliver positive signals or because they lack adequate MHC-negative signals. The NK defence system appears important against some herpes viruses, which downregulate MHC expression in the cells they infect. NK cells are also important in resistance to mouse and human cytomegalovirus, and possibly to HIV, influenza virus, and Ebola viruses. A distinct NK cell population, NKT cells, may provide some antigen-specific innate immune protection against certain viruses. Many other leukocytes are involved in innate defence, including macrophages, DC, neutrophils and perhaps T cells expressing γδ T-cell receptors for antigen.

In addition to IFN-α/β, several other host proteins function in antiviral defence. These include natural antibody, which may play a role in defence against some virus infections, as well as the complement proteins. Some viruses may be directly inactivated

by complement activation or be destroyed by phagocytic cells that bind and ingest complement-bound virions. Several cytokines and chemokines induced by virus infection also play a role in defence. These include the cytokines TNF-α, IFN-γ, IL-12, IL-6, and chemokines such as MIP-1α. In particular, IL-12 is a potent inducer of IFN-γ from NK cells. Inflammatory chemokines may also play an important role in innate antiviral defence by orchestrating macrophage, neutrophil, DC, and NK responses at the site of infection. Not only are these components of innate immunity involved in mediating initial protection against viruses, several components (such as the TLRs and type I IFN and IL-12) serve to shape the nature and effectiveness of the subsequent adaptive response to viral antigens.

Adaptive immunity to viruses:

Innate immunity generally serves to slow, rather than stop, viral infection, allowing time for the adaptive immune response to begin. The two major divisions of adaptive immunity, antibody and T-cell-mediated, are mainly directed at different targets. Antibodies usually function by binding to free viral particles, and in so doing block infection of the host cell. In contrast, T cells act principally by recognizing and destroying virus-infected cells. As all viruses replicate within cells and many of them spread directly between cells without re-entering the extracellular environment, resolution of infection is reliant more on T-cell function than on antibody. Antiviral antibody, however, does assume considerably more importance as an additional immune-protective barrier against reinfection. It is the presence of antibody at portals of entry – most often mucosal surfaces – that is of particular relevance to influenza and HIV infections. Accordingly, vaccinologists try to design vaccines that optimally induce mucosal antibody.

Initiation of adaptive immunity is closely dependent upon early innate mechanisms that activate antigen-presenting cells (APC). APC and lymphocytes are drawn into lymphoid tissues by chemokine and cytokine signals and retained there for a few days in order to facilitate effective interactions between these cells. The architecture of the secondary lymphoid tissues supports the coordinated interactions between cells of the adaptive immune system through a network of supportive stromal cells and local chemokine gradients. The induction events occur in lymph nodes draining the infection site, or in the spleen if virus enters the bloodstream. The passage of viral antigens to lymph nodes usually occurs in DCs. Some viruses are able to compromise the function of APC, such as HSV and measles virus, which can inhibit DC maturation.

B-cell activation occurs following antigen encounter in the B-cell follicles, and possibly the T-cell zones, in the spleen or lymph nodes. Some activated B cells become short-lived plasma cells while others move the edges of the B-cell follicles and interact with antigen-specific helper CD4 T cells via presentation of antigenic peptides on B-cell MHC class II molecules. These activated B cells initiate germinal centre (GC) reactions, which ensure somatic hypermutation and affinity maturation for the selection of high-affinity, antibody-producing long-lived plasma cells as well as memory B cells. Recent advances have greatly improved our understanding of the signals that control the

generation of these important B-cell subsets, particularly at the molecular level. We now know that upregulation of the transcription factors Blimp-1, XBP-1, and IRF-4 dictates plasma cell formation, whereas Pax-5 expression delineates B cells destined for GC reactions and the memory B-cell lineage.

Antibody binding to epitopes expressed by native proteins at the surface of free virions usually blocks viral attachment or penetration of target cells. Sometimes the consequence is viral lysis (with complement proteins also involved), opsonization, or sensitization for destruction by Fc receptor-bearing cells that mediate antibody-dependent cellular cytotoxicity (ADCC). Occasionally, however, Fc receptor binding of antibody-bound virus may facilitate infection and result in more severe tissue damage. This occurs in dengue fever and may happen in some instances in HIV infection.

As indicated previously, antibody may function most effectively to prevent reinfection, especially at mucosal surfaces. The antibody involved in humans is predominantly secretory immunoglobulin A (IgA), but serum-derived IgG may also be protective, particularly in sites such as the vaginal mucosa.[15] Both antibody isotypes act mainly to block infection of epithelial cells, although in some instances the antibody may transport antigen from within the body across epithelial cells to the outside. Mucosal antibody persists for a much shorter period than does serum antibody, which explains in part why immunity to mucosal pathogens is usually of much shorter duration than is immunity to systemic virus infections.

Like B-cell responses, T-cell responses to viral infections also begin within the lymphoid tissues. Specific CD8 cytotoxic T lymphocyte (CTL) precursors recognize antigen in the context of MHC class I–peptide antigen complexes on professional APC, such as DC. The CD8 T cells become activated, proliferate, and differentiate into effectors. Expansion of these naïve antigen-specific precursors is considerable, often exceeding 10 000-fold, and results in an effector population that can account for 40% or more of a host's total CD8 T-cell population. Various factors, including antigen and APC, co-stimulatory molecules (such as CD28 and 4-1BB) and inflammatory cytokines (such as IFN-α/β and IL-12) are required to program the development of functional effector lymphocytes. The CTL effectors enter the efferent lymph and bloodstream and access almost all body locations, including both primary and subsequent sites of infection. However, effectors do not stay activated for long once the virus is cleared, and approximately 95% die by a process termed activation-induced cell death. Following this contraction phase, the remaining cells differentiate into memory cells, which remain as a more or less stable population in the host for many years. They represent an expanded pool of CTL precursors that can be activated upon secondary encounter with antigen, and provide enhanced protection upon reinfection with the same virus.

T-cell immunity against a particular virus commonly involves both CD4 and CD8 T-cell subsets. Both CD4 and CD8 T cells recognize peptides derived from viral antigens bound to surface MHC proteins (class II and class I, respectively). Complexes of viral peptides bound to MHC class II proteins are generated by APC from scavenged and

processed virus-infected cells or viral particles. Antigen–MHC class I complexes are expressed on the surface of infected cells, and antigen can also be transferred to APC from infected cells by a process known as cross-priming. Recent experiments in mice have also demonstrated a role for transfer of antigen between DC as they migrate from infected tissues to the lymphoid tissues. Curiously, although many peptides derived from viral proteins have an appropriate motif that permits MHC binding, the majority of CD8 T cells, and possibly CD4 T cells, are often specific for a few immunodominant epitopes.

During the past few years there have been major advances in the techniques to quantify antigen-specific T-cell responses. The most revolutionary of these has been the use of MHC class I and class II tetramers to directly visualize antigen-specific CD8 and CD4 T-cell responses, respectively. Many recent studies have used MHC class I tetramers to analyse virus-specific CD8 T-cell responses both in animal models and in humans. These studies demonstrated the significant size of CD8 T-cell responses to viruses and that the majority of the activated CD8 T cells seen at the peak of the response are virus-specific.

CTL function by recognizing virus-infected cells and killing them. This often involves perforins and cytotoxic granules containing granzymes. Effector CTL can also induce death in target cells following engagement of Fas ligand on the CTL with Fas on target cells. Both pathways lead to apoptosis of the target cell, involving the degradation of nucleic acids, including those of the virus. Alternatively, CD8 T cells also mediate defence through the release of various cytokines following antigen recognition. Some of the cytokines and chemokines most highly produced by CTL include IFN-γ, TNF-α, lymphotoxin-α, and RANTES. These cytokines can have multiple antiviral effects on infected cells and the cells around them, including purging of virus from infected cells without killing the cell. This is particularly important for viruses like HSV which infects non-rejuvenating cells such as nerve cells.

CD4 T cells are also involved in antiviral defence. They are important, though not always essential, for controlling infections such as HSV, influenza virus, HIV, and many others. CD4 T cells participate in antiviral immunity in several ways. First, the subset acts as helper cells for the induction of both antiviral antibodies and CD8 T-cell responses to most virus antigens. CD4 T cells also function as antiviral effector cells, and generate stable memory cell populations similar to those of CD8 T cells. The differentiation of CD4 T cells into effectors occurs in a manner very similar to that with CD8 T cells. At present less is known about the size and specificity of CD4 T-cell responses, but reports indicate that CD4 T cells undergo less expansion during virus infections, resulting in an effector pool smaller than that observed with CD8 T cells. CD4 T cells are activated by recognizing viral peptides. However, these are larger than those involved in CD8 T-cell recognition and are associated with class II MHC molecules present on more specialized cells such as APC. Thus, CD4 T cells rarely recognize viral epitopes present on cells as a consequence of viral gene expression within that cell, dictating their function as helper cells for B cells and CD8 T cells, and as producers of cytokines for help and viral clearance.

In some instances, CD4 T cells can perform cytotoxic functions, though not as effectively as CD8 CTL. More commonly, however, effector CD4 T cells act by synthesizing and releasing numerous cytokines following their reaction with antigen. Subsets of CD4 T effectors produce different groups of cytokines. The type most often involved in antiviral defence are designated T-helper 1 (Th1) cells, and primarily produce IFN-γ, LTα, TNF-α, and IL-2 to help orchestrate the inflammatory response and act directly or indirectly in antiviral defence. Conversely, Th2 effectors produce an array of cytokines that may downregulate the protective function of Th1 cells, such as IL-4, IL-5, and two anti-inflammatory cytokines, IL-10 and transforming growth factor-β (TGF-β). Th2 T cells play a protective function against some parasite infections, though in some virus infections an exuberant Th2 response may be associated with immunopathology or impaired immunity. Indeed, blocking the Th2 cytokine IL-10 was recently shown to assist in the clearance of chronic viral infection. Additionally, an IL-17-producing subset of effector CD4 T cells has also been described (Th17), with potential roles in immune pathogenesis.

Immune evasion and immunity to chronic viral infections:

Many, if not all, viruses employ evasion strategies to circumvent aspects of the immune system, allowing them time to replicate further or escape detection. One such mechanism may involve killing or infecting APC. Viruses may also delay or prevent apoptosis induced by CTL within infected cells. Other viral evasion measures aimed at the CD8 T-cell-mediated antiviral defence system serve to inhibit antigen processing, thereby minimizing effector CTL induction. Many viruses also downregulate MHC molecules on the surface of infected cells to escape CTL killing. In addition, viruses may produce various mimics or modulators/inhibitors of cytokines, chemokines, or other components of the immune system or their receptors. Viruses also resort to antigenic hypervariability to escape antibody or T-cell recognition. This can occur during transmission from host to host (i.e., influenza virus), or within hosts during chronic infection through the generation of viral escape mutants (i.e., HIV).

The success of many viral pathogens rests in their ability to subvert the host immune response. The most successful human viruses can escape the immune system and persist for the life of the host. Two well-studied examples of this are CMV and EBV. T-cell responses to these viruses are prominent and readily detectable in people, yet the immune system is unable to clear either pathogen completely. However, these viruses generally remain undetectable in immune-competent individuals. Other viral infections, such as those caused by the herpes viruses HSV and VZV, are marked by periods of latency, where no virus can be detected. Yet periods of viral reactivation, often triggered by stress, can lead to episodes of disease. These are controlled by the immune response which plays a central role in controlling herpes virus latency.

Many of the most medically important human viruses are associated with persistent viremia. These include chronic infections such as HIV, HCV, HBV, and human

T-lymphotropic virus (HTLV), among others. Such viral infections are marked by high levels of persisting antigen and can result in skewed T-cell immunodominance hierarchies, altered tissue localization of immune cells, and severely impaired T-cell function. This altered T-cell function is hierarchical and appears to correlate directly with antigen levels, resulting in functional T-cell defects ranging from reduced cytokine production and altered proliferative capacity (exhaustion) to death (deletion) of the responding T cells. This is in stark contrast to normal memory T-cell development which occurs in the absence of persisting antigen. Recent studies have demonstrated that signalling through programmed death (PD)-1 on effector CTL causes exhaustion during chronic infections. This pathway may be essential for preventing excessive immunopathology by effector T cells, yet appears to contribute directly to failed immunity to HIV infection, and other chronic human viral infections. These studies implicate this pathway as a potential therapeutic target.

Probable questions:

1. State the entry points of virus in human body.
2. Describe the innate and adaptive immunity of human body against viruses.
3. Why certain viruses are regarded as successful pathogens?

Suggested reading and references:

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Unit- VI

Principles of immunity in relation to bacteria

Objectives:

In this section we will discuss on principles of immunity in relation to bacteria.

Introduction:

Infectious diseases are a leading cause of morbidity and mortality worldwide and are a major challenge for the biomedical sciences. Improved sanitary conditions, clean water supplies and vector control are by far the most effective measures to reduce the incidence of infectious disease. However, the development of vaccines and therapeutics is also important, and this requires an understanding of the host immune system. Recently, much progress has been made towards discovering the mechanisms of microbial pathogenesis and host–microbe symbiosis. And knowledge about the immune system has also been steadily increasing. Yet many challenges remain, perhaps the most daunting being effective vaccine development. Indeed, it is not known how to elicit protective immunity against most pathogens in a safe and practical manner. To achieve this and other goals, such as the safe and efficient blockade of autoimmune and allergic immune responses, further developments in basic research are clearly required.

Host–microbe interactions:

All metazoan hosts exist in close association with microbial communities that colonize them. The 'rules of engagement' of host–microbe interactions are incompletely understood, and defining these is clearly important for understanding the evolution and functioning of the immune system.

The host as a set of niches colonized by microorganisms:

Mammalian hosts provide a number of niches that can be colonized by microorganisms, including the skin, intestine, upper and lower respiratory tract, urogenital tract and internal organs. Some of these niches (for example, the colon and the skin) are colonized constitutively by an endogenous microbiota. Other niches (for example, the internal organs and the lower respiratory tract) are normally kept sterile (in an immunocompetent host). The effect of microbial colonization on host fitness depends on the microbial adaptation strategy. These effects can be positive, as is the case for the many intestinal bacteria that provide a range of benefits to the host. In other cases, microbial colonization can be detrimental to the host, and these colonizing bacteria are referred to as pathogens. Such negative effects can depend on the status of the host's immune system: for example, certain pathogens, known as opportunistic pathogens, affect only immunocompromised individuals.

Virulence factors:

The adaptation of bacteria to particular host niches depends on the activity of various adaptation factors; for pathogens, these are known as virulence factors. Adaptation factors are often encoded on mobile genetic elements (for example, plasmids and genomic islands) that can be transmitted within and between bacterial species, although there are important exceptions (for example, in *Mycobacterium* spp.). The role of virulence factors is to enable adaptation to the specific environments in the host niches and to promote transmission to another host. In this way, some common themes of virulence-factor activity (and therefore pathogenicity) can be identified. Depending on the niche that they colonize, bacterial pathogens have virulence factors that allow a range of activities: penetration of surface epithelia, attachment to cell surfaces and/or the extracellular matrix, invasion of intracellular compartments, acquisition of iron, evasion of host-defence mechanisms and transmission to another host. Different strategies of pathogenic microbial adaptation are associated with varying degrees of damage to the tissues of the host. Regardless of the degree of virulence, at least some symptoms of infectious disease are side-effects of microbial adaptation to host niches.

Recognition of microorganisms by the immune system:

The detrimental effects of microbial infections led to the evolution of a variety of host-defence mechanisms. In jawed vertebrates, there are two types of defence: innate and adaptive (also known as acquired). The main distinction between these is the receptor types used to recognize pathogens. Innate immune recognition is mediated by pattern-recognition receptors (PRRs), which are germline encoded, and each receptor has broad specificities for conserved and invariant features of microorganisms. By contrast, adaptive immune recognition is mediated by antigen receptors: the genes encoding these receptors are assembled from gene segments in the germ line, and somatic recombination of these segments enables the generation of a diverse repertoire of receptors with random but narrow specificities. Antigen receptors are clonally distributed on T and B lymphocytes, which allows clonal selection of pathogen-specific receptors and is the basis for immunological memory. (That is, each lymphocyte expresses antigen receptors of a single specificity, so only specific populations of lymphocytes are selected to expand in response to a pathogen.) Therefore, the innate immune system and the adaptive immune system deal with the molecular diversity of pathogens in fundamentally different ways.

Innate immune system:

Innate immune recognition (also known as pattern recognition) is based on the detection of molecular structures that are unique to microorganisms. Pattern recognition is unusual in that each host receptor (PRR) has a broad specificity and can potentially bind

to a large number of molecules that have a common structural motif or pattern. The targets of PRRs are sometimes referred to as pathogen-associated molecular patterns (PAMPs), although they are present on both pathogenic and non-pathogenic microorganisms. PAMPs are well suited to innate immune recognition for three main reasons. First, they are invariant among microorganisms of a given class. Second, they are products of pathways that are unique to microorganisms, allowing discrimination between self and non-self molecules. Third, they have essential roles in microbial physiology, limiting the ability of the microorganisms to evade innate immune recognition through adaptive evolution of these molecules. Bacterial PAMPs are often components of the cell wall, such as lipopolysaccharide, peptidoglycan, lipoteichoic acids and cell-wall lipoproteins. An important fungal PAMP is β-glucan, which is a component of fungal cell walls. The detection of these structures by the innate immune system can signal the presence of microorganisms. The recognition of viruses also partly follows this principle. However, because all viral components are synthesized within host cells, the main targets of innate immune recognition in this case are viral nucleic acids. Discrimination between self (host) and viral nucleic acids occurs on the basis of specific chemical modifications and structural features that are unique to viral RNA and DNA, as well as on the cellular compartments where viral (but not host-derived) nucleic acids are normally found (discussed later). Nevertheless, this discrimination is not perfect and can fail under certain conditions, which can result in the development of autoimmune diseases.

An important aspect of pattern recognition is that PRRs themselves do not distinguish between pathogenic microorganisms and symbiotic (non-pathogenic) microorganisms, because the ligands of the receptors are not unique to pathogens. Yet, despite humans being colonized by trillions of symbiotic bacteria, homeostasis is somehow maintained under normal conditions. Furthermore, innate immune recognition of symbiotic microorganisms has an important role in maintaining intestinal homeostasis. And dysregulation of these interactions can lead to the development of inflammatory bowel disease and other disorders.

PRRs and their functions:

There are several functionally distinct classes of PRR. The best characterized class is Toll-like receptors (TLRs). TLRs are transmembrane receptors that recognize viral nucleic acids and several bacterial products, including lipopolysaccharide and lipoteichoic acids. The full range of TLR functions in antimicrobial defence has not yet been determined, but TLRs are known to elicit inflammatory and antimicrobial responses after activation by their microbial ligands.

In terms of the inflammatory response, TLRs activate tissue-resident macrophages to produce pro-inflammatory cytokines, including tumour-necrosis factor (TNF), interleukin-1β (IL-1β) and IL-6, which coordinate local and systemic inflammatory responses. TNF and IL-1β, in turn, activate the local endothelium to induce

vasodilation and increase the permeability of the blood vessel, allowing serum proteins and leukocytes to be recruited to the site of infection. In addition, an increase in the amount of tissue factor (also known as coagulation factor III) on the endothelium leads to a local coagulation cascade that helps to prevent microbial dissemination through the blood. Furthermore, IL-1β, together with IL-6, activates hepatocytes to produce acute-phase proteins, including collectins and pentraxins. These proteins, in turn, activate complement and opsonize pathogens for phagocytosis by macrophages and neutrophils. In this way, TLRs indirectly elicit an antimicrobial response.

TLRs also directly trigger such a response, by inducing macrophages to produce antimicrobial proteins and peptides. In mouse macrophages, the activation of TLRs results in transcription of the gene encoding inducible nitric-oxide synthase (iNOS; also known as NOS2), which has an important role in antimicrobial defence. Interestingly, iNOS is not produced in response to the activation of TLRs on human macrophages. Instead, human keratinocytes synthesize vitamin D, which is crucial for antimicrobial activity, partly because of vitamin-D-receptor-dependent induction of the gene encoding the antimicrobial peptide LL37 (also known as CAMP). Sunlight (a source of UVB radiation) is necessary for vitamin D synthesis, so the difference in vitamin D requirement for antimicrobial defence might reflect the nocturnal and diurnal lifestyles of mice and humans, respectively.

Another well-characterized PRR is dectin 1, a transmembrane receptor that binds to β-glucan and is present on dendritic cells and macrophages. Dectin 1 is a member of a large family of C-type lectins, many of which are present on these same cell types but have unknown functions. Dectin 1 contains an atypical immunoreceptor tyrosine-based activation motif (ITAM) that engages the protein tyrosine kinase SYK, thereby activating a signalling pathway that involves CARD9, Bcl-10 and MALT1. This PRR has an important role in antifungal defence, being involved in the phagocytosis of fungal pathogens, the induction of an antimicrobial response (such as activation of NADPH oxidase) and the production of cytokines.

In addition to transmembrane receptors on the cell surface and in endosomal compartments, there are intracellular (cytosolic) receptors that function in the pattern recognition of bacterial and viral pathogens. These include NLRs and the intracellular sensors of viral nucleic acids RIG-I (retinoic-acid-inducible gene I; also known as DDX58), MDA5 (melanoma differentiation-associated gene 5; also known as IFIH1) and DAI (DNA-dependent activator of interferon-regulatory factors; also known as ZBP1). NLRs are a large family of about 20 intracellular proteins with a common protein-domain organization but diverse functions. All NLRs contain a nucleotide-binding oligomerization domain (NOD) followed by a leucine-rich-repeat domain at the carboxy terminus. At the amino terminus, NLRs have one of three domains and are thereby categorized into three subfamilies: a caspase-recruitment domain (CARD), present in proteins in the NOD subfamily; a pyrin domain, in the NALP subfamily; or a BIR domain (baculoviral inhibitor-of-apoptosis-protein repeat-containing domain), in the NAIP

subfamily. The N-terminal domains engage distinct signalling pathways, which define the functional properties of the family members.

The proteins of the NOD subfamily — NOD1 and NOD2 — are both involved in sensing bacterial peptidoglycans, although they recognize structurally distinct peptidoglycan fragments. The sensing of peptidoglycan by NOD1 or NOD2 triggers the production of pro-inflammatory cytokines and chemokines and the recruitment of neutrophils to the site of infection. In addition, these NOD proteins contribute to the initiation of the adaptive immune response, and mutations in NOD2 have been implicated in the pathogenesis of Crohn's disease. NOD2 is also crucial for the production of antimicrobial peptides known as defensins by Paneth cells (which are present in the small intestine), and NOD proteins can presumably activate antimicrobial responses in other cell types.

The NALP subfamily of NLRs has 14 members, and at least some of these are involved in the induction of the inflammatory response mediated by the IL-1 family of cytokines, which includes IL-1β, IL-18 and IL-33. These cytokines are synthesized as inactive precursors that need to be cleaved by the pro-inflammatory caspases: that is, caspase 1, caspase 4 and caspase 5 in humans, and caspase 1, caspase 11 and caspase 12 in mice. These caspases are activated in a multi-subunit complex called the inflammasome. There are several types of inflammasome, categorized according to their composition and the involvement of a particular NALP or NAIP. The individual inflammasomes are activated in response to a variety of bacterial infections, by mechanisms that have been poorly defined. Why IL-1-family members are activated by such an elaborate mechanism is puzzling. Unlike other pro-inflammatory cytokines, IL-1β production is regulated by two distinct signals: TLR-induced transcription and inflammasome-mediated processing of the precursor protein. It is possible that, in addition to IL-1-family members, the inflammasomes process antimicrobial peptides or proteins that have not yet been characterized. NALPs might also contribute to antimicrobial defence by inducing the apoptosis of infected cells. Whether they can also directly induce the expression of antimicrobial genes is unknown.

Intracellular recognition of viral infections is mediated by two types of viral nucleic-acid sensor. Viral RNA in the cytosol is detected by the RNA-helicase-family proteins RIG-I and MDA5, whereas viral DNA is detected by the recently identified protein DAI. RIG-I and MDA5 recognize different types of viral RNA: single-stranded RNA containing 5′ triphosphate and double-stranded RNA, respectively. These structural features are absent from cellular (host) RNAs, which contain either short hairpin structures, in the case of transfer RNAs and ribosomal RNAs, or a 5′-cap structure, in the case of messenger RNA. These structural differences allow discrimination between viral and self RNAs. Activation of RIG-I or MDA5 results in the production of type I interferons (IFNs; IFN-α and IFN-β) and thereby the induction of antiviral immunity. Interestingly, a crucial adaptor involved in RIG-I and MDA5 signalling is associated with the mitochondrial membrane, but the reason for this is unclear at present. The details of how viral DNA is recognized in the cytosol, and the signalling pathways induced by the

engagement of DAI, are not yet known. It is, however, clear that the RNA-sensing pathway and the DNA-sensing pathway converge on the protein kinase TBK1 (TANK-binding kinase 1) and the transcription factor IFN-regulatory factor 3. Type I IFNs are therefore elicited by the engagement of either type of sensor. This results in antiviral immune responses in both cases, through inducing the expression of numerous IFN-inducible genes, the products of which have a broad range of antiviral activities.

Adaptive immune system:

Adaptive immune recognition is mediated by two types of antigen receptor: T-cell receptors and B-cell receptors. The genes encoding antigen receptors are assembled from variable and constant fragments through recombination-activating gene (RAG)-protein-mediated somatic recombination, a process that yields a diverse repertoire of receptors. This diversity is further increased by additional mechanisms, such as non-templated nucleotide addition, gene conversion and (in the case of B cells) somatic hypermutation, generating a highly diverse repertoire of receptors with the potential to recognize almost any antigenic determinant in a specific manner.

There are two types of lymphocytes that express antigen receptors: conventional lymphocytes and innate-like lymphocytes. In the case of conventional lymphocytes — that is, conventional T cells (most αβ T cells) and B cells (also known as B2 cells) — antigen receptors are assembled essentially at random. By contrast, for innate-like lymphocytes — that is, B1 cells, marginal-zone B cells, natural-killer T cells and subsets of γδ T cells — the diversity of antigen receptors is restricted and not entirely random. Their specificities are skewed towards a predefined set of ligands.

The specificities of the receptors of conventional lymphocytes are not predetermined and neither, therefore, is the site where these cells might encounter their cognate antigen (that is, the antigen specifically recognized by the receptor) or the effector response they need to elicit on activation. So these lymphocytes circulate through the lymph nodes, which drain most of the body's tissues and organs, and the spleen, which filters the blood, until they encounter an antigen that they are specific for. Microbial antigens are taken up by antigen-presenting cells in the peripheral tissues and are delivered to the lymph nodes or spleen through the lymph or blood, respectively, where they are recognized by conventional lymphocytes. Because the specificity of each antigen receptor is not directly linked to the origin of the antigen, conventional lymphocytes need to be able to differentiate into several types of effector cell, depending on the class of pathogen they recognize (discussed later). The differentiation of conventional lymphocytes into a particular effector-cell type and their localization to the site of infection are regulated by the instructions provided by the innate immune system, generally in the form of cytokines and chemokines, respectively.

There are two types of conventional αβ T cell: T-helper (T_H) cells, which are marked by the co-receptor CD4 on the cell surface; and cytotoxic T cells, which express

CD8. These cells recognize antigenic peptides bound to major histocompatibility complex (MHC) class II and class I molecules, respectively. Conventional B cells can recognize almost any antigen by binding to a specific three-dimensional molecular determinant (or epitope).

Innate-like lymphocytes differ from conventional lymphocytes in several important ways. Although the antigen receptors of innate-like lymphocytes are assembled in a similar manner to those of conventional lymphocytes, their assembly process is not entirely random. Receptor diversity is biased towards a characteristic set of specificities for each subset of innate-like lymphocytes. Accordingly, the effector functions of these lymphocytes and the sites where they reside are often predetermined. The effector responses of innate-like lymphocytes therefore do not generally require the same types of instruction that are provided by the innate immune system to conventional lymphocytes.

The innate-like B cells known as B1 cells reside in the peritoneal and pleural cavities and produce mainly antibodies of the IgM class with specificities skewed towards some common bacterial polysaccharides and some self antigens. Innate-like T cells recognize non-classical MHC molecules (also known as MHC class Ib molecules), which can present bacteria-specific ligands: for example, bacterial lipids or formylated peptides in the case of the CD1 and H–2M3 families, respectively. In a way, these MHC-like molecules function as PRRs, presenting microbial ligands to specialized T cells. Some non-classical MHC molecules might themselves be ligands for T-cell receptors, without presenting any other molecules. In this case, the expression of these molecules is thought to be inducible by the engagement of PRRs on specific cell types, such as mucosal epithelial cells.

Modules of the innate immune system:

Unlike the adaptive immune system, the innate immune system is not a single entity. It is a collection of distinct subsystems, or modules, that appeared at different stages of evolution and carry out different functions in host defence. Some of the main modules found in mammals and how these function in innate host defence are described in this section.

I. **Mucosal epithelia**

All metazoans have mucosal epithelia, one of the most ancient and universal modules of innate immunity. Together with the skin, the mucosal epithelia are the main interface between the host and the microbial world (including both pathogenic and symbiotic microorganisms). Mucosal epithelia have many important functions in protecting the host from pathogen invasion, as well as in establishing a symbiotic relationship with the human microbiota. Accordingly, mucosal epithelial cells and skin keratinocytes have specialized antimicrobial functions: for example, producing antimicrobial peptides, which limit the viability

and multiplication of pathogens and symbiotic microorganisms that colonize these sites. The production of these antimicrobial molecules is induced by engagement of TLRs and NOD proteins and, presumably, other PRRs. Epithelial cells at the mucosal surface also produce mucins, which help to prevent the attachment and entry of pathogens.

II. **Phagocytes**

The phagocytic uptake of pathogens is crucial for host defence and is carried out by macrophages and neutrophils. These phagocytes are equipped with multiple antimicrobial mechanisms that are activated on initial contact with pathogens. They have a crucial role in defence against both intracellular bacteria and extracellular bacteria, as well as fungal pathogens. Phagocytosis is facilitated by opsonins, which are host products of the acute-phase response and the complement systems, through their ability to bind to both the cell walls of microorganisms and the opsonin receptors present on phagocytes.

III. **Acute-phase proteins and complement**

A variety of secreted proteins that function in the circulation and tissue fluids — acute-phase proteins and the complement system — constitute another module. Acute-phase proteins are secreted by hepatocytes in response to the pro-inflammatory cytokines IL-1β and IL-6, and the serum concentration of acute-phase proteins increases markedly at the early stages of infection. A key component of this response is the secreted PRRs: collectins, ficolins and pentraxins. Their main functions are opsonizing microbial cells for phagocytosis and activating the complement system. Whereas collectins and ficolins initiate the lectin pathway of complement activation, pentraxins activate the classical pathway, which is also induced by antibodies. Complement activation itself has several consequences, including the following: opsonization of pathogens, through the covalent attachment of C3 fragments; recruitment of phagocytes to the site of infection, through the release of proteolytic fragments of C4 and C5 that have chemotactic activity; and direct killing of pathogens, through the formation of the membrane-attack complex, which is the terminal component of the complement cascade.

IV. **Inflammasomes**

Inflammasomes are protein complexes that activate pro-inflammatory caspases. The activation of caspase 1, in particular, is required for processing the IL-1 family of cytokines, including IL-1β, IL-18 and IL-33. These complexes might also process proteins other than pro-inflammatory cytokines. Inflammasomes are activated by the NALP and NAIP subfamilies of NLRs in response to bacterial infections and some forms of cellular stress. IL-1-family cytokines have diverse functions in inflammation and host defence.

V. **Natural killer cells**

Natural killer (NK) cells are specialized in defence against intracellular pathogens, mainly viruses. These cells have two main functions: inducing the apoptosis of infected cells and producing cytokines, particularly IFN-γ. They express two types of receptor, activating and inhibitory, and these receptors recognize their cognate, host-encoded ligands on infected (target) cells. The balance of expression of activating and inhibitory ligands by a target cell is thought to determine whether it is killed or spared by a particular NK cell. The mechanisms that control the production of these ligands are poorly understood but might involve cell-autonomous viral recognition by intracellular sensors of infection or cell-autonomous detection of excessive cellular stress. Recognition of viral infection by the infected cells themselves, through RIG-I or MDA5, and by plasmacytoid dendritic cells, through TLRs, also controls NK-cell activity, by eliciting the production of type I IFNs either directly or indirectly through the expression of IL-15. IL-15 also regulates NK-cell maintenance.

VI. **Type I IFNs and IFN-induced proteins**

Type I IFNs and IFN-induced proteins have a crucial role in defence against viruses. Type I IFNs are produced in response to viral infections, and these proteins trigger the expression of more than 100 genes, the products of which have diverse antiviral activities. Type-I-IFN production can be elicited in two ways: first, by intracellular sensors of infection; and, second, by TLR3, TLR7 and TLR9 (which are located intracellularly, on endosomes). The first mode of production is ubiquitous and occurs in virally infected cells. It results in autocrine or paracrine IFN-mediated signalling, which confers an antiviral state on the infected cell and neighbouring cells. By contrast, the second mode of production involves the engagement of TLR7 or TLR9, which results in specialized type-I-IFN-producing cells, known as plasmacytoid dendritic cells, producing systemic levels of IFN-α. In almost all cases, type I IFNs are produced in response to viral or bacterial nucleic acids. The only exception to this seems to be IFN-β production in response to TLR4 ligands, which are not nucleic acids.

VII. **Eosinophils, basophils and mast cells**

Eosinophils, basophils and their products form a host-defence module involved in protection against multicellular parasites, such as helminths. Mast cells are also a component of this module, although their function is not restricted to protection against parasites. Mast cells reside in mucosal and connective tissues, whereas eosinophils and basophils are recruited to the sites of infection from the circulation. During bacterial infection, mast cells can be activated directly by TLRs. The way in which parasites activate mast cells, basophils and eosinophils is largely unknown. Recently, however, one of the main parasite-associated cell-wall components, chitin, was found to induce eosinophil recruitment. Interestingly, the

main defensive strategy that components of this module use against parasites does not seem to target these pathogens directly (although direct effects do occur). Instead, it is the host tissues, particularly the mucosal epithelia, smooth muscles and vasculature, that are the main targets of the immune response. These tissues are affected by the mediators released by mast cells and basophils in a way that limits the spread of parasites and promotes their expulsion from the host. The function of this module is regulated by several cytokines, including IL-4, IL-5, IL-9 and IL-13.

Innate control of adaptive immune responses:

In addition to direct activation of innate host-defence mechanisms, some PRRs are coupled to the induction of adaptive immune responses. As discussed earlier, conventional lymphocytes (most αβ T cells and B2 cells) express antigen receptors with random specificities and therefore recognize antigens that lack any intrinsic characteristics indicative of their origin. Therefore, conventional lymphocytes require instructions indicating the origin of the antigen they recognize. These instructions come from the innate immune system in the form of specialized signals inducible by PRRs, which can sense infection because of their specificity for products of microbial origin. Therefore, the basic principle of innate control of adaptive immunity is based on establishing an association between the antigens recognized by lymphocytes and the microbial products (that is, PAMPs) recognized by PRRs.

For T cells, this association is interpreted by dendritic cells. Dendritic cells reside in most peripheral tissues, where they monitor the tissue environment for the presence of pathogens by using various PRRs. When a pathogen is encountered by a dendritic cell, it is taken up by phagocytosis, and its protein constituents are processed into antigenic peptides, which are presented at the cell surface by MHC class I and/or class II molecules. For MHC class II molecules, the antigenic peptides selected for presentation derive from the phagosome in which the pathogen was internalized in response to the triggering of TLRs or other PRRs. A similar mechanism might also operate for MHC class I molecules. Therefore, the association between an antigen and a PAMP is established as a result of their presence in the same phagocytosed 'cargo' (for example, a bacterial cell). PRRs also activate dendritic cells, inducing them to produce cytokines and express cell-surface signals and to migrate to the lymph nodes through the lymphatic vessels that drain the site of infection. When these dendritic cells reach the lymph nodes, they present the pathogen-derived antigens, together with PRR-induced signals (cytokines and cell-surface-associated molecules), to T cells. This results in T-cell activation and, in the case of T_H ($CD4^+$) cells, differentiation into one of several types of effector T_H cell.

For B cells, the association between an antigen and a PAMP can be established directly, when the two are physically linked in a single molecule or particle. This presumably occurs through co-engagement of a B-cell receptor and a PRR. In the extreme

case, a TLR ligand (for example, lipopolysaccharide or flagellin) is itself recognized by the B-cell receptor and by a corresponding TLR expressed by a B cell. Antigens of this class, which combine ligands for both innate and adaptive immune recognition, are called T-independent antigens, because they can elicit B-cell responses without 'help' from T_H cells. When an antigen and a PAMP are not physically linked, their association is established through effector T_H cells that have previously been activated by dendritic cells. Antigens of this class (usually proteins) are called T-dependent antigens.

The antigen receptors of innate-like lymphocytes are skewed towards the recognition of microbial products, so the activation of these cells does not require the same elaborate mechanisms as for conventional lymphocytes. Indeed, B1 cells can be activated directly by PRRs and are programmed to produce antibodies with a broad specificity for common bacterial antigens. Innate-like T cells recognize microbial antigens (such as lipids, glycolipids and formylpeptides) presented by non-classical MHC molecules. In certain cases, these cells recognize MHC-like molecules that do not seem to present any antigens but whose expression is inducible by PRRs. In such cases, the production of T-cell-receptor ligands in response to microbial products might be sufficient to signal the presence of infection.

T_H cells can differentiate into several types of effector cell: T_H1, T_H2 and T_H17 cells (Fig. 1). These cells are characterized by the production of distinct sets of cytokines. T_H1 cells produce IFN-γ and activate macrophages and other cell types to trigger defence against intracellular pathogens. T_H1-cell-derived IFN-γ also instructs B cells to produce antibodies of the IgG2 subclass. T_H2 cells are involved in protection against multicellular parasites and produce IL-4, IL-5 and IL-13. These cytokines control the function of eosinophils, basophils and the mucosal epithelia. IL-4 also instructs B cells to produce antibodies of the IgE class, which are important in defence against parasites through their effects on mast-cell and basophil activation. Finally, T_H17 cells produce IL-17, which induces non-haematopoietic cell types, including epithelial cells, to produce chemokines that recruit neutrophils to the site of infection. T_H17-cell responses are involved in protection against extracellular bacteria and fungi. The differentiation of naive T_H cells (which have not previously encountered their cognate antigen) into the three effector-cell lineages, T_H1, T_H2 and T_H17 cells, is controlled by transcriptional master regulators, in this case T-bet, GATA-binding protein 3 (GATA3) and retinoic-acid-receptor-related orphan receptor-γt (RORγt), respectively. The expression of these master regulators is controlled by cytokines produced by antigen-presenting cells (such as dendritic cells) in response to PRR activation.

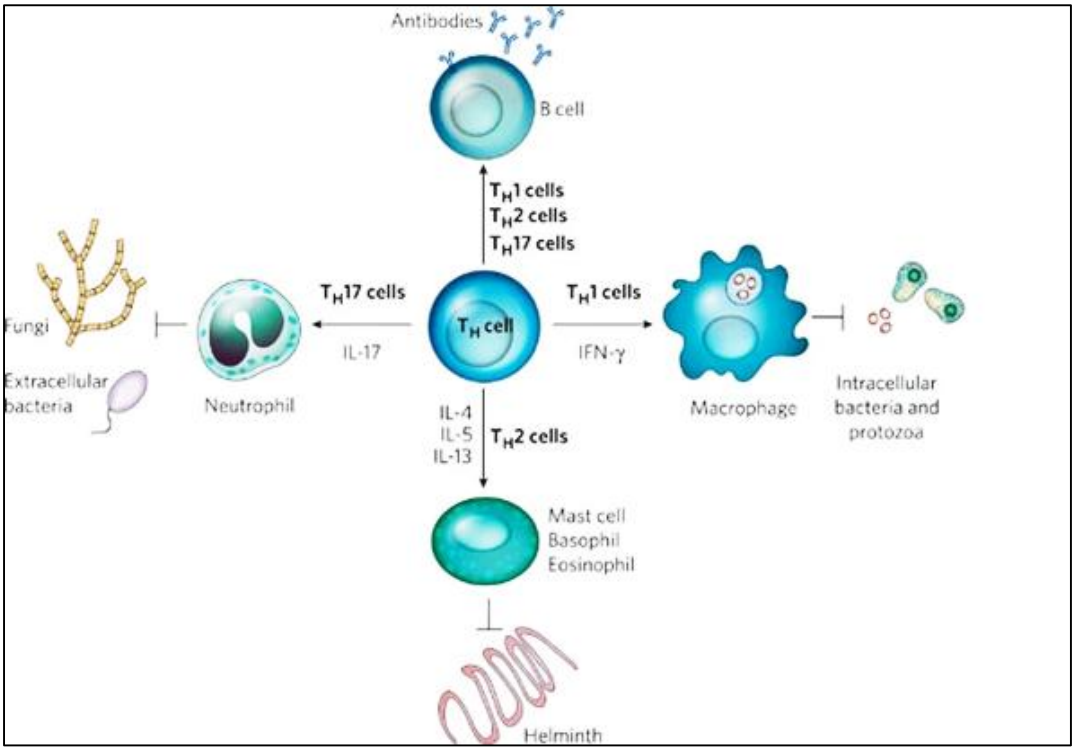

Figure1: When circulating 'naive' T_H cells first recognize their cognate antigen, they differentiate into one of several effector-cell lineages (listed in bold), depending on the infecting pathogen. T_H1, T_H2 and T_H17 cells are the known types of effector T_H cell; however, other types of effector T_H cell probably exist. Each T_H-cell lineage is characterized by the cytokines that are produced and by the innate immune effector mechanism that is activated (denoted by arrows). It is possible (but has not been proved) that every module of the innate immune system is controlled by a dedicated effector T_H-cell lineage

The effector response in each case is thus dictated by the innate immune system. In terms of T_H cells, TLR engagement induces IL-12 production, which directs T_H cells to differentiate into T_H1 cells. By contrast, TLR-induced IL-6, together with transforming growth factor-β (from an unknown cellular source), induces differentiation into T_H17 cells. And dectin-1 engagement results in the production of IL-23, which is required for T_H17-cell function and/or maintenance. The mechanisms of T_H2-cell generation are unknown but presumably follow a similar principle, with the dedicated cytokines likely to be IL-4 and thymic stromal lymphopoietin (TSLP), produced in response to engagement of an unidentified sensor after helminth infection. For other cell types, type I IFNs (which are produced in response to TLR engagement or RIG-I, MDA5 or DAI engagement during viral infections) regulate the function of cytotoxic T cells and NK cells, either directly or indirectly by inducing IL-15 production.

Importantly, the adaptive immune response ultimately results in an antigen-specific activation of the effector mechanisms of the innate immune system. Thus, the effector T_H cells produce the appropriate effector cytokines that activate a specific

module of the innate immune system, including activation of macrophages by T_H1 cells, activation of neutrophils by T_H17 cells and activation of eosinophils, mast cells and basophils by T_H2 cells. Similarly, to NK cells, cytotoxic T cells induce apoptosis of infected cells, except that the T-cell response is antigen specific. Likewise, antibodies activate the modules of the innate immune system in a class-dependent (and antigen-dependent) manner. IgG activates complement and opsonizes pathogens to aid their phagocytosis by macrophages and neutrophils, whereas IgE activates mast cells and basophils. Each of the innate effector responses can therefore be activated either directly, by the appropriate PRRs at the early stages of infection, or indirectly, by T cells and antibodies (in an antigen-specific manner) at the later, effector, stages of the immune response. Furthermore, each effector mechanism of the adaptive immune system might have evolved to activate the appropriate host-defence module of the innate immune system.

The relative contributions of the innate immune system and the adaptive immune system during bacterial infections have been investigated extensively. One important principle that has emerged from these studies is that, although innate host defence is crucial for controlling an infection, it is often insufficient for pathogen clearance. For example, to clear a *Listeria monocytogenes* infection requires functional T-cell responses. It therefore seems that the innate immune system in vertebrates evolved to depend, to some extent, on antigen-specific (adaptive) immunity. This might explain why the mammalian innate immune system, unlike that of arthropods, is not self-sufficient at affording protection against many infections. It should be noted, however, that our understanding of host defence might be biased because almost all studies are based on symptomatic infections. Asymptomatic infections are presumably common, and many of these infections might be cleared efficiently by innate host-defence mechanisms.

Probable questions:

1. What are virulence factors?
2. Justify the inclusion of pathogen-associated molecular patterns in innate immune system.
3. Briefly describe pattern-recognition receptors highlighting their function.
4. How adaptive immune system play its role in microbial defence mechanism?
5. What are the different components of innate immune system? Explain briefly.
6. Elucidate innate control of adaptive immune system.

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Unit- VII

Principles of immunity in relation to protozoa

Objectives:

In this section we will discuss on principles of immunity in relation to protozoa.

Introduction:

Resistance to parasitic protozoa appears to be similar to resistance against other infectious agents, although the mechanisms of resistance in protozoan infections are not yet as well understood. Resistance can be divided into two main groups of mechanisms: (1) nonspecific mechanism(s) or factor(s) such as the presence of a nonspecific serum component that is lethal to the parasite; and (2) specific mechanism(s) involving the immune system. Probably the best studied nonspecific mechanisms involved in parasite resistance are the ones that control the susceptibility of red blood cells to invasion or growth of plasmodia, the agents of malaria. Individuals who are heterozygous or homozygous for the sickle cell haemoglobin trait are considerably more resistant to *Plasmodium falciparum* than are individuals with normal haemoglobin. Similarly, individuals who lack the Duffy factor on their red blood cells are not susceptible to *P vivax*. Possibly both the sickle cell trait and absence of the Duffy factor have become established in malaria-endemic populations as a result of selective pressure exerted by malaria. Epidemiologic evidence suggests that other inherited red blood cell abnormalities, such as thalassaemia and glucose-6-phosphate dehydrogenase deficiency, may contribute to survival of individuals in various malaria-endemic geographical regions. A second well-documented example of a nonspecific factor involved in resistance is the presence in the serum of humans of a trypanolytic factor that confers resistance against *Trypanosoma brucei brucei*, an agent of trypanosomiasis (sleeping sickness) in animals. There is evidence that other nonspecific factors, such as fever and the sex of the host, may also contribute to the host's resistance to various protozoan parasites. Although nonspecific factors can play a key role in resistance, usually they work in conjunction with the host's immune system.

Different parasites elicit different humoral and/or cellular immune responses. In malaria and trypanosome infections, antibody appears to play a major role in immunity. In both *T cruzi* and *T brucei gambiense* infections, antibody-dependent cytotoxic reactions against the parasite have been reported. Although antibody has been shown to be responsible for clearing the African trypanosomes from the blood of infected animals, recent evidence suggests that the survival time of infected mice does not necessarily correlate with the ability of the animal to produce trypanosome-specific antibody. In other words, resistance as measured by survival time may not solely involve the specific humoral immune system. Recent data suggest that cellular immunity is required for resistance to malaria. for example, vaccine trials with a sporozoite antigen indicated that

both an active cellular response and sporozoite-specific antibody may be needed for successful immunization.

Cellular immunity is believed to be the single most important defence mechanism in leishmaniasis and toxoplasmosis. In animals infected with *Toxoplasma*, the activated macrophage has been shown to play an important role in resistance. Accordingly, resistance to the protozoan parasites most likely involves nonspecific factors as well as specific humoral and/or cellular mechanisms. Cytokines are involved in the control of both the immune response and pathology. It has become apparent that there are subsets of both helper (h) and cytotoxic (c) T-cells that produce different profiles of cytokines. For example, the Th-1 subset produces gamma interferon (IFN-α), and interleukin-2 (IL-2) and is involved in cell-mediated immunity. In contrast the Th-2 subset produces IL-4 and IL-6, and is responsible for antibody-mediated immunity. The induction of a particular T-cell subset is key to recovery and resistance. The Th-1 subset and increased IFN-g are important in resistance to *Leishmania*, *T cruzi* and *Toxoplasma* infections, whereas the Th-2 response is more important in parasitic infections in which antibody is a key factor. It is important to recognize that the cytokines produced by one T-cell subset can up or downregulate the response of other T-cell subsets. IL-4 will downregulate Th-1 cells and exacerbate infection and/or susceptibility of mice to *Leishmania*. The cytokines produced by T and other cell types do not act directly on the parasites but influence other host cell types. The response of cells to cytokines includes a variety of physiological changes, such as changes in glucose, fatty acid and protein metabolism. For example, IL-1 and tumour necrosis factor will increase gluconeogenesis, and glucose oxidation. It should be noted that cytokines influence the metabolism not only of T-cells, but also a variety of other cell types and organ systems. Cytokines can also stimulate cell division and, therefore, clonal expansion of T and B-cell subsets. This can lead to increased antibody production and/or cytotoxic T-cell numbers. The list of cytokines and their functions is growing rapidly, and it would appear that these chemical messages influence all phases of the immune response. they are also clearly involved in the multitude of physiological responses (fever, decreased food intake, etc.) observed in an animal's response to a pathogen, and in the pathology that results.

Unlike most viral and bacterial infections, protozoan diseases are often chronic, lasting months or years. When associated with a strong host immune response, this type of chronic infection is apt to result in a high incidence of immunopathology. The question also arises of how these parasites survive in an immunocompetent animal. The remainder of this chapter treats the mechanisms responsible for pathology, particularly immunopathology, in protozoan disease, and the mechanisms by which parasites evade the immune responses of the host. Finally, because of the very rapid advances in our knowledge of the host-parasite relationship (due primarily to the development of techniques in molecular biology), it is necessary to briefly mention the potential for developing vaccines to the pathogenic protozoa.

Pathology:

The protozoa can elicit humoral responses in which antigen-antibody complexes in the region of antibody excess activate Hageman blood coagulation factor (Factor XII), which in turn activates the coagulation, fibrinolytic, kinin and complement systems. It has been suggested that this type of immediate hypersensitivity is responsible for various clinical syndromes in African trypanosomiasis, including blood hyper viscosity, edema, and hypotension. Similar disease mechanisms would be expected in other infections by protozoa involving a strong humoral immune response.

Immune complexes have been found circulating in serum and deposited in the kidneys and other tissues of humans and animals infected with protozoans. These parasite antigen-antibody complexes, plus complement, have been eluted from kidney tissue in cases of malaria and African trypanosomiasis. Antigen and antibody have been directly visualized in the glomeruli of infected animals by light and electron microscopy. Inflammatory cell infiltrates accompany these deposits, and signs of glomerulonephritis are usually seen. African trypanosomes and presumably their antigens are also found in a variety of extravascular locations. Immune complexes, cellular infiltrates, and tissue damage have been detected in these tissues.

Another important form of antibody-mediated pathology is autoimmunity. Autoantibodies to a number of different host antigens (for example, red blood cells, laminin, collagen, and DNA) have been demonstrated. These autoantibodies may play a role in the pathology of parasitic diseases in two ways. First the antibodies may exert a direct cytotoxic effect on the host cells; for example, autoantibodies that coat red blood cells produce haemolytic anaemia. Alternatively, autoantibodies may be pathogenic through a build-up of antigen-antibody complexes in the kidneys or other tissues, leading to glomerulonephritis or other forms of immediate hypersensitivity. A particularly good example of a protozoan infection in which autoimmunity appears to be an important contributor to pathogenesis is *T cruzi* infection. In this case, there is substantial evidence that host and parasite share cross-reacting antigens. Antibodies and cytotoxic lymphocytes to these antigens appear to be harmful to host tissue. This type of experimental data, combined with the fact that the parasite itself seems not to cause the tissue pathology, lead one to conclude that autoimmunity may play a key role in pathogenesis.

Cellular hypersensitivity is also observed in protozoan diseases. For example, in leishmaniasis (caused by *Leishmania tropica*), the lesions appear to be caused by a cell-mediated immune response and have many, if not all, of the characteristics of granulomas observed in tuberculosis or schistosomiasis. In these lesions, a continuing immune response to pathogens that are able to escape the host's defence mechanisms causes further influx of inflammatory cells, which leads to sustained reactions and continued pathology at the sites of antigen deposition. During a parasitic infection, various host cell products (cytokines, lymphokines, etc.) are released from activated cells of the immune system. These mediators influence the action of other cells and may be directly involved

in pathogenesis. An example is tumour necrosis factor (TNF), which is released by lymphocytes. TNF may be involved in the muscle wasting observed in the chronic stages of African trypanosomiasis. TNF has also been implicated in the cachexia and wasting in *Leishmania donovani* infection, cerebral malaria in *P falciparum* in children and decreased survival in *T cruzi*-infected mice. It is apparent that mediators involved in resistance to protozoan parasites may also lead to pathology during a chronic infection. There appears to be a delicate balance between the factors involved in resistance to infectious agents and those which ultimately produce pathology and clinical disease.

Numerous authors have suggested that toxic products produced by parasitic protozoa are responsible for at least some aspects of pathology. For example, the glycoproteins on the surface of trypanosomes have been found to fix complement. This activation of complement presumably results in the production of biologically active and toxic complement fragments. In addition, trypanosomes are known to release proteases and phospholipases when they lyse. These enzymes can produce host cell destruction, inflammatory responses, and gross tissue pathology. Furthermore, it has been hypothesized that the trypanosomes contain a B-cell mitogen that may alter the immune response of the host by eliciting a polyclonal B-cell response that leads to immunosuppression. Finally, it has recently been shown that the African trypanosomes also contain an endotoxin which is presumably released during antibody- mediated lysis. Parasitic protozoa have also been reported to synthesize (or contain) low-molecular-weight toxins. For example, the trypanosomes produce several indole catabolites; at pharmacologic doses, some of these catabolites can produce pathologic effects, such as fever, lethargy, and even immunosuppression. Similarly, enzymes, B-cell mitogen, etc., are presumably released by many if not all of the other parasitic protozoa. There has been limited work on the role of these protozoal products in pathogenesis. However, parasitic protozoa are generally not known to produce toxins with potencies comparable to those of the classic bacterial toxins (such as the toxins responsible for anthrax and botulism). One possible exception is the African trypanosomes which are suggested to contain an endotoxin.

Immune Escape:

Parasite escape mechanisms may include a number of different phenomena. In antigenic masking, the parasite becomes coated with host components and so fails to be recognized as foreign. In blocking, noncytotoxic antibody combines with parasite antigens and inhibits the binding of cytotoxic antibodies or cells. The parasite may pass part of its life cycle in an intracellular location, for example, in erythrocytes or macrophages, in which it is sheltered from intracellular digestion and from the cytotoxic action of antibody and/ or lymphocytes. Some parasites practice antigenic variation, altering their surface antigens during the course of an infection and thus evading the host's immune responses. Finally, the parasite may cause immunosuppression, reducing

the host's immune response either to the parasite specifically or to foreign antigens in general. These strategies are discussed in more detail below:

Escape Mechanisms: Escape mechanisms are strategies by which parasites avoid the killing effect of the immune system in an immunocompetent host. Escape mechanisms used by protozoal parasites include the following.

Antigenic Masking: Antigenic masking is the ability of a parasite to escape immune detection by covering itself with host antigens.

Blocking of Serum Factors: Some parasites acquire a coating of antigen-antibody complexes or noncytotoxic antibodies that sterically blocks the binding of specific antibody or lymphocytes to the parasite surface antigens.

Intracellular Location: The intracellular habitat of some protozoan parasites protects them from the direct effects of the host's immune response. By concealing the parasite antigens, this strategy also delays detection by the immune system.

Antigenic Variation: Some protozoan parasites change their surface antigens during the course of an infection. Parasites carrying the new antigens escape the immune response to the original antigens.

Immunosuppression: Parasitic protozoan infections generally produce some degree of host immunosuppression. This reduced immune response may delay detection of antigenic variants. It may also reduce the ability of the immune system to inhibit the growth of and/or to kill the parasites.

Masking and Mimicry:

Various species of trypanosomes have host immunoglobulins associated with their cell surfaces. There are several reports that these antibodies are not bound to the trypanosomes through their variable regions, but presumably through the Fc portion of their molecule. These antibodies may mask the parasite-that is, prevent immune recognition by the host. However, no evidence other than the presence of immunoglobulins on the surface of the trypanosomes supports this hypothesis. Mimicry, in which the parasite has the genetic information to synthesize antigens identical to those of its host, has not been demonstrated in parasitic protozoa.

Blocking:

It has been hypothesized that in some cases antigen-antibody complexes in serum of infected animals bind to the parasite's surface, mechanically blocking the actions of cytotoxic antibodies or lymphocytes and directly inhibiting the actions of lymphocytes. This type of immune escape mechanism has been proposed for tumor cells and for the parasitic helminths. Because the trypanosomes carry immunoglobulins on their cell

surfaces, they may use a similar mechanism; however, no direct evidence has yet been reported.

Intracellular location:

Many protozoan parasites grow and divide within host cells. For example, *Plasmodium* parasites grow first in hepatocytes and then in red blood cells. *Leishmania* and *Toxoplasma* organisms are capable of growing in macrophages; one genus of parasitic protozoa, *Theilera*, not only multiplies in lymphocytes but appears even to stimulate the multiplication of the infected lymphocytes. Although some parasites, such as Plasmodium, are restricted to a limited number of host cell types, others, such as *T cruzi* and *Toxoplasma*, appear to be able to grow and divide in a variety of different host cells.

An intracellular refuge may protect a parasite from the harmful or lethal effects of antibody or cellular defence mechanisms. For example, *Plasmodium* may be susceptible to the actions of antibody only during the brief extracellular phases of its life cycle (the sporozoite and merozoite stages). It should be remembered that *Plasmodium* actually resides in a membrane-bound vacuole in the host cell. Thus, plasmodia are shielded from the external environment by at least two host membranes (the outer cell membrane and an inner vacuole membrane). Although intracellular plasmodia are very well protected from the host's immune response early in their growth, this strategy does create physiologic problems for the parasite. For example, the parasite must obtain its nutrients for growth through three membranes (two host and one parasite), and must eliminate its waste products through the same three membranes. Plasmodia solve this problem by appropriately modifying the host cell membranes. Parasitic proteins are incorporated into the red blood cell outer membrane. The host eventually responds to these antigens, and this response ultimately leads to the increased removal of infected host cells.

The existence of extracellular phases in the malaria life cycle is important, since immunization against these stages is the rationale for the development of our current vaccine candidates. The protective antigens on these extracellular stages have been purified as potential antigens for a vaccine. However, this approach has problems. For example, the sporozoite stage is exposed to protective antibody for only a brief period, and even a single sporozoite that escapes immune elimination will lead to an infection. Second, the antigenic variability of different isolates and the ability of different strains to undergo antigenic variation are not fully known. Therefore, the effectiveness of the vaccine candidates must still be demonstrated. However, a large synthetic peptide containing antigenic sequences from 3 different proteins of *P falciparum* has been shown to reduce the clinical incidence of malaria by 31% in field trials. There is therefore optimism that a vaccine against *P falciparum* may be available in the near future.

A number of parasitic protozoa reside in macrophages. Although these organisms are protected from external immune threats, they must still evade digestion by the

macrophage. Three strategies have been suggested. First, the parasite may prevent the fusion of lysosomes with the phagocytic vacuole. The actual mechanism responsible for this inhibition is not yet understood, but it has been shown to occur in cells infected with *Toxoplasma*. A second mechanism is represented by the ability of *T cruzi* to escape from the phagocytic vacuole into the cytoplasm of the macrophage. Finally, it is possible that some parasites can survive in the presence of lysosomal enzymes, as can the leprosy bacillus. One of the best-studied examples of a protozoan parasite able to survive in the phagolysosome is *Leishmania*. It has been suggested that the resistance of this parasite to the host's hydrolytic enzymes is due to surface components that inhibit the host's enzymes and/or to the presence of parasitic enzymes that hydrolyze the host's enzymes. As previously noted, at least one protozoan parasite, *Theilera*, is capable of growing directly in lymphocytes. Therefore, this parasite may escape the host's immune response by growing inside the very cells required for the response.

Antigenic Variation:

Three major groups of parasitic protozoa are known to be able to change the antigenic properties of their surface coat. The African trypanosomes can completely replace the antigens in their glycocalyx each time the host exhibits a new humoral response. These alterations in serotype are one important way in which the African trypanosomes escape their host's defence mechanism. Although less well-characterized, similar changes are reported to occur in *Plasmodium*, *Babesia*, and *Giardia.*

It has been estimated that African trypanosomes have approximately 1,000 different genes coding for surface antigens. These genes are located on various chromosomes; however, to be expressed, the gene must be located at the end of a chromosome (telomeric site). The rate at which variation occurs in a tsetse-fly-transmitted population appears quite high. It has been shown that 1 in 10 cells appears to be capable of switching its surface antigen. The order in which the surface coat genes are expressed is not predictable. Much information is available on the nucleotide sequence of the genes coding the coat proteins; however, neither the factor(s) that induces a cell to switch its surface antigens nor the specific genetic mechanism(s) involved in the switch are fully understood. The antibody response does not induce the genetic switch, but merely selects variants with new surface antigens out of the original population. Considerably less information is available on the phenomenon of antigenic variation in malaria or babesiosis. However, antigen variation could be a major problem in reference to the development of a blood stage (merozoite) vaccine for malaria. Finally, antigenic variation has been observed in *Giardia lamblia*. A number of different gene families coding for surface proteins in *Giardia* have been identified. Antigenic variation has been suggested to assist *Giardia* in escaping the host's immune response.

Immunosuppression:

Immunosuppression of the host has been observed with almost every parasitic organism carefully examined to date. In some cases, the suppression is specific, involving only the host's response to the parasite. In other cases, the suppression is much more general, involving the response to various heterologous and nonparasitic antigens. It has not yet been proven that this immunosuppression allows the parasites to survive in a normally immunocompetent host. However, one can postulate that immunosuppression could permit a small number of parasites to escape immune surveillance, thus favouring establishment of a chronic infection. This mechanism might be particularly effective in parasites undergoing antigenic variation, since it could allow the small number of parasites with new surface antigens to go undetected initially. Immunosuppression experimentally induced by various extraneous agents has certainly been shown to produce higher parasitaemia, higher infection rates, or both. Therefore, the hypothesis that parasite-induced immunosuppression increases the chance for a parasite to complete its life cycle makes sense.

It should be noted that immunosuppression can be pathogenic itself. A reduced response to heterologous antigens could favour secondary infections. Humans suffering from malaria or trypanosomiasis have been shown to be immunosuppressed to a variety of heterologous antigens. Secondary infections may often be involved in death from African trypanosomiasis.

A variety of mechanisms have been suggested to explain the immunosuppression observed in protozoan infections. The most common mechanisms proposed are (1) the presence in the infected host of parasite or host substances that non-specifically stimulate the growth of antibody-producing B cells, rather than stimulating the proliferation of specific antiparasitic B-cells; (2) proliferation of suppressor T-cells and/or macrophages that inhibit the immune system by excretion of regulatory cytokines; and (3) production by the parasite of specific immune suppressor substances.

Probable questions:

1. What do you mean by resistance to a pathogen?
2. Discuss the effectiveness of cellular immunity against pathogenic protozoa.
3. Briefly describe hypersensitivity of host in response to protozoan toxins.
4. What are the different methods of immune escape adopted by pathogenic protozoa?

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Unit- VIII

Principles of immunity in relation to helminths

Objectives:

In this section we will discuss on principles of immunity in relation to helminths.

Introduction:

Helminth infections can elicit a spectrum of clinical manifestations mirroring diversity in host immune responses. For example, in lymphatic filariasis, most infected individuals remain clinically asymptomatic despite harbouring significant worm burdens; this is thought to reflect the induction of parasite-specific tolerance in the immune system. Others exhibit acute manifestations, including fever and lymphadenopathy, and this is thought to reflect inflammatory processes induced by incoming larvae, dying worms, or superadded infections. Individuals who mount a strong but inappropriate immune response end up with lymphatic damage and subsequent immune-mediated pathology—hydrocele and elephantiasis. Finally, a group of infected individuals mount exuberant immune responses that often result in unusual pathology, such as tropical pulmonary eosinophilia. Thus, the clinical manifestations of lymphatic filariasis exemplify the spectrum of host–parasite interactions that occur during helminth infections.

Prototypical Host Responses to Helminths:

The canonical host immune response to all helminths is of the T-helper 2 (Th2) type and involves the production of cytokines interleukin (IL)-4, IL-5, IL-9, IL-10, and IL-13; the antibody isotypes immunoglobulin G1 (IgG1), IgG4, and IgE; and expanded populations of eosinophils, basophils, mast cells, type 2 innate lymphoid cells, and alternatively activated macrophages. However, it is also being increasingly recognized that while the predominant response is Th2 in nature, a large regulatory component involving both regulatory cytokines and cells are also part of this repertoire. The Th2 response induced by helminth parasites is quite stereotypical, but its initiation, progression, and culmination of this response requires interaction with many different cell types, most notably (i) epithelial/stromal cells, (ii) innate lymphoid cells (ILCs), (iii) dendritic cells (DCs) and macrophages; (iv) T cells; (v) B cells; (vi) eosinophils; (vii) mast cells/basophils; and (viii) neutrophils. In addition, the host–helminth interactions can lead to a variety of modulated immune responses that are mediated largely by the induction of regulatory T cells (Tregs) and alternatively activated macrophages (AAMs).

Helminths and Epithelial Cells:

Epithelial cells are the first barrier layer exposed to or breached by most helminths, and the capacity of these cells to respond by initiating an "alarm" response has recently been recognized.4 These epithelial cells mount a prototypical response comprising chemokines and cytokines, such as IL-1, IL-25, IL-33, and thymic stromal lymphopoietin (TSLP), as well as alarmins, such as uric acid, ATB, HMGB1, and S100 proteins. These signals program DCs to mount Th2 cell–mediated immunity and in doing so boost type 2 innate lymphoid cell (ILC2), basophil, and mast cell function. Epithelial cells produce chemokines, including CCL17 and CCL22 (acting on ILC2, basophils, Th2 cells, and Tregs), and eotaxins, such as CCL11, CCL24, and CCL26 (acting on eosinophils and Th2 cells). They also produce prostaglandin D2 (PGD2), which acts on the CRTH2 receptor to recruit ILC2, basophils, mast cells, and Th2 cells. More recently, tuft cells, a specialized secretory cell type of the intestinal epithelium, has been identified as a major player in anthelmintic immunity. In addition, epithelial cells in the intestine, for instance, are in constant contact with both beneficial and pathogenic bacteria and hence ideally located for immunological surveillance of the intestinal lumen. This recognition of signals by intestinal epithelial cells is essential to mucosal homeostasis, implicating these cells as central modulators of inflammatory responses. Finally, the production of mucus and mucus-associated bioactive molecules (Mucin5AC, trefoil factor-2, and resistin-like molecule-β [RELMβ]) are important in promoting protection against intestinal helminth infection.

Helminths and Innate Lymphoid Cells:

The ILC family includes ILC1, which predominantly express IFN-γ; ILC2, which predominantly express IL-5 and IL-13; and ILC3; which predominantly express IL-22 and/or IL-17.5 ILC2 are defined by their expression of the IL-33 receptor (IL-33R) and the transcriptional regulators, Id2, RORα, GATA-3, and Bcl11b. Unlike T cells, ILC2 rely on cytokines to drive activation rather than on cognate interactions mediated by antigen-specific receptors. ILC2 are a critical innate source of type 2 cytokines, including moderately large quantities of IL-5 and IL-13, but also of IL-4, IL-9, granulocyte macrophage–colony-stimulating factor (GM-CSF), and amphiregulin. These cytokines potently induce eosinophilia, mucus production from goblet cells, activation of AAM, muscle contractility, mastocytosis, and tissue repair. They are dependent on IL-2 and IL-7 for their development and activation. In addition, the transcription factors GATA-3 and RORα have been found to be essential for the development of ILC2. Although the function of ILC2 and Th2 cells appear to be largely overlapping, the kinetic differences in the ability to secrete cytokines rapidly and in profuse amounts allows for a coordinated interaction between the two cell types. Moreover, ILC2 can directly regulate the activation of T cells through their expression of major histocompatibility complex (MHC) Class II molecules and the accessory molecules, CD80 and CD86, albeit less efficiently compared with DCs. Finally, recent reports have linked ILC2 with metabolic homeostasis, obesity, and dietary

stress, providing an indirect link by which helminths might modulate host metabolic function.

Helminths and Dendritic Cells:

DCs are professional antigen-presenting cells (APCs) that play an essential role in presenting antigen to T cells to initiate immune responses. Although the role of DCs in inducing Th1, Th17, and Treg responses is well established, their role in inducing Th2 responses has remained relatively unclear. Nevertheless, a series of studies have shown that DCs are required for optimal Th2 responses in vivo. Thus in vivo depletion of DCs has been shown to inhibit the induction of Th2 responses to *S. mansoni* or *Heligmosomoides polygyrus*. Helminth products can prime DCs for the induction of Th2 responses by interaction with pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs) and C-type lectin receptors (CLRs). This interaction, which depends on TLR and CLR signalling, can promote Th2 responses by suppressing antigen presentation, co-stimulation, and/or expression of Th1-promoting cytokines by directly interfering with these pathways. DCs that drive Th2 responses typically exhibit specialized markers, such as CD301b, PDL2, and CD11b, and several receptors for the Th2-related cytokines IL-4R, IL-13R, IL-25R, TSLP-R, and IL-33R. Additionally, the extracellular signal-regulated kinase (ERK) and signal transducer and activator of transcription 4 (STAT4) pathway upregulates the costimulatory molecules, CD40, OX40L, and Jagged. Activation of the major transcription factors interferon regulatory factor 4 (IRF4) and KLF4 inhibits IL-12 production and increased IL-10 secretion. In addition, DCs expressing FcεRIII can induce murine IgG1-related Th2 responses. These factors typically act individually or in concert to orchestrate Th2 responses in helminth infections. Although Th2 cell–mediated immunity requires IRF4-dependent CD301b+ CD11b+ DCs in the mouse, Langerhans cells are the predominant inducers of Th2 cells ex vivo in humans. The modulation of DC function by helminth antigens appears to be generalizable and has been shown to impair their ability to respond to other infectious stimuli (e.g., *Mycobacterium tuberculosis*).

Helminths and Macrophages:

Macrophages are the other important class of APCs that can serve as protective effector cells in bacterial and protozoan infections by their production of nitric oxide and other mediators. Helminth interaction with macrophages induces a population of cells preferentially expressing arginase instead of nitric oxide as a result of increased activation of arginase-1 by IL-4 and IL-13.6 These AAMs are characterized by their ability to upregulate arginase-1, chitinase 3-like proteins 3 and 4 (also known as Ym1 and Ym2, respectively), and RELM-α. These AAMs are known to be important in wound healing and have been postulated to play a potential role in repairing wound damage that occurs during tissue migration of helminth parasites. In fact, there appears to be two distinct populations of AAMs, one derived from blood and functioning in an immune regulatory

role and the other derived from tissue-resident macrophages apparently responsible for much of the fibrosis seen in chronic helminth infections. By virtue of the expression of regulatory molecules, such as IL-10, TGF-β, and programmed cell death 1 ligand 2 (PDL2), these AAMs may have a predominantly regulatory role in helminth infections. These anti-inflammatory macrophages function through arginase-1, PDL2, triggering receptor expressed on myeloid cells 2 (TREM2) and RELM-α to inhibit classic macrophage inflammation and recruitment and T-cell responses. Similarly, macrophage-derived human resistin is induced by helminth infection and promotes inflammatory responses and increased susceptibility. Helminths and T Cells Typically, infections with helminths induce a robust Th2 response manifested by enhanced expression of IL-4, IL-5, IL-9, IL-10, and IL-13 in response to live parasites, parasite antigens, or mitogens. The central player in Th2 immunity is certainly the CD4+ Th2 cell. It is clear that IL-4Rα, a component of both the IL-4 and IL-13 receptors, is at the epicentre of Th2 immunity, since IL-4 and IL-13, together or individually, are absolutely critical for resistance to most helminth parasites. Recent work has reported that the Th2 cell population is heterogeneous, containing both IL-5+ and IL-5− Th2 cells that express IL-4 and IL-13. In addition, IL-4 and IL-13 production is spatially separated, with IL-13 expression being marked in tissues and IL-4 expression being pronounced in the lymph nodes within the Th2 cell compartment. Finally, induction of GATA-3 and downregulation of T-box expressed in T cells (T-bet) has been shown to be an important step in T resistin-like molecule cell differentiation to the Th2 phenotype in helminth infections. Interestingly, chronic helminth infections are associated with downmodulation of parasite antigen-specific proliferative responses as well as IFN-γ and IL-2 production but with intact IL-4 responses to parasite antigens and global downregulation of both Th1 and Th2 responses to live parasites. Finally, the receptor NLRP3 has been shown to be a key transcription factor in Th2 differentiation. Although the role of tissue resident memory T (TRM) cells is well established in viral and bacterial infections, very little is known about the role of these cells in helminth infections. However, it has been shown that tissue-resident Th2 cells can exert innate (TCR-independent and IL-33–dependent) functions upon appropriate stimuli and confer protection against helminth infection. In addition, although multifunctionality (ability to produce two or more cytokines) has not been well described in the Th2 cell compartment, helminth infections are known to be associated with an antigen-dependent enhancement of mono- and dual-functional Th2 cells and its reversal after treatment. Of interest, a stable subset of parasite induced T-bet+, GATA-3+, Th1/Th2 hybrid T cells has been described to develop directly from naïve precursors and to play a role in limiting pathological inflammation in animal models of helminth infection. Recently, a new subset of T cells expressing IL-9 and IL-10, but not IL-4 (and therefore different from Th2 cells), has been described in allergic inflammation and in response to intestinal parasites. These cells appear to be under the control of TGF-β and IL-4 and are dependent on STAT6, GATA-3, IRF4, and PU.1. Th9 cells have been recently shown to be associated with host protection in *Nippostrongylus brasiliensis* and Trichuris muris infection. Finally, Th9 cells have also been shown to be predominantly associated with lymphatic pathology in filariasis. T-follicular helper (Tfh) cells are a subset of CD4 T

cells that migrate to B-cell follicles after activation and promote germinal centre formation and B-cell isotype switching. These cells, which form an independent lineage of CD4 T cells, have been recently identified to be the predominant IL-4 producing T cells early in helminth infection. In addition, Tfh are major producers of IL-21, a cytokine that plays a crucial role in supporting polarized Th2 responses in vivo. Th17 cells, another subset of CD4 T cells, express the prototypical cytokine—IL-17. In terms of helminth infections, the role of Th17 cells has been primarily studied in animal models of *S. mansoni*, where it has been strongly associated with infection induced, immune-mediated pathology. More recently, it has also been demonstrated in human infections, in which children with *S. hematobium*–associated pathology has higher Th17 responses compared with those who are pathology-free. Similarly, a strong association of Th17 responses with pathological responses has also been demonstrated in lymphatic filariasis. Finally, Th22 cells are yet another subset of CD4 T cells that typically secrete IL-22. To date, only a few studies have examined the role of Th22 cells in helminth infections. IL-22 was shown to be induced in the intestinal mucosa after infection with *T. trichiura* or *Necator americanus* in humans, whereas the frequency of Th22 cells was shown to be higher in individuals with filarial infection compared with endemic healthy controls.

Helminths and B Cells:

Helminth interactions with B cells occur both at the B-cell cytokine level and at the level of antibody production. Interactions at the cellular level primarily result in B-cell activation and cytokine production, most notably by the induction of IL-10. B cells have been shown to be important for the Th2 responses to certain helminths, with IL-2 producing B cells supporting optimal development of effector and memory Th2 cells and LTα1β2- expressing B cells supporting the recruitment of a Th2 promoting DCs. Immune regulation by B cells has also been recognized in schistosome infection, where B-cell deficiency leads to enhanced Th2 cell–dependent immunopathology. However, it is at the level of antibody production that B cells play a profound role in helminth infections. Susceptibility to secondary infection is increased in the absence of B cells in infection with *Litomosoides sigmodontis*, *S. mansoni*, *T. muris*, and *Heligmosomoides polygyrus bakeri*. IgG is reported as an antibody isotype that is important for protection against intestinal helminths, and IgM (typically produced in a T cell–independent manner) has been linked to timely elimination of filarial parasites. One of the most consistent findings in helminth infections, both in mice and humans, is the elevated level of IgE that is observed after exposure to helminths. Most of the IgE produced is not antigen specific, perhaps representing nonspecific potentiation of IgE-producing B cells or deregulation of a normally well-controlled immune response. Interestingly, these IgE antibodies persist many years after the infection has been treated, indicating the presence of long-lived memory B cells or plasma cells in helminth infections. IgE production both in mice and humans is absolutely dependent on IL-4 or IL-13. Other isotypes that are commonly elevated in humans with chronic helminth infection are IgG4 and IgG1, the former being

most dependent on both IL-4 and IL-10. Recent studies have highlighted the role of regulatory B cells in suppression of immune responses to helminth parasites. This B-cell function involves the secretion of IL-10 and IL-35 and is similar to the regulatory activity of B cells in autoimmune diseases. Helminths and Eosinophils Blood and tissue eosinophilia is characteristic of helminth infection and is mediated by IL-5 (probably in concert with IL-3 and GM-CSF). Recruitment of eosinophils to the site of infection occurs very early in experimental helminth infection—as early as 24 hours after exposure. Kinetics of blood eosinophilia in humans is harder to determine but is postulated to occur as early as 2–3 weeks after infection, as demonstrated in experimental infections of volunteers. Both basal eosinophil levels and tissue accumulation during helminth infection appear to be under the influence of ILC2. Apart from the rapid kinetics of recruitment, eosinophils in blood and tissue also exhibit morphological and functional changes attributable to eosinophil activation. Eosinophils possess a range of immunomodulatory factors that are released upon cell activation, including cytokines, growth factors, and chemokines. Unlike T and B cells, eosinophils can rapidly release cytokines within minutes in response to stimulation, since most of the cytokines are stored in a preformed fashion in secretory vesicles. Moreover, eosinophils can participate in the regulation of IgE and goblet cell mucus production; they also serve as effector cells in protective immune responses and as regulatory cells influencing both innate and adaptive immunity in helminth infections.

Helminths and Basophils/Mast Cells:

Basophils are an important component of the immune response to helminth infections. Basophils are capable of secreting a variety of mediators, including histamines, cytokines, chemokines, and lipid mediators that promote Th2 responses. Basophils in humans and mice also readily generate large quantities of IL-4 in IgE-dependent and IgE-independent manners. Basophils appear to play an important role in protective immunity to secondary infection (similar to eosinophils) with *N. brasiliensis*, *H. polygyrus bakeri*, and *L. sigmodontis*; they also play an active role in resistance to primary infection (through secretion of IL-4 and IL-13) with *T. muris* and *T. spiralis*. In addition, basophils have been shown to be critical APCs for driving Th2 cell differentiation in different models of helminth infection. Mast cells may contribute to inflammatory reactions directed against invasive helminth parasites. These cells express high affinity Fcε receptors that are sensitized with parasite antigen– specific IgE and can be triggered by parasite antigens. It has been postulated that cytokines and other mediators released by sensitized mast cells contribute to (i) the recruitment and activation of effector eosinophils; (ii) increased local concentrations of antibody and complement; and (iii) enhanced mucus hypersecretion and increased peristalsis of the gastrointestinal (GI) tract that plays an important role in resistance to certain GI nematode infections. More recently, a role for mast cells (in an IgE-independent manner) in mediating the secretion of epithelial-

derived cytokines (IL-25, IL-33, and TSLP) and optimal migration of DCs was shown in *H. polygyrus bakeri* infection.

Helminths and Neutrophils:

Although neutrophils are typically considered more important in bacterial and fungal infections, a number of studies have revealed that neutrophils can act in conjunction with macrophages to contain or kill helminth parasites. Thus, neutrophils are major components of the granulomas forming around filarial parasites and the cysts containing larvae of intestinal helminths. Neutrophils have been demonstrated to collaborate with macrophages in the immobilization and killing of *S. stercoralis* larvae in a process that is complement dependent and involving neutrophil extracellular traps (NETs). Similarly, neutrophils contribute in the early antifilarial response through oxidative burst, degranulation, and NETosis and protect against infective larvae in skin. A seminal study reported that neutrophils adopt an "N2" phenotype during experimental infection with *N. brasiliensis* in the lung and express the genes for IL-13, IL-33, RELM-α, and Ym1. These "N2" neutrophils can train macrophages to acquire a memory phenotype that protects against secondary infection. Finally, it was also shown that even during primary infection, the absence of neutrophils resulted in greater worm burdens because of lack of immunity in the lungs. Thus, neutrophils appear to play an unexpected role in immunity to helminths that certainly merits further investigation.

Probable questions:

1. Elucidate the response of following components of host's immune system against helminth invasion: i) Epithelial cells ii) Innate lymphoid cells iii) Dendritic cells iv) Macrophages v) B cells vi) Basophils vii) Neutrophils.

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Unit IX

T-cell receptor organ and functions of immune response

Objective: In this unit we will learn about T-cell receptor organ and its functions in immune response.

Introduction

T cells are a type of white blood cell called lymphocytes. They help your immune system fight germs and protect you from disease. There are two main types. Cytotoxic T cells destroy infected cells. Helper T cells send signals that direct other immune cells to fight infection.

- **Different types of T cells**

There are two main types of T cells:

I. ***Cytotoxic T cells***: Cytotoxic T cells are also called CD8+ cells because they have a CD8 receptor on their membranes. These cells get their name from "cyto," which means cell, and "toxic," which means poisonous or harmful. Cytotoxic T cells kill cells infected with viruses and bacteria, and they also destroy tumor cells.

II. ***Helper T cells***: Helper T cells are also called CD4+ cells because they have a CD4 receptor on their membranes. Unlike cytotoxic T cells, helper T cells don't kill cells directly. Instead, they send signals that tell other cells in your immune system how to coordinate an attack against invaders. Helper T cells signal cytotoxic T cells; B cells and another type of white blood cell called a macrophage.

- **Location of T cells**

T cells exist in different places depending on the point in the cell cycle. T cells start in your bone marrow, mature in your thymus and eventually relocate to your lymph tissue or bloodstream.

I. ***Bone marrow:*** T cells start in the spongy tissue inside your bone called marrow. Like all blood cells, they start as hematopoietic stem cells. These cells have the potential to develop into any type of blood cell.

II. ***Thymus*****:** T cells move to an organ called your thymus (located in your upper mid-chest) to mature. At this stage, the immature T cells are called thymocytes. Your thymus is like boot camp for T cells. Once inside, T cells go through testing to be sure they can bind correctly to MHC and won't attack your body's healthy cells. They also receive the right receptor, either CD4 (helper T cells) or CD 8 (cytotoxic T cells). Only T cells that pass all these tests go out into your body.

III. ***Lymph tissue and bloodstream*:** Fully mature T cells travel to tissue and organs in your lymph system, like your spleen, tonsils and lymph nodes. They may also circulate in your bloodstream.

- **Structure of the T-cell receptor**

TCR is a heterodimeric membrane protein that belongs to the immunoglobulin superfamily. TCR includes two types of receptors, αβ chain receptors and γδ chain receptors, and about 95% of human peripheral blood T lymphocytes express αβ chain receptors. Most TCRs are composed of highly variable alpha subunits and beta subunits linked by disulfide bonds. This type of T cells is called αβ T cells. A minority containing both gamma and delta subunits are called γδ T cells. The extracellular portion of each chain consists of two domains. The overall structure is similar to that of the antigen-binding fragment (Fab) of an immunoglobulin (Ig). The TCR domains furthest from the membrane resemble the Ig variable (V) regions, while the TCR domains closest to the membrane resemble the Ig constant (C) regions. Antigen binding occurs at sites created by the V domains of the αβ or γδ chains. The three-dimensional (3-D) structure of the extracellular portion of the TCR has been determined, with great similarity to Ig.

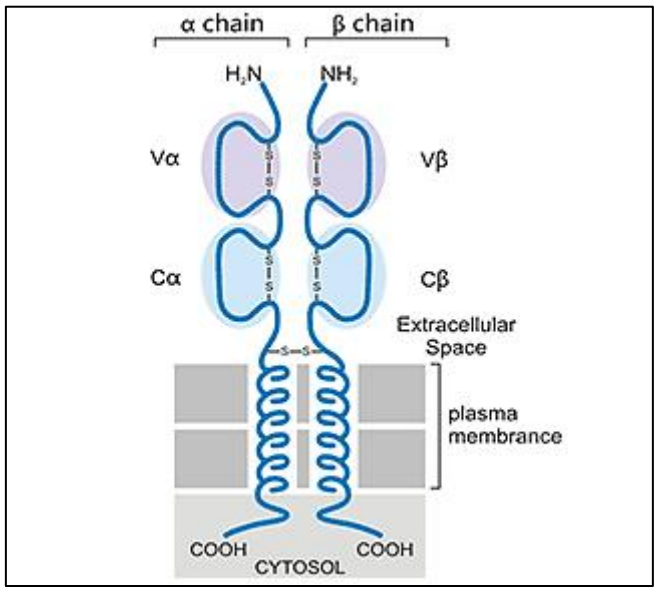

Figure 1. Schematic diagram of the structure of αβ chain receptor (TCR) (Source: https://www.cusabio.com/receptor/T-Cell-Receptor)

TCR recognizes antigens with specificity and diversity. The V region (Vα, Vβ) of TCR has three hypervariable regions, CDR1, CDR2, and CDR3. Among them, CDR3 has the largest variation, which directly determines the antigen-binding specificity of TCR. The diversity of TCRs is formed by the principles of gene mixed arrangement and combinatorial design. It is estimated that its total amount reaches as many as 1015-1018, which endows individuals with almost unlimited antigen recognition and response capabilities, ensuring that individuals can have an effective immune response to foreign antigens (pathogens) in a variable environment. At the same time, T cells also follow the principle of clonal selection, that is, when TCR binds to a cognate antigen, it will lead to the proliferation of T cells to form antigen-specific T cell clones.

- **Activation of T cells**

Helper-T-cell activation

Helper T cells do not directly kill infected cells, as cytotoxic T cells do. Instead, they help activate cytotoxic T cells and macrophages to attack infected cells, or they stimulate B cells to secrete antibodies. Helper T cells become activated by interacting with antigen-presenting cells, such as macrophages. Antigen-presenting cells ingest a microbe, partially degrade it, and export fragments of the microbe—i.e., antigens—to the cell surface, where they are presented in association with class II MHC molecules. A receptor on the surface of the helper T cell then binds to the MHC-antigen complex. But this event alone does not activate the helper T cell. Another signal is required, and it is provided in one of two ways: either through stimulation by a cytokine or through a costimulatory reaction between the signaling protein, B7, found on the surface of the antigen-presenting cell, and the receptor protein, CD28, on the surface of the helper T cell. If the first signal and one of the second signals are received, the helper T cell becomes activated to proliferate and to stimulate the appropriate immune cell. If only the first signal is received, the T cell may be rendered anergic—that is, unable to respond to antigen.

A discussion of helper-T-cell activation is complicated by the fact that helper T cells are not a uniform group of cells but rather can be divided into two general subpopulations—T_H1 and T_H2 cells—that have significantly different chemistry and function. These populations can be distinguished by the cytokines they secrete. T_H1 cells primarily produce the cytokines gamma interferon, tumour necrosis factor-beta, and interleukin-2 (IL-2), while T_H2 cells mainly synthesize the interleukins IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13. The main role of the T_H1 cells is to stimulate cell-mediated responses (those involving cytotoxic T cells and macrophages), while T_H2 cells primarily assist in stimulating B cells to make antibodies.

Once the initial steps of activation have occurred, helper T cells synthesize other proteins, such as signaling proteins and the cell-surface receptors to which the signaling proteins bind. These signaling molecules play a critical role not only in activating the particular helper T cell but also in determining the ultimate functional role and final differentiation state of that cell. For example, the helper T cell produces and displays IL-2 receptors on its surface and also secretes IL-2 molecules, which bind to these receptors and stimulate the helper T cell to grow and divide.

- **Results of helper-T-cell activation**

The overall result of helper-T-cell activation is an increase in the number of helper T cells that recognize a specific foreign antigen, and several T-cell cytokines are produced. The cytokines have other consequences, one of which is that IL-2 allows cytotoxic or regulatory T cells that recognize the same antigen to become activated and to multiply. Cytotoxic T cells, in turn, can attack and kill other cells that express the foreign antigen in association with class I MHC molecules, which—as explained above—are present on

almost all cells. So, for example, cytotoxic T cells can attack target cells that express antigens made by viruses or bacteria growing within them. Regulatory T cells may be similar to cytotoxic T cells, but they are detected by their ability to suppress the action of B cells or even of helper T cells (perhaps by killing them). Regulatory T cells thus act to damp down the immune response and can sometimes predominate so as to suppress it completely.

- **Function of T cells**

i. T cells are key fighters in what's known as your adaptive immune system. Think of your adaptive immune system as a specialized smart system that's constantly monitoring for threats. Once it detects an intruder, your adaptive immune system builds a customized defence to fight it.

ii. Each T cell is unique in that it's designed to fight only one type of intruder. Once your immune system identifies the threat, it locates the specific T cell designed to defeat it and recruits that T cell for battle. The T cell copies itself, making more T cells to defeat the intruder. These T cells that join the fight are called effector cells. When your immune system is working properly, these effector T cells destroy the threat, helping rid you of infection and disease.

iii. Some of the T cells become memory cells instead of effector cells. Unlike effector T cells, memory T cells aren't fighters. Instead, they remember the intruder so that if it returns, your immune system recognizes it and quickly mounts a defence.

- **Difference between T cells and antibodies**

Both T cells and antibodies protect you from pathogens, but they play different roles in your immune system. B cells are the other type of white blood cell (lymphocytes). It's B cells (not T cells) that make antibodies, a specific type of protein that kills harmful invaders. While B cells send antibodies to kill harmful cells, cytotoxic T cells kill harmful cells directly.

Cell-mediated immune mechanisms

In addition to their importance in cooperating with B cells that secrete specific antibodies, T cells have important, separate roles in protecting against antigens that have escaped or bypassed antibody defences. It is a known fact that antibodies do not necessarily protect against viral infections, because many viruses can spread directly from cell to cell and thus avoid encountering antibodies in the bloodstream. It is also known that persons who fail to make antibodies are very susceptible to bacterial infections but are not unduly liable to viral infections. Protection in these cases results from cell-mediated immunity, which destroys and disposes of body cells in which viruses or other

intracellular parasites (such as the bacteria that cause tuberculosis and leprosy) are actively growing, thus depriving microorganisms of their place to grow and exposing them to antibodies.

Cell-mediated immunity has two mechanisms.

i. One involves activated helper T cells, which release cytokines. In particular, the gamma interferon produced by helper T cells greatly increases the ability of macrophages to kill ingested microbes; this can tip the balance against microbes that otherwise resist killing. Gamma interferon also stimulates natural killer cells.

ii. The second mechanism of cell-mediated immunity involves cytotoxic T cells. They attach themselves by their receptors to target cells whose surface expresses appropriate antigens (notably ones made by developing viruses) and damage the infected cells enough to kill them.

Cytotoxic T cells may kill infected cells in a number of ways. The mechanism of killing used by a given cytotoxic T cell depends mainly on a number of costimulatory signals. In short, cytotoxic T cells can kill their target cells either through the use of pore-forming molecules, such as perforins and various components of cytoplasmic granules, or by triggering a series of events with the target cell that activate a cell death program, a process called apoptosis. In general, the granular cytotoxic T cells tend to kill cells directly by releasing the potent contents of their cytotoxic granules at the site of cell-to-cell contact. This renders the cell membrane of the target cell permeable, which allows the cellular contents to leak out and the cell to die. The nongranular cytotoxic T cells often kill cells by inducing apoptosis, usually through the activation of a cell-surface protein called Fas. When a protein on the surface of the cytotoxic T cell interacts with the Fas protein on the target cell, Fas is activated and sends a signal to the nucleus of the target cell, thus initiating the cell death process. The target cell essentially commits suicide, thereby destroying the virus within the cell as well.

Probable questions:

1. Where are T cells located?
2. How do T cells work in the immune system?
3. What is the difference between T cells and antibodies?
4. State function of T-cell.
5. What are the different types of T cells?
6. Describe the structure of T-cell receptor with diagram.
7. Describe the mechanism of helper T cell activation/ Outline the steps in activating cytotoxic T cells in the lymphoid tissue.

8. Which cell is responsible for cell-mediated immunity?
9. Discuss about the cell mediated immunity.
10. What is cell-mediated immunity and how does it defend against invading pathogens and foreign cells?

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Unit X

Antigen-antibody reaction and its role in clinical parasitology

Objective: In this unit we will discuss about Antigen-antibody reaction and its role in clinical parasitology.

Introduction

The interactions between antigens and antibodies are known as antigen–antibody reactions. The reactions are highly specific, and an antigen reacts only with antibodies produced by itself or with closely related antigens. Since these reactions are essentially specific, they have been used in many diagnostic tests for the detection of either the antigen or the antibody in vitro. The antigen and antibody reactions also form the basis of immunity against microbial diseases in vivo. In the host, it may cause tissue injury in hypersensitivity reactions and in autoimmune diseases.

Antigen-antibody reactions are fundamental to the immune system and play a crucial role in the diagnosis and treatment of parasitic infections

- **What is an Antigen (Ag)?**

(Anti= against; gen=thing that produces or causes) Any foreign substances that when entering our body sometimes self-elicit a series of immune responses and are precisely called **immunogens**. Whereas some of them don't directly elicit an immune response but require the help of some other molecules (carrier proteins) to do so and are called **hapten**. The immunogens and hapten are collectively called **antigens**.

– They can be proteins, peptides, lipids, or, polysaccharides.

– Antibody binding site is called an **epitope**.

– Abbreviation: "**Ag**"

- **What is an Antibody (Ab)?**

An antibody is simply the component produced by the immune system in response to antigens. So basically, antigens are the generator of antibodies. They interact with each other to induce an immune response.

– Also called immunoglobulins (**Ig**)

– Y-shaped

– Glycoproteins

– Produced by plasma **B-cells**

– Antigen binding site is called **paratope**.

– Types: **IgG**, **IgA**, **IgM**, **IgE**, **IgD**

General Features of Antigen–Antibody Reactions

Antigen and antibody bind through noncovalent bonds in a manner similar to that in which proteins bind to their cellular receptors, or enzymes bind to their substrates. But antigen– antibody reactions differ from the latter as there is no irreversible chemical alteration in either of the participants, i.e., antigen or the antibody. The antigen and antibody binding are reversible and can be prevented or dissociated by high ionic strength or extreme pH. Following is some of the general features of these interactions:

Strength of Ag-Ab interaction:

1. Affinity:

Affinity measures the strength of interaction between an epitope and an antibody's antigen binding site. It is defined by the same basic thermodynamic principles that govern any reversible biomolecular interaction:

$$K_A = \frac{[Ab\text{-}Ag]}{[Ab]\,[Ag]}$$

- K_A = affinity constant
- [Ab] = molar concentration of unoccupied binding sites on the antibody
- [Ag] = molar concentration of unoccupied binding sites on the antigen
- [Ab-Ag] = molar concentration of the antibody-antigen complex

In other words, K_A describes how much antibody-antigen complex exists at the point when equilibrium is reached. The time taken for this to occur depends on rate of diffusion and is similar for every antibody. However, high-affinity antibodies will bind a greater amount of antigen in a shorter period of time than low-affinity antibodies. K_A can therefore vary widely for antibodies from below 10^5 mol^{-1} to above 10^{12} mol^{-1}, and can be influenced by factors including pH, temperature and buffer composition.

- ✓ Combined strength of total non-covalent interactions between single Ag-binding site of Ab and single epitope is affinity of Ab for that epitope.
- ✓ Low affinity Ab: Bind Ag weakly and dissociates readily.
- ✓ High affinity Ab: Bind Ag tightly and remain bound longer.

2. Avidity:

Antibodies and antigens are multivalent, meaning they possess more than one binding site. The measure of the total binding strength of an antibody at every binding site is termed avidity. Avidity is also known as the functional affinity.

Avidity is determined by three factors.

i. The binding affinity: The strength of the relationship at a singular binding site.

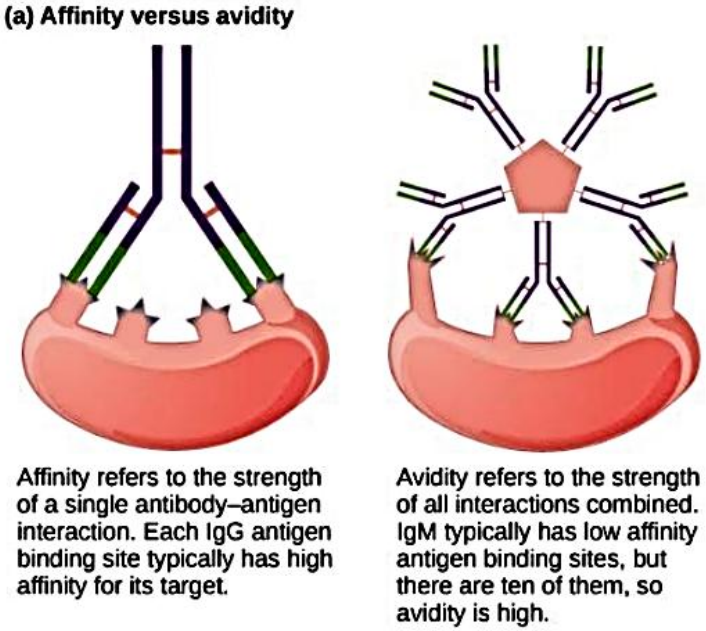
ii. The valency: The total number of binding sites involved.

iii. The structural arrangement: The structure of the antigen and antibody involved.

iv. All antibodies are multivalent e.g. IgGs are bivalent and IgMs are decavalent. The greater an immunoglobulin's valency (number of antigen binding sites), the greater the amount of antigen it can bind. Similarly, antigens can demonstrate multivalency because they can bind to more than one antibody. Multimeric interactions between an antibody and an antigen help their stabilization.

v. A favourable structural arrangement of antibody and antigen can also lead to a more stable antibody-antigen complex

vi. Strength of multiple interactions between multivalent Ab and Ag is avidity. Avidity is better measure of binding capacity of antibody than affinity. High avidity can compensate low affinity.



3. Cross reactivity:

• Antibody elicited by one Ag can cross react with unrelated Ag if they share identical epitope or have similar chemical properties.

Chemical Bonds Responsible for the Antigen-Antibody Reaction

The interaction between the Ab-binding site and the epitope involves exclusively non-covalent bonds, in a similar manner to that in which proteins bind to their cellular receptors, or enzymes bind to their substrates. The binding is reversible and can be prevented or dissociated by high ionic strength or extreme pH. The following intermolecular forces are involved in Ag–Ab binding:

1. **Electrostatic bonds:** This results from the attraction between oppositely charged ionic groups of two protein side chains; for example, an ionized amino group (NH4+) on lysine in the Ab, and an ionized carboxyl group (COO_) on an aspartate residue in the Ag.
2. **Hydrogen bonding:** When the Ag and Ab are in very close proximity, relatively weak hydrogen bonds can be formed between hydrophilic groups (e.g., OH and C=O, NH, and C=O, and NH and OH groups).
3. **Hydrophobic interactions**: Hydrophobic groups, such as the side chains of valine, leucine, and phenylalanine, tend to associate due to Van der Waals bonding and coalesce in an aqueous environment, excluding water molecules from their surroundings. As a consequence, the distance between them decreases, enhancing the energies of attraction involved. This type of interaction is estimated to contribute up to 50% of the total strength of the Ag–Ab bond.
4. **Van der Waals bonds:** These forces depend upon interactions between the "electron clouds" that surround the Ag and Ab molecules. The interaction has been compared to that which might exist between alternating dipoles in two molecules, alternating in such a way that, at any given moment, oppositely oriented dipoles will be present in closely apposed areas of the Ag and Ab molecules.

Factors Affecting Antigen-Antibody Interaction

Many factors affect Ag-Ab reactions. Some of the common factors are:

1. **Temperature:** It depends on the chemical nature of epitopes, paratopes, and, bonds involved. Eg. hydrogen bonds are stable at low temperatures and hydrophobic bonds are stable at high temperatures.
2. **pH:** Optimal pH range is 6.5 to 8.5. Extreme pH values change the conformation of antibodies and inhibit the reaction.
3. **Ionic strength:** The effect of ionic is important in blood group serology. Here the reaction is significantly influenced by sodium and chloride ions. In normal saline solution, Na+ and Cl− cluster around the complex and partially neutralize charges, potentially interfering with antibody binding to antigen. This could be problematic when low-affinity antibodies are used.

4. **Enzyme Treatment:** Many proteolytic enzymes are used to enhance the Ag-Ab reactions. Some of the commonly used ones are papain, ficin, bromelin.
5. **Concentrations of Ag and Ab:** Increase in the concentration of antigen and antibody enhances the reaction.
6. **No. of antigen-binding sites:** More the no. of antigen-binding sites on the antibody, the more the chances of interaction. For eg., IgM is a pentamer and hence has 10 binding sites whereas IgG is a monomer and hence has only 2 binding sites so IgM will bind more efficiently with antigens.
7. **Structural arrangement:** If the structure of epitope and paratope is such that they could fit well as lock and key then it enhances the interaction between antigen and antibody.

Stages of Antigen–Antibody Reactions in clinical parasitology

The antigen–antibody reaction occurs in two stages: primary and secondary.

1. **Primary Stage**

Primary stage is the initial interaction between antigen and antibody. It is rapid and reversible, but without any visible effects. The ionic bonds, hydrogen bonds, van der Waals forces, and hydrophobic interactions are the weaker intermolecular forces that bind antigen and antibodies together in this primary stage. Covalent binding, which is a stronger intermolecular force between antigen and antibody, however, does not occur in this stage.

2. **Secondary Stage**

Secondary stage is an irreversible interaction between antigen and antibody, with visible effects, such as agglutination, precipitation, neutralization, complement fixation, and immobilization of motile organisms. The binding between antigen and antibody during this stage occurs by covalent binding. A single antibody is capable of causing different types of antigens–antibody reactions, and a single antigen is capable of inducing production of different classes of immunoglobulins, which differ in their biological properties. The results of agglutination, precipitation, neutralization, and other tests are usually expressed as a titer. Titer is defined as the highest dilution of serum that gives a positive reaction in test. Higher titer means greater level of antibodies in serum. For example, a serum with a titer of 1/128 contains more antibodies than a serum with a titer of 1/8.

Types of Antigen-Antibody Interaction

Ag-Ab reactions are basically of two types:

1. In Vivo (Occurring in natural condition): It includes the general antibody-mediated immune response occurring in our body against any antigen.

2. In Vitro (Done in artificial conditions): It includes a series of serological tests performed in laboratories to detect antigens or antibodies in case of many diseases.

In Vivo Reactions	**In vitro Reactions**
Agglutination	Agglutination
Precipitation	Precipitation
Complement fixation	Complement fixation
Neutralization	Neutralization
Ab Dependent Cell-Mediated Toxicity	ELISA
Immobilization	Radioimmunoassay (RIA)
Opsonization	Western Blotting
	Immunochromatograhy (ICT)
	Immunofluorescence

- **Agglutination**

The interaction between antibody and a particulate antigen results in visible clumping called agglutination. Antibodies that produce such reactions are called agglutinins. Better agglutination takes place with IgM antibody than with IgG antibodies. Excess of an antibody also inhibits agglutination reaction; this inhibition is called prozone phenomenon.

1. Agglutination is more sensitive than precipitation for the detection of antibodies.

2. Agglutination occurs optimally when antigens and antibodies react in equivalent proportions.

The prozone phenomenon may be seen when either an antibody or an antigen is in excess. Incomplete or monovalent antibodies do not cause agglutination, though they combine with the antigen. They may act as blocking antibodies, inhibiting agglutination by the complete antibody added subsequently.

Application of Agglutination reaction:

1. Cross-matching and grouping of blood.

2. Identification of Bacteria. E.g. Serotyping of Vibrio cholera, Serotyping

of Salmonella Typhi and Paratyphi.

3. Serological diagnosis of various diseases. E.g Rapid plasma regains (RPR) test for

Syphilis, Antistreptolysin O (ASO) test for rheumatic fever.

4. Detection of unknown antigen in various clinical specimens. E.g. detection

of Vi antigen of Salmonella Typhi in the urine.

Haemagglutination

It is a passive agglutination reaction that involves RBCs as carrier particles.
– RBCs of sheep, humans, chicks, etc. are used
– Used in Blood Typing
– In the detection of parasitic infections and viral diseases such as influenza, mumps, and measles.

- **Precipitation**

It is a type of antigen-antibody reaction, in which the antigen occurs in a soluble form. When a soluble antigen reacts with its specific antibody, at an optimum temperature and PH in the presence of electrolyte antigen-antibody complex forms insoluble precipitate. This reaction is called a precipitation reaction. A lattice is formed between the antigens and antibodies; in certain cases, it is visible as an insoluble precipitate. Antibodies that aggregate soluble antigens are called precipitins. The interaction of antibody with soluble antigen may cause the formation of insoluble lattice that will precipitate out of solution. Formation of an antigen-antibody lattice depends on the valency of both the antibody and antigen. The antibody must be bivalent; a precipitate will not form with monovalent Fab fragments. The antigen must be bivalent or polyvalent; that is, it must have at least two copies of same epitope or different epitopes that react with different antibodies present in polyclonal sera. Antigen and antibody must be in an appropriate concentration relative to each other. Antigen access: Too much antigen prevents efficient crosslinking/lattice formation.

Application of Precipitation reaction:

1. Detection of unknown antibody to diagnose infection e.g. VDRL test for syphilis.

2. Standardization of toxins and antitoxins.

3. Identification of Bacteria e.g. Lancified grouping of streptococci.

4. Identification of bacterial component e.g Ascoli's thermoprecipitin test

for Bacillus anthracis.

- **Complement fixation**

Complement fixation is a method that demonstrates antibody presence in patient serum. Complement fixation is a classic method for demonstrating the presence of antibody in patient serum. The complement fixation test consists of two components. The first component is an indicator system that uses combination of sheep red blood cells, complement-fixing antibody such as immunoglobulin G produced against the sheep red blood cells and an exogenous source of complement usually guinea pig serum. When these elements are mixed in optimum conditions, the anti-sheep antibody binds on the

surface of red blood cells. Complement subsequently binds to this antigen -antibody complex formed and will cause the red blood cells to lyse.

The second component is a known antigen and patient serum added to a suspension of sheep red blood cells in addition to complement. These two components of the complement fixation method are tested in sequence.

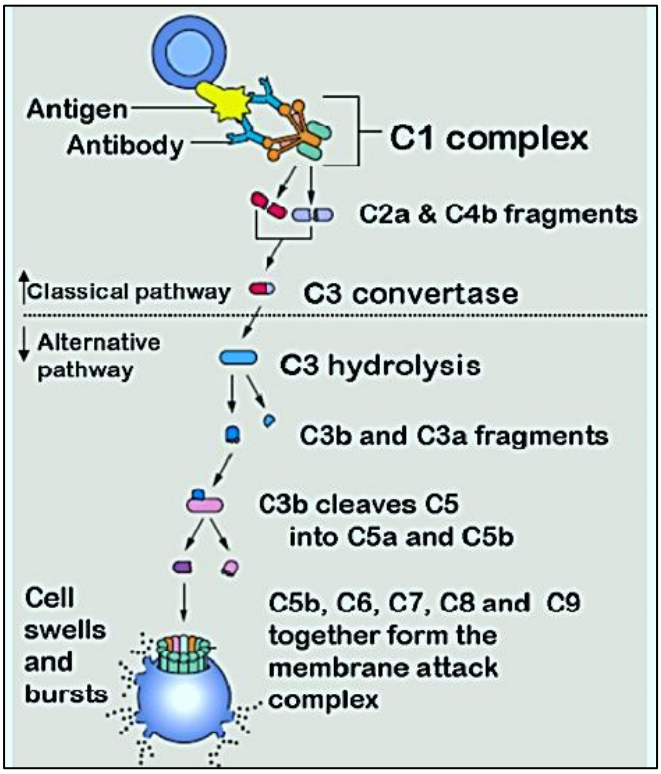


Fig: The complement pathway: Complement binds to antigen-antibody complex and leads to cell lysis (Source: rnlkwc.ac.in/pdf)

Patient serum is first added to the known antigen, and complement is added to the solution. If the serum contains antibody to the antigen, the resulting antigen-antibody complexes will bind all of the complement. Sheep red blood cells and the anti -sheep antibody are then added. If complement has not been bound by an antigen -antibody complex formed from the patient serum and known antigens, it is available to bind to the indicator system of sheep cells and anti-sheep antibody. Lysis of the indicator sheep red blood cells signifies both a lack of antibody in patient serum and a negative complement fixation test. If the patient's serum does contain a complement-fixing antibody, a positive result will be indicated by the lack of red blood cell lysis.

- **Enzyme-Linked Immunosorbent Assay (ELISA)**

It is one of the sensitive techniques for the detection of the presence of antigen or antibody and quantification as well in case of clinical diagnosis of many diseases such as AIDS, Ebola, Pernicious anemia, different parasitic infections, etc. Enzymes are used for labeling.

– Detects free antigens or antibodies.

– Reagents: Coating buffer, Blocking buffer, Wash Buffer, Substrates, Sample and, Assay Diluents.

– Types: Direct, Indirect, and, Sandwich ELISA.

- **Hemolysis**

When Ag-Ab interactions result in the rupture or lysis of RBC, it is called hemolysis which results in the release of Haemoglobin. It can be catalyzed by enzymes called hemolysins. It is the demonstrable endpoint of some reactions.

- **Immunofluorescence**

It is a type of immunoassay technique in which fluorescent dyes are used for the visualization of Ag-Ab reactions.

– Detects surface antigens or antibodies.

– Fluorescent dyes such are fluorescein isothiocyanate and lissamine rhodamine used.

– Types: Direct and Indirect.

- **Neutralization**

In neutralization, the biological effects of viruses and toxins are neutralized by homologous antibodies called neutralizing antibodies.

Types: - Virus Neutralization Tests (Eg. Viral hemagglutination inhibition test)

– Toxin Neutralization Tests (Eg. Schick test, antistreptolysin O test, etc.)

- **Radioimmunoassay (RIA)**

It is a type of immunoassay in which radioisotopes are used for labeling the antigen or antibody to detect the formation of the Ag-Ab complex.

– Can determine very small quantities of Ag and Ab in the serum.

– Used for the quantification of hormones, drugs, and, viral antigens.

- **Sensitization**

The first step in the Ag-Ab interactions involves the formation of the Ag-Ab complex and it is called sensitization. It is not observable and is observed only after agglutination of the formed complex using different reagents. IgG antibodies can sensitize red cells to the corresponding antigens and hence are called sensitizing antibodies.

- **Western Blotting**

It is called so because of its similarity to Southern Blotting.

– Protein separation is done by electrophoresis.

– Used in the detection of proteins.

– Confirmatory test in the diagnosis of HIV.

Applications of Antigen-Antibody Interaction

1. The most common use is the determination of blood groups i.e. blood typing.
2. Rapid diagnosis test kits used for pregnancy detection as well as detection of several diseases such as malaria, dengue, etc. are based on this principle. They require very little time for the tests.
3. Serological ascertainment of exposure to infectious agents.

4. Quantification of drugs, hormones, viral antigens, etc.
5. Detection of presence or absence of proteins in serum.
6. To study the characteristics of different immunodeficiency diseases.
7. To perform confirmatory tests for infections such as Western Blotting for HIV infection.

Probable questions:

1. State the characteristics of antigen antibody interaction.
2. Discuss the antigen antibody reaction and its role in the host defense.
3. What is an antigen and antibody?
4. What is antigen-antibody affinity? What is the significance of antigen affinity?
5. What is antibody avidity? Which factors are responsible for determination of avidity?
6. Describe different types of chemical bonds responsible for the antigen-antibody reaction.
7. State the role of Van der Waals bonds/ Hydrophobic interactions in antigen antibody interaction.
8. Discuss about the factors which affects antigen-antibody interaction.
9. Describe the stages of antigen–antibody reactions in clinical parasitology.
10. What is agglutination? What is prozone?
11. In clinical parasitology where the agglutination reaction is applied?
12. What is hemagglutination?
13. Write short notes on antigen antibody precipitation reaction? Where the precipitation reaction is applied?
14. State the role of complement fixation in clinical parasitology?
15. How antigen-antibody reactions play a crucial role in the diagnosis and treatment of parasitic infections?
16. Describe different types of In vitro Antigen-Antibody Interaction.
17. Describe briefly different types of In vivo Antigen-Antibody Interaction.
18. Describe the applications of Antigen-Antibody interaction.

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Unit XI

Basic immunological changes due to parasitic infection

Objective: in this unit we will discuss about basic immunological changes due to parasitic infection.

Introduction

Parasitic infections are one of the world's major causes of human illness and suffering. Parasitic diseases lower the productivity of the human work force, and some of the apathy encountered in regions where these diseases are endemic may be directly traced to such infections. Manifestations of disease range from the fevers of malaria to physical deformities, such as "river blindness" and elephantiasis, resulting from infections by certain filarial worms. In the extreme, death results, either as a direct consequence of the parasitic infection or from viral, bacterial, nutritional or other diseases to which the body, weakened by the ravages of parasitic infections

Protozoan parasitic diseases are commonly transmitted by vectors (vector-borne diseases), contaminated food (food-borne diseases), or water (water-borne diseases), and most of them are associated with economically disadvantaged populations (Barrow and Dujardin, 2020). poor water supply and sanitary conditions (Omarova and Tussupova, 2018) and host immunodeficiency (Andreani and Lodge, 2012). Furthermore, many of these protozoan parasites are zoonotic, which makes their control and prevention even more complex, requiring an integrative and multi-disciplinary approach (Chomel, 2008).

Several important distinctions can be made between the different groups of parasites in terms of their size, structure, reproduction, mode of transmission, and location in the host, all of which are relevant to their relationship with the host immune system.

- **Protozoa,** like the other major groups of micro parasites (viruses and bacteria), are small in relation to host cells, and many take advantage of their small size to live intracellularly. All protozoans share the capacity to replicate within the host, so that parasite load and infection severity are potentially independent of the number of infection events.

- **Worms and arthropods** are macro parasites. Not only are they large in relation to host cells, but characteristically (with some important exceptions) they do not replicate within the host. In contrast to the microparasites, levels and severity of infection with macro parasites reflect

Immunological changes in host due to parasitic infection

(Davidson, 1985)

The biologic interaction between parasite and host has been intensively studied in the last decade. Many parasites induce an exuberant immune response from the host; unfortunately, because of generations of interaction and genetic alteration this response may be ineffective or may interfere with normal immune response. Additionally, parasites are relatively large, some have resistant body walls, and many possess the ability to migrate away from an inflamed area and thus avoid being sequestered. These differences set parasitic infection apart from infection with most viruses and bacteria, in which the immune response is frequently protective.

The effectors of the immune response to parasitic infection, and their success in restricting or eradicating parasitic infestation, are reviewed in the following discussions.

1. Immunoglobulins

Some cestodes, especially in their larval stages, may be eradicated by complement-fixing IgG antibodies, and this protection may be transferred by purified immunoglobulin to athymic animals (Chandra, 1983.). Antibodies to many antigens especially those directed against metazoan parasites, may cross-react with other parasitic antigens, lending some broader protective immunity. Even intraluminal organisms may invoke a humoral antibody response.

Giardiasis has long been known to be associated with humoral immunodeficiency states. This and the apparent resistance to giardiasis of individuals living in endemic areas has suggested a role for humoral immunity in this infection. Circulating immunofluorescent antibodies have been demonstrated against trophozoites in what seems to be a specific response, but it is not known whether this antibody is protective. True protective effects of IgA have been difficult to demonstrate. Probably the clearest evidence is in murine infection with *Taenia taeniaeformis*, a cestode, but protection has been postulated in *Trichinella* and *Nippostrongylus* infection. In giardiasis there is some evidence that IgA may prevent adherence of trophozoites.

Increased levels of specific and nonspecific IgE are found in many **helminth infections**. IgE initiates mast cell and basophil degranulation, but in general this response has not been shown to be effective. Antibodies can also inhibit the invasion of red cells by protozoan parasites such as *Plasmodium (Butcher et al. 1978)* and *Babesia.* Antibody-mediated neutralization of enzymes and other destructive molecules released by parasites may be protective. Finally, reaginic IgE antibodies are often elevated in parasitized hosts; these are considered helpful in the elimination of parasites, presumably via mast cell degranulation and immediate hypersensitivity responses. In the case of many intestinal parasites, e.g., *Nippostrongylus brasiliensis,* the initial damage by antibody is followed by T-cell participation resulting in expulsion of the worm. (Chandra,

1982). Additionally, the elevated levels of this antibody seem to be unusual in that they rarely cause severe anaphylactic responses, or even worsening of asthma in persons in endemic areas, although visitors seem to be more susceptible to these problems.

2. Complement

Schistosomula may initiate activation of both the classical and alternative complement pathways; the alternative pathway activation may kill larvae in the absence of antibody.

3. Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)

Although phagocytic cells have some direct activity against parasitic organisms, the most effective protection in some parasitic infections is provided by the mechanism of antibody-dependent cellular cytotoxicity (ADCC). Macrophages, neutrophils, and eosinophils may demonstrate direct toxicity or phagocytosis toward parasites previously coated with antibody derived from activated B lymphocytes, possibly with the assistance of "helper" T-Iymphocytes. The clearest example of ADCC is seen in the *in vitro* killing of schistosomula. Schistosomula may be killed by incubation with antibodies and eosinophils, but not by eosinophils alone. Although most of the *in vitro* studies have demonstrated the efficacy of eosinophils in ADCC, there is evidence that neutrophils and macrophages are cytotoxic in a similar manner. The actual attachment of the cytotoxic cells is most frequently mediated by IgG, although IgE may also be effective. ADCC is also felt to be protective in trichinosis and some filarial diseases.

4. Eosinophils

The role of eosinophils in parasitic infection is complex. There is a qualitative difference in eosinophils collected from persons with eosinophilia such that there is a correlation between the peripheral eosinophil count and killing of schistosomula in vitro: this appears to be true regardless of the cause of the eosinophilia. Eosinophils are attracted to an area by eosinophil chemotactic factors (ECF), which are elaborated by a variety of intravascular stimuli including complement and antigen-stimulated leukocytes.

Additionally, eosinophils may phagocytose immune complexes and act as effector cells mediating local type I reactions, primarily in tissue stage parasites, especially larval forms. Damage to schistosomula by eosinophils and antibodies is mediated by major basic protein (MBP), found in eosinophilic granules. *In vivo* studies reveal that immunity can be inhibited by the use of an anti-eosinophilic serum, whereas antisera against other effector cells cause no such effect.

5. Macrophages

Macrophages participate both in antigen-specific and nonspecific immune responses, but principally in the latter. Phagocytes of the host provide a home for the survival and proliferation of many protozoa, e.g., *Toxoplasma gondii, Trypanosoma cruzi,* and *Leishmania* species. The parasites evolve methods of dealing with the intracellular environment, which is generally inimical to pathogens. In fact, for some parasites

pathogenicity may depend on the ability to grow in activated macrophages. In specific instances, there is failure of phagosome fusion with lysosomes (for example, *T. gondiii,* escape into cytosol, (for example, *T. cruzi* and *Mycobacterium leprae)*, resistance to lysosomal enzymes, and reduced expression of surface H-2 molecules (for example, *Leishmania* species).

The initial steps in the interaction between macrophages and parasites involve recognition, adhesion, and phagocytosis. The activation of complement by parasites may generate chemotactic molecules that attract phagocytes at the site of action. For some organisms, e.g., *Leishmania* and *Trypanosoma,* ingestion in vitro can proceed in the absence of serum factors. Pretreatment of phagocytes with chymotrypsin, which does not influence complement-mediated phagocytosis of red cells but blocks the attachment and phagocytosis of *T. cruzi,* suggests that C3b receptors are not essential for ingestion.

The formation of a micro filamentous mesh is an important prerequisite because this step is blocked by cytochalasin B. The parasitophorous vacuole is lined by a membrane derived essentially from the plasma membrane of the host cell. The size of the vacuole relative to that of the parasite can vary considerably. Fusion of vesicles as well as influx of fluid into secondary lysosomes can alter the size of the vacuole, but the significance of these processes and of vacuole size for the survival of intracellular parasites is not known.

The next steps of fusion with primary or secondary lysosomes, generation of microbicidal activity, parasite inactivation, and digestion or extrusion are enhanced by macrophage activation. Certain parasites, on their part, have evolved mechanisms for evasion of the lethal effects of phagosome-lysosome fusion. Some of these mechanisms are inhibition of phagolysosome formation, resistance to the intralysosomal milieu, and escape to extravacuolar spaces. The cytotoxic mechanisms of macrophages include hydrolytic enzymes and synthesis of highly reactive oxygen metabolites including the superoxide anion, hydrogen peroxide,

6. T Cells

Frequently, sequestration of the organism is T-cell dependent, a classical delayed hypersensitivity response. This isolation protects the host from parasitic toxins and may also inhibit the growth and reproduction of the parasite. Additionally, T cells may have direct activity against parasitic cells, especially if they display histocompatibility antigens. "Helper" T cell may help sensitize B cells to specific antigens. T-cell-deficient mice are very slow to recover from some forms of malaria, but it is unclear whether this is due to a deficiency in delayed hypersensitivity, cytotoxicity or helper T cell.,

Evasion and avoidance of the immuune response

Parasites have developed some fascinating ways of evading the host's immune response, to which they are exposed in most situations for much of their life cycle. Some examples are presented in the following paragraphs.

I. Localization in Immunologically Deprived Sites

Visceral larva migrants frequently inhabit the posterior chamber of the eye; *Echinococcus* and *Trichinella* form cystic structures that afford some protection from the immune system.

II. Imitation of Host Antigens

Adult *Schistosoma* species, which by the nature of their location in the blood stream are continually exposed to the immune system, have the ability to incorporate host antigens on their surface and thus can be unrecognized as outsiders by the immune system.

III. Antigenic Variation

The African trypanosome survives in the human host by periodically altering its surface antigenic coat, thus aborting the developing immune response of the host. Research has suggested that a single clone of trypanosomes may produce more than 100 variable antigenic types. There are suggestions that other species of parasites may have a similar ability, including some malarias and even *Entamoeba histolytica*.

IV. Antigenic Depletion

In antigenic depletion, shedding of antigens from the surface of the organism provides protection from the immune response; *Leishmania* and some schistosomes practice this deception.

V. Induction of Immunosuppression

Many blood-borne parasites and some helminths have been shown to cause diminished T- and B-cell responses. An actual alteration in the histologic appearance of the lymph node may be seen. Frequently, excess "nonspecific immunoglobulins are found, caused by a polyclonal B-cell activation, which may block effector sites from activation with "protective" immunoglobulins. The resulting immunosuppression is not antigen-specific, making the individual susceptible to other infections, and making response to vaccination more difficult. In addition to modification of lymphocyte function, other protective cells may be affected. Children infected with visceral larva migrants have been shown to have defects in neutrophil function, possibly secondary to high levels of IgE.

VI. Adaptation to Intracellular Environment

Some protozoan organisms have developed special resistance to the generally hostile environment found in macrophages, and this resistance allows them to proliferate within the cells. *Toxoplasma gondii* can prevent the fusion of lysosomes with its parasitophorous vacuole; *T. cruzi* escapes the vacuole and reproduces intracytoplasmically; and

Leishmania species are resistant to lysosomal enzymes, possibly because of their surface saccharide coats.

Probable questions:

1. State the role of IgE antibody during parasitic infection in host body/ What is the changes occur in IgE antibody concentration in host body during parasitic infection?
2. What is ADCC? What ADCC play role in parasitic infection?
3. Describe the role of eosinophils in parasitic infection.
4. State the role of macrophage in parasitic infection.
5. When a parasite attacks a host body, what are the changes occur in T cells?
6. Describe the mechanism of interaction between macrophage and parasite in host body.
7. Describe different immunological changes in host due to parasitic infection.
8. Describe evasion and avoidance techniques of different parasite from host's immune response.

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Unit XII

Basic immunological changes due to antigen vaccination

Objective: In this unit we will discuss about basic immunological changes due to antigen vaccination.

Introduction

Vaccination is getting or giving a vaccine (injection, oral or nasal spray) to prevent infectious disease.

Immunity means your body has been exposed to a germ (or part of it, or something that mimics it) and your immune system has developed antibodies to fight it. When you are immune to a germ, it will not make you sick if you encounter it again.

Immunization is the process of your body becoming resistant to a germ (pathogen) after you get a vaccine.

Inoculation is introducing a pathogen or vaccine to the body to help someone develop immunity to a disease.

Pathogens are what we commonly call germs: the viruses, bacteria, fungi, parasites and other microbes that can enter our bodies and cause infection and illness.

Antigens are anything present in the body that the immune system recognizes as an invader and tries to fight. Vaccines put antigens into the body.

Antibodies are Y-shaped proteins produced by immune cells in the body to fight pathogens. Most people have hundreds of millions of different kinds of antibodies.

- ## Immunizing agents

Immunization refers to the process by which a person becomes protected against a disease through exposure to immunizing agents. Immunizing agents are classified as active or passive, depending on the process by which they confer immunity; prevention of disease through the use of immunizing agents is called immunoprophylaxis. The development of an immune response to an immunizing agent depends on several factors, including the type of agent, the recipient's age, prior exposure to the antigen, and the presence of immune-compromising conditions.

Active immunization is the inherent production of antibodies against a specific agent after exposure to the antigen through vaccination. Active immunizing agents are typically referred to as vaccines. For many active immunizing agents, a protective immune response is typically achieved within a few weeks of vaccination. For example, it takes about two weeks after vaccination to reach the protective levels of humoral antibodies that are associated with immunity to influenza infection.

Passive immunization involves the transfer of pre-formed antibodies (including mAbs) to provide immediate, temporary protection from infection or to reduce the severity of illness caused by the infectious agent. Passive immunization can occur by trans placental transfer of maternal antibodies to the developing fetus, or it can be provided by administration of a passive immunizing agent. Protection provided by passive immunization is immediate upon administration but temporary and typically of shorter duration compared to active immunization because the transferred antibodies degrade more quickly over time.

- ## Vaccines

Vaccines are complex biologic products designed to induce a protective immune response effectively and safely. They have historically been developed and tested for their ability to elicit a humoral (antibody-based) immune response. An ideal vaccine is: safe with minimal adverse effects; effective in providing lifelong protection against disease after a single dose that can be administered at birth; inexpensive; stable during shipment and storage; and easy to administer. Some vaccines come closer to fulfilling these criteria than others. Although each vaccine has its own benefits, risks, indications and contraindications, all vaccines offer protection against the disease for which they were created.

All vaccines undergo a very rigorous development process. The first steps in the development of a vaccine include the identification of the microorganism or toxin that causes an important burden of disease in the population. Once the pathogen is identified, research is initiated into the possibility of developing a vaccine to reduce the disease incidence, or severity, or both.

- ## Type of vaccine:

Vaccines can be classified according to the type of active component (antigen) they contain and their replication capacity. Generally, vaccines are most often categorized in two groups – live attenuated vaccines and non-live vaccines:

I. **Live attenuated vaccines** contain whole, weakened bacteria or viruses that have the capacity to replicate within a host. Thus, the stimulus to the immune system of a vaccine recipient more closely resembles that associated with natural infection, resulting in longer lasting and broader immunity than can be achieved with other vaccine types. Because of the strong immunogenic response, live attenuated vaccines, except those administered orally, typically produce immunity in most recipients with one dose; however, a second dose helps to make sure that almost all vaccine recipients are protected, because some individuals may not respond to the first dose. Live vaccines require careful storage and handling to avoid inadvertent inactivation.

II. **Non-live vaccines** contain whole inactivated (killed) bacteria or viruses, their parts, or products secreted by bacteria that are modified to remove their pathogenic effects (toxoids). Non-live vaccines also include messenger ribonucleic acid (mRNA) vaccines. Non-live vaccines cannot cause the disease they are designed to prevent. Because the immune response to non-live vaccines may be less than that induced by live organisms, they often require adjuvants and multiple doses. The initial doses prime the immune system and are called primary vaccination or the primary series. As protection following primary vaccination diminishes over time, periodic supplemental doses (booster doses) may be required to increase or boost antibody levels.

III. **Inactivated Vaccines:** Many of the first vaccines were created this way. After isolating the virus or bacterium, manufacturers grow a large population of these germs and treat them with heat or chemicals to inactivate them before putting them into vaccines.

Though inactivated vaccines may cause fewer or less intense side effects, they may result in less robust immunity than a live attenuated vaccine and may require booster shots.

Examples of inactivated vaccines: hepatitis A, some forms of flu, polio and rabies vaccines

IV. **Subunit (Recombinant, Polysaccharide or Conjugate) Vaccines:** After isolating and growing the germs, vaccine manufacturers isolate a part of it (such as a protein, a sugar or part of its surface) that is harmless by itself but is recognized by the immune system as an invading antigen. This part (which is harmless on its own) is manufactured and put it into a vaccine that activates the immune system against the entire pathogen if it enters the body. Because vaccine makers only use a part of the pathogen, these vaccines can be safer for people with weakened immune systems.

Examples of subunit vaccines: pertussis (whooping cough), hepatitis B, shingles, meningitis and human papillomavirus (HPV) vaccines

V. **Messenger RNA (mRNA) Vaccines:** Some vaccines contain instructions to your cells to create a part of a virus, which cannot cause disease on its own. Messenger RNA is a molecule that tells our bodies to make proteins. mRNA from a virus tells our cells to make harmless proteins just like those on a harmful virus.

The presence of the pathogen part in your body triggers your immune system to create antibodies that protect you from that virus.

Examples of mRNA vaccines: the two-dose COVID vaccines

VI. **Toxoid Vaccines:** Some bacteria release toxic substances. Some of these toxins can be made into toxoids by isolating and modifying them so they are not harmful. A vaccine that contains a small amount of toxoid can help a person's immune system learn to fight the bacterium.

Examples of toxoid vaccines: tetanus and diphtheria vaccines

VII. Viral Vector Vaccines: Viral vector vaccines use a harmless deliverer (vector), such as a type of adenovirus. To make the vaccine, an adenovirus is loaded with information that informs cells how to manufacture a look-alike, harmless part of the disease-causing virus.

The vaccines deliver "directions" from the vector, telling cells how to create copies of the harmless part of the virus. The cells make copies of the part, which accumulate in the body, triggering the immune system to create antibodies to fight them.

VIII. Cancer Vaccines: Exciting new developments in research are using vaccines to supercharge the immune system to fight certain kinds of cancer. For instance, a vaccine for pancreatic cancer uses principles of immunotherapy to help the immune system recognize and destroy cancerous cells

- ## How vaccines work

Administration of a vaccine antigen triggers an inflammatory reaction that is initially mediated by the innate immune system and subsequently expands to involve the adaptive immune system through the activation of T and B cells. While the majority of vaccines have been studied to provide protection through the induction of humoral immunity (primarily through B cells), some vaccines, such as Bacille Calmette-Guérin (BCG) and live herpes zoster vaccines, act principally by inducing cell-mediated immunity (primarily though T cells). Long-term protection requires activation of both T and B cells. Although humoral immunity is the basis most often used as a marker of how well a vaccine works study of cellular immune markers of protection is an area of active research.

After vaccination, the antigen enters your body. Special cells called antigen-presenting cells, or APCs, circulate throughout the body looking for invaders.

When an APC encounters the antigen from the vaccine, it consumes it. Then, the APC displays a part of the antigen on its outer wall, like a trophy that other cells in the immune system can detect. The APC, with the antigen part on its outer surface, reports to areas of the immune system such as the lymph nodes, where cells called T-cells are found. The T-cells, which include helper T-cells, are activated and alert other cells to fight the antigen.

B-cells are another weapon of the immune system. These cells recognize the antigen when activated by the T-cells, or simply by encountering antigens in the body.

Others are plasma B-cells, which produce antibodies the body uses against the pathogen. Antibodies are Y-shaped proteins created in huge numbers by the plasma B-cells. They interact with and bind to antigens, and either destroy them or make it so that they cannot enter a cell and cause disease.

Some vaccines contain a weakened (attenuated) form of a particular bacterium or virus, which enters cells in the body. Killer T-cells, informed of the antigen's presence by the APCs, then destroy those infected cells.

Memory cells such as memory B-cells, memory helper T-cells and memory killer T-cells make sure that the immune system will recognize if the real pathogen appears. When the immune system is triggered a second time by the same antigen, it responds with more speed and strength to destroy the invader, creating even more antibodies and memory cells to fight it.

Herd immunity refers to the immunity of a population against a specific infectious disease. The resistance of that population to the spread of an infectious disease is based on the percentage of people who are immune and the probability that those who are still susceptible will come into contact with an infected person. The proportion of the population required to be immune to reach herd immunity depends on a number of factors, the most important one being the transmissibility of the infectious agent either from a symptomatically infected person or from an asymptomatically colonized person.

Probable questions:

1. What is vaccination and immunization?
2. Differentiate between vaccination and immunization.
3. What is inoculation of vaccination process.
4. What is active immunization and passive immunization?
5. Describe different types of vaccines with example.
6. What is live attenuated vaccine? Give example.
7. What is killed vaccine? Give example.
8. What is inactivated vaccine? Give example.
9. What Messenger RNA (mRNA) Vaccines? Give example.
10. Comment on subunit vaccine.
11. Describe the working procedure of vaccine after administration in the host body.

12. What is memory cell?
13. What is plasma cell?
14. What is herd immunity?
15. Briefly describe the immunological changes in host body due to administration of immunizing agents.

Suggested reading:

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Unit XIII

Basic immunological changes due to immunopathology

Objective: In this unit we will learn about basic immunological changes due to immunopathology.

Introduction

Immunopathology refers to the study of the damage caused to an organism by its own immune system, often in response to an infection or other stimulus.

Defects or malfunctions in either the innate or adaptive immune response can provoke illness or disease. Such disorders are generally caused by an overactive immune response (known as hypersensitivity reactions), an inappropriate reaction to self (known as autoimmunity) or ineffective immune responses (known as immunodeficiency).

- **Hypersensitivity reactions**

1. Hypersensitive reaction may develop through a humoral or a cell mediated immune response.

2. Anaphylactic reactions initiated by antibody or antigen antibody complexes are referred to as immediate hypersensitivity because the symptoms are manifest within minutes or hours after a sensitized recipient encounters antigen.

3. Delayed-type hypersensitivity (DTH) is named in recognition of the delay of symptoms until days after exposure.

4. Two immunologists, P. G. H. Gell and R. R. A. Coombs, proposed a classification scheme to discriminate among the various types of hypersensitivity. Hypersensitivities are classically divided into four categories (types I–IV) that differ by the immune molecules and cells that cause them, and the way they induce damage.

- Type I: immediate hypersensitivity.
- Type II: cytotoxic or antibody-dependent hypersensitivity.
- Type III: immune complex disease.
- Type IV: delayed-type hypersensitivity.

I. Type I hypersensitivity

Type I hypersensitivity is the most common type of hypersensitivity reaction. It is an allergic reaction provoked by re-exposure to a specific type of antigen, referred to as an allergen.

There are two stages to type 1 hypersensitivity: the sensitization stage and the effect stage.

Unlike the normal immune response, the type I hypersensitivity response is characterized by the secretion of IgE by plasma cells. IgE antibodies bind to receptors on the surface of tissue mast cells and blood basophils, causing them to be "sensitized". During the sensitization stage, the person encounters the antigen but does not experience any symptoms. Next stage is effect stage. During the effect stage, the person has exposure to the antigen again. As the body now recognizes the antigen, it is able to produce a response that results in the symptoms that people typically experience with an allergic reaction.

Exposure to the same allergen cross-links the bound IgE on sensitized cells resulting in degranulation and the secretion of active mediators such as histamine, leukotrienes, and prostaglandins that cause vasodilation and smooth-muscle contraction of the surrounding tissue.

Common environmental allergens inducing IgE-mediated allergies include

- food products, such as nuts, shellfish, and soy
- animal sources, such as cats, rats, or bee stings
- environmental sources, such as mold, latex, and dust, pollen
- allergic conditions, such as allergic rhinitis, allergic asthma, and conjunctivitis

However, these types of reactions are more frequently seen in children than adults.

Treatment of type I reactions generally involves trigger avoidance, and in the case of inhaled allergens, pharmacological intervention with bronchodilators, antihistamines and anti-inflammatory agents.

Type I reactions underlie all atopic disorders (eg, atopic dermatitis, allergic asthma, rhinitis, conjunctivitis) and many allergic disorders (eg, anaphylaxis, some cases of angioedema, urticaria, latex and some food allergies). The terms atopy and allergy are often used interchangeably but are different:

- **Atopy** is an exaggerated IgE-mediated immune response; all atopic disorders are type I hypersensitivity disorders.
- **Allergy** is any exaggerated immune response to a foreign antigen regardless of mechanism.

Thus, all atopic disorders are considered allergic, but many allergic disorders (eg, hypersensitivity pneumonitis) are not atopic.

Atopic disorders most commonly affect the nose, eyes, skin, and lungs. These disorders include conjunctivitis, extrinsic atopic dermatitis (the most common type of

eczema), immune-mediated urticaria, some forms of angioedema, acute latex allergy, some allergic lung disorders (eg, allergic asthma, IgE-mediated components of allergic bronchopulmonary aspergillosis), allergic rhinitis, and allergic reactions to venomous stings.

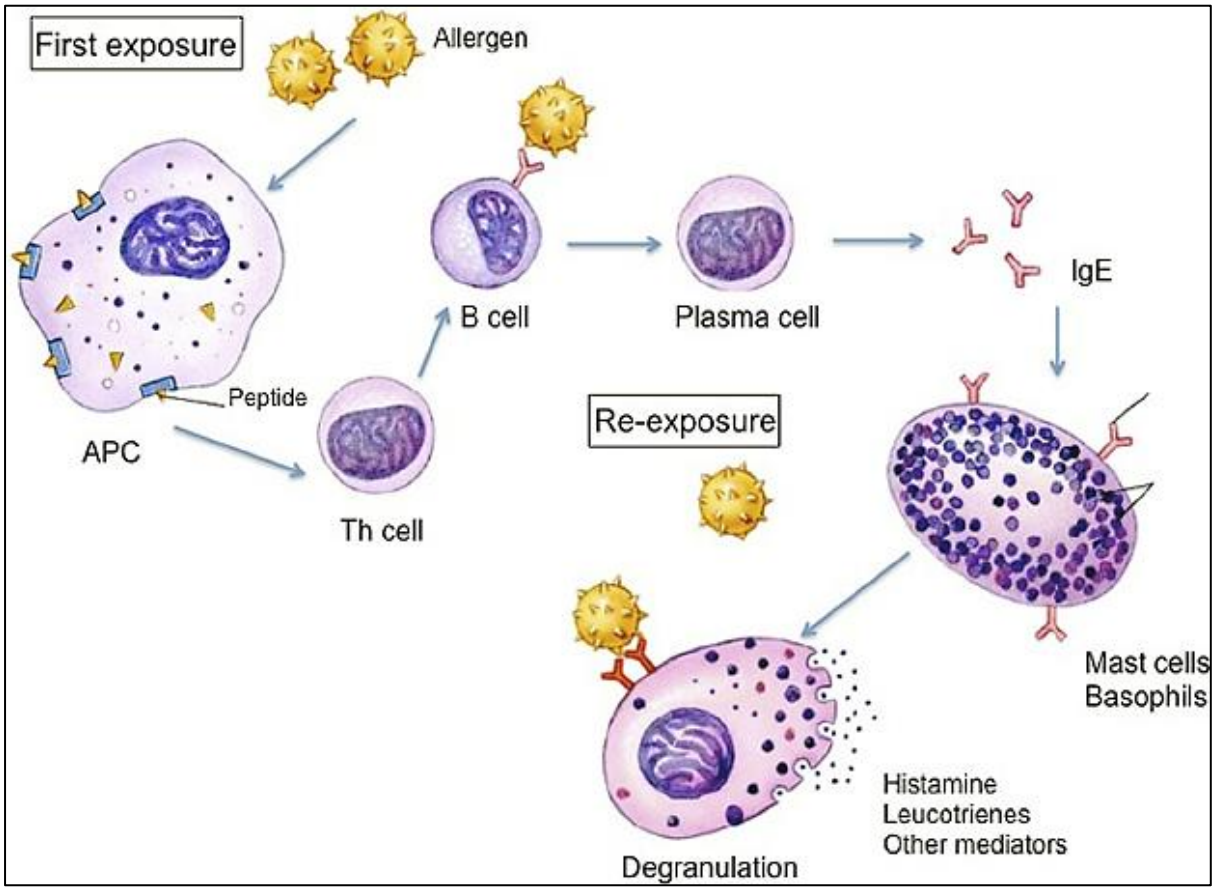


Fig: Type I hypersensitivity reaction (Source: https://www.onlinebiologynotes.com/)

II. Type II hypersensitivity

Type II hypersensitivity reaction refers to an antibody-mediated immune reaction in which antibodies (IgG or IgM) are directed against cellular or extracellular matrix antigens, resulting in cellular destruction, functional loss, or tissue damage.

This can cause long-term damage to cells and tissues, resulting in conditions such as:

- the blood disorder immune thrombocytopenia if there are not enough platelets
- autoimmune hemolytic anemia if the red blood cells burst
- autoimmune neutropenia if the body destroys neutrophils
- autoimmune conditions such as Graves' disease, Hashimoto thyroiditis
- It also include hyperacute graft rejection of an organ transplant

Mechanism of Type II Hypersensitivity Reactions

- The reaction is completed in two phases – sensitization phase and effector phase.
- **A sensitization phase** leads to production of antibodies that recognize substances or metabolites that accumulate in cellular membrane structures. **In the effector phase**, target cells become coated with antibodies which lead to cellular destruction.
- Antibody bound to a surface antigen can induce the death of the antibody-bound cell by three distinct mechanisms – by activation of the complement system, cell destruction by antibody dependent cell mediated cytotoxicity (ADCC) or by the process of opsonization.

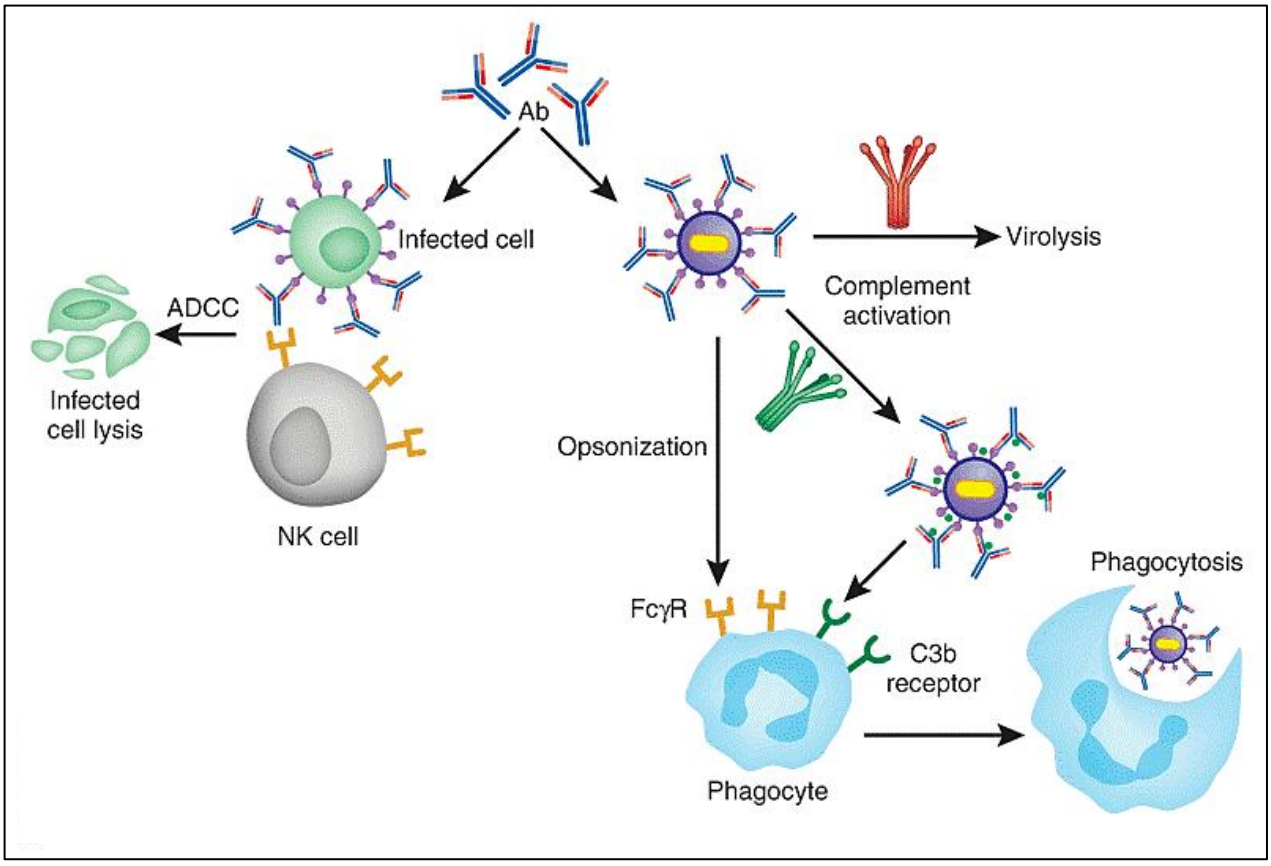


Fig: Type II hypersensitivity reaction (Source: https://www.onlinebiologynotes.com/)

- First, IgG or IgM antibodies coating target cells can bind to F_c receptors present on cells such as macrophages and neutrophils and mediate phagocytosis.
- IgG or IgM antibodies can also activate complement via the classical pathway. This leads to deposition of C3b, which can mediate phagocytosis. Complement activation also leads to production of the membrane attack complex (MAC), which forms pores in the cellular membrane resulting in cytolysis.

- Finally, IgG antibodies can bind FcγRIII on NK cells and macrophages, thus mediating release of granzymes and perforin and resulting in cell death by apoptosis (ADCC).
- Alternatively, the antigen-antibody complex thus formed may be non- cytolytic but impair the normal functioning of the cell in which the antibody interrupts the cell receptor function. Such antibodies are referred to as" antireceptor antibodies".

Examples of Type II (antibody-mediated immune reaction) Hypersensitivity

1. **Rhesus incompatibility (Rh hemolytic disease)**

During subsequent pregnancies, when Rh –ve mother conceive Rh +ve fetus, small numbers of fetal erythrocytes that pass across the placenta stimulate a memory response which results in IgG antibodies destroying the fetal erythrocytes (hemolytic disease of the newborn).

2. **Transfusion Reactions**

Natural antibodies to major blood group antigens (A, B) bind to transfused erythrocytes carrying the target antigens resulting in massive hemolysis.

3. **Cell Destruction due to Autoantigens**

Antibodies to a variety of self-antigens such as basement membranes of lung and kidney (Goodpasture's Syndrome), the acetylcholine receptor (Myasthenia Gravis) and erythrocytes (Autoimmune Hemolytic Anemia) can result in tissue damaging reactions.

4. **Drug Induced Hemolytic Anemia**

Drugs such as penicillin, cephalosporin and streptomycin can absorb non-specifically to surface proteins on erythrocytes and cause IgG-mediated damage to such red cells.

III. Type III hypersensitivity

- Type III hypersensitivity is primarily mediated by antibodies of the IgG and IgM classes which combine with soluble antigen that are not bound to cell surfaces. The antigens may be self or foreign (i.e., microbial). Tissue damage is caused mainly by complement activation and release of lytic enzymes from neutrophils.
- The reaction can take hours, days, or even weeks to develop, depending on whether or not there is immunological memory of the precipitating antigen. The response can also become chronic, particularly in autoimmune reactions, where antigen persists.
- Type III hypersensitivity as in other cases of hypersensitivity occur when the mechanism of self-tolerance is breached and some self-reactive immune cells

are activated to mount reactions against auto antigens such as the DNA from an auto cell.

The mechanism of both the types can be summarized as follows:

1. Antigen-antibody complexes are formed when antibodies bind to antigens.
2. In case the complex is not cleared by normal process of phagocytosis; the immune complexes persist in the circulation.
3. The immune complexes subsequently deposit in tissues.
4. The tissue deposited complexes activate the classical complement cascade.
5. The complement fragments (e.g. C_3a and C_5a) that form during complement activation activate a variety of potent mediators of inflammation causing an influx of neutrophils and monocytes to the site of deposition.
6. The attracted neutrophils attempt to engulf the immune complexes. Since the complexes are deposited over the tissues, the neutrophils do not succeed.
7. Consequently, the neutrophils release a number of substances like prostaglandins, lysosomal enzymes, and free oxygen radicals over the complexes causing damage to the tissues at the site of immune complex deposition.
8. Additionally, the binding of the Fc region of antibody in the immune complex may bind to the Fc receptor on platelets causing aggregation, blood clots and blockage of blood vessels leading to haemorrhages at the site.

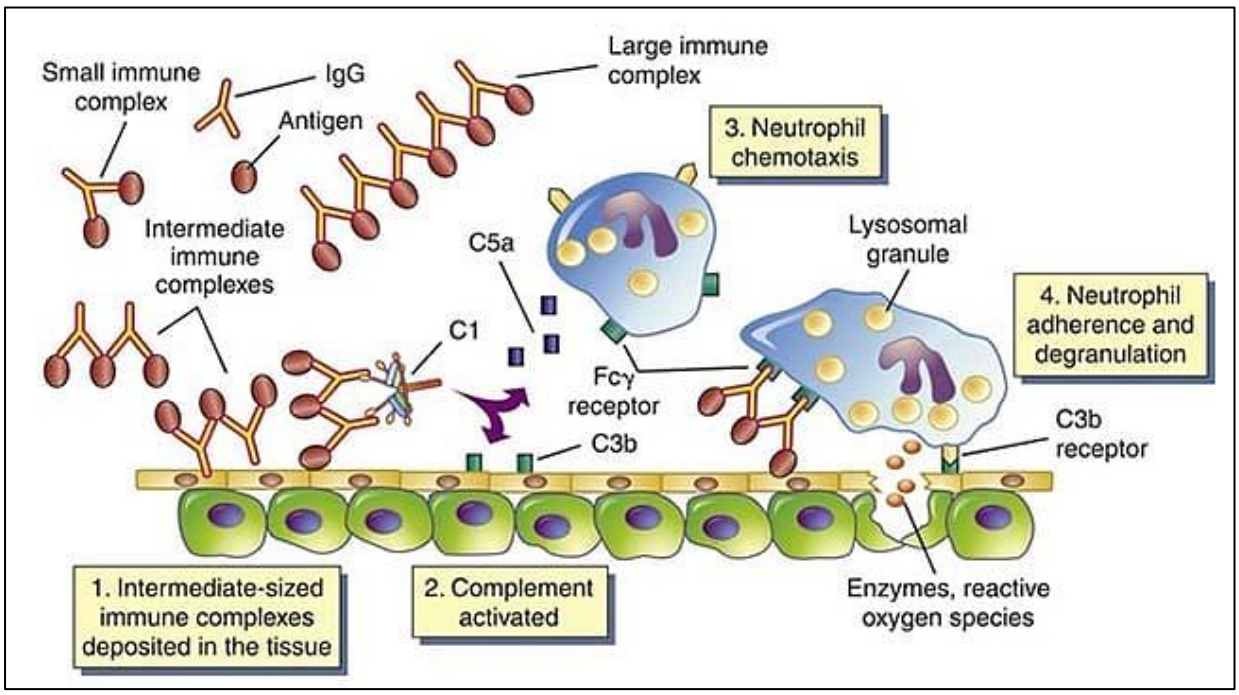


Fig: Type III hypersensitivity reaction (Source: https://www.onlinebiologynotes.com/)

Examples of Type III (Immune Complex) Hypersensitivity

1. **Systemic Lupus Erythematosus**: Antibodies are made that bind to certain nuclear antigens which deposit mainly in the kidneys, skin, and joints.
2. **Post-streptococcal Glomerulonephritis**: While fighting a Streptococcal infection, the patient makes antibody that reacts against the pathogen but also cross-react with glomerular antigen which cause antigen-antibody complexes to lodge on glomerular membrane.
3. **Drug Induced Serum Sickness:** Since most drugs are poor immunogens, they act as a hapten by combining with tissue protein in the host and induce immune responses against the drug-host protein complex.
4. **Farmer's Lung** and **Bird fancier's disease:** Pulmonary diseases resulting from inhalation of bacterial spores and avian serum/faecal proteins respectively

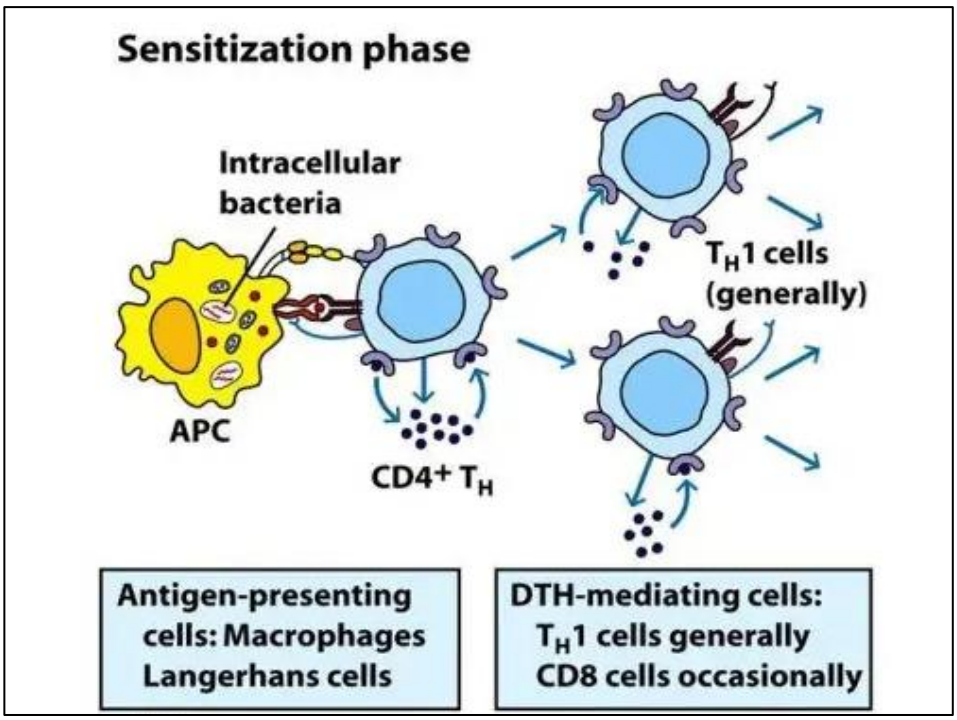
IV. Type IV hypersensitivity

- Type IV hypersensitivity reaction also known as cell mediated hypersensitivity or delayed type of hypersensitivity is the T lymphocytes mediated destruction of cells along with dendritic cells, macrophages and cytokines playing major roles.
- The reaction is mediated by specific subsets of CD4+ helper T cells (Th-1 and Th-17 cells) or by CD8+ cytotoxic T cells.
- Type IV hypersensitivity occurs 24 hours after contact with an antigen, usually starting at 2 or 3 days and often last for many days.
- For this reason, type IV hypersensitivity reaction is termed as "delayed hypersensitivity".
- Type IV hypersensitivity is unique in that, unlike the first three types of hypersensitivity which are antibody mediated, type IV hypersensitivity is cell mediated and also a delayed reaction.

Mechanism of Type IV (Cell Mediated) Hypersensitivity

- In type IV hypersensitivity reaction, when a sub-population of CD4 Th1 cells encounter certain type of antigens, they produce cytokines which induce a localized inflammatory reaction mediated by non-specific inflammatory cells most prominently macrophages.
- The antigens involved may be either intracellular pathogens such as *Mycobacterium tuberculosis*, *Listeria monocytogens, Histoplasma capsulatum,* Herpes Simplex virus etc. or contact antigens such as Nickel salts, Poison ivy etc.
- The reaction is accomplished in two phases: the initial sensitization phase and the later effector phase.

- In the sensitization phase, the primary contact with the antigen is established. During this period, specific Th cells are sensitized and are clonally expanded.



- In the effector phase, a subsequent exposure to the same antigen induces the delayed type hypersensitivity response.

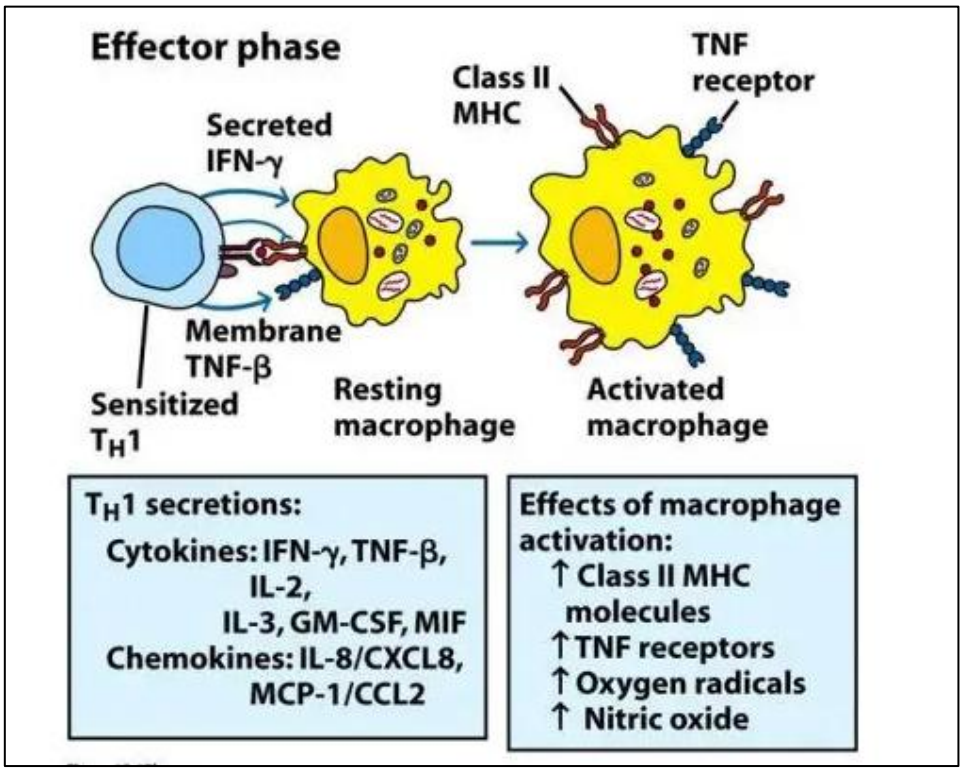


Fig: Type IV hypersensitivity reaction

- Cytokines such as IL-2 and interferon gamma is released inducing the further release of other Th1 cytokines, which mediates the immune response activating macrophages and other non-specific inflammatory cells.
- Activated CD8+ T cells on the other hand, destroy target cells on contact, whereas activated macrophages produce hydrolytic enzymes and on presentation with certain intracellular pathogens, transform into multinucleated giant cells.

Variants of Type IV (Cell Mediated) Hypersensitivity

1. **Contact Hypersensitivity**

- Occurs after sensitization with simple chemicals (eg, nickel, formaldehyde), plant materials (poison ivy), some cosmetics, soaps, and other substances.
- Small molecules enter the skin and acting as haptens attach to body proteins which induce cell-mediated hypersensitivity particularly in the skin.
- On subsequent exposure to the agent, the sensitized person develops erythema, itching, vesication, eczema, or necrosis of skin within 12-48 hours.

2. **Tuberculin -type Hypersensitivity**

- Occurs due to sensitization of soluble antigens of microorganisms during many infectious diseases.
- It is exemplified by tuberculin reaction in which when a small amount of tuberculin is injected into the skin of a person previously exposed to *Mycobacterium tuberculosis*, mononuclear cells accumulate in the subcutaneous tissue along with abundance of CD4 Th1 cells leading to induration and redness developing at its peak in 24–72 hours.

Examples of Type IV (Cell Mediated) Hypersensitivity

1. **The tuberculin reaction (Mantoux test):** This is a 'recall' response to purified mycobacterial antigens and is used as the basis of a diagnostic skin test for an immune response to tuberculosis.
2. **Granuloma formation:** The inability to kill intracellular pathogens in macrophages often results in a chronic stimulation of the pathogen specific T cells. The cytokines produced are responsible for 'walling off' the macrophages containing the persistent antigens and thus the production of granulomas.
3. **Allergic contact dermatitis:** Environmental chemicals, metals or topical medications causing epidermal necrosis, inflammation, skin rash and blisters.

4. **Type-1 diabetes:** The killing of the pancreatic islet cells by cytotoxic T cells resulting in insulin deficiency.

The key features of the four types of hypersensitivity reaction.

type	Mediated by	Timeframe	Examples
Type I hypersensitivity	IgE antibody	Immediate (minutes)	Allergy, anaphylaxis, atopy
Type II hypersensitivity	IgG or IgM antibody (cytotoxic)	Hours to days	Haemolytic disease of the newborn, autoimmune haemolytic anaemia, Goodpasture's syndrome
Type III hypersensitivity	Antigen-antibody immune complexes	Hours to days/weeks	Serum sickness, RA, SLE, post-streptococcal glomerulonephritis
Type IV hypersensitivity	T cells	Delayed (24 to 72 hours)	Contact dermatitis, tuberculin skin test

Autoimmunity

Normally the function of immune system in our body is to recognize foreign elements and to destroy them before they could harm us either by humoral immune response or cell mediated immune responses. Thus, the immune system defends the body against infections and certain other diseases by identifying, attacking, and destroying the foreign substances. Sometimes the immune system makes a mistake and starts attacking the body's own tissues or organs. This is called autoimmunity. Autoimmunity is the system of immune responses of an organism against its own healthy cells and tissues. It refers to the body's development of intolerance of the antigens on its own cells i. e. there is an immune response to one's own tissue antigens. This type of body response results in a disease state characterized by a specific antibody or cell-mediated immune response against the body's own tissues (autoantigens). So, we can say that autoimmunity is the breakdown of mechanisms responsible for self-tolerance and induction of an immune response against components of self. Any disease that results from such an abnormal immune response is termed as autoimmune diseases. There are many autoimmune diseases one example being type 1 diabetes in which the Islets cells (produce Insulin) in the pancreas are destroyed by the immune system. An autoimmune disease is a case of mistaken identity resulting in failure of the immune system to differentiate between self and non-self-components; in which the body reacts against its own constituents of

tissues. It is not yet known exactly what causes the autoimmune diseases; therefore, the treatment of such disease is very challenging one.

Organ specific or localized autoimmune diseases

This is again subdivided into two categories:

i) Autoimmune diseases which are mediated by direct cellular damage occur when lymphocytes or antibodies bind to cell-membrane antigens, causing cellular lysis and/or an inflammatory response in the affected organ. Gradually, the damaged cellular structure is replaced by connective tissue (scar tissue), and the function of the organ declines.

ii) Autoimmune disease which are mediated by stimulating or blocking autoantibodies. In some autoimmune diseases, antibodies act as agonists, binding to hormone receptors instead of the normal ligand.

- **Examples of organ-specific autoimmune disease mediated by cell damage**

a) Hashimoto's thyroiditis

Hashimoto's thyroiditis, also known as chronic lymphocytic thyroiditis and it is an autoimmune disease in which the thyroid gland is gradually destroyed. In earlier period it does not show any symptoms but over time the thyroid may enlarge forming a painless goitre.

Symtoms: weight gain, constipation, depression, and general pains and after many years the thyroid typically swollen in size. Potential complications include thyroid lymphoma. Antibodies are formed to a number of thyroid proteins, including thyroglobulin and thyroid peroxidase, both of which are involved in the uptake of iodine. Binding of the auto-antibodies to these proteins interferes with iodine uptake and leads to decreased production of thyroid hormones (hypothyroidism). In Hashimoto's thyroiditis, which is most frequently seen in middle-aged women, an individual produces auto-antibodies and sensitized TH1 cells specific for thyroid antigens.

b) Goodpasture's syndrome

In this disease, auto-antibodies specific for certain basement-membrane antigens bind to the basement membranes of the kidney glomeruli and the alveoli of the lungs. Subsequent complement activation leads to direct cellular damage and an ensuing inflammatory response mediated by a buildup of complement split products. The damage to the glomerular and alveolar basement membranes leads to progressive kidney damage and pulmonary hemorrhage. Death may ensue within several months of the onset of symptoms.

Symptoms: The first signs of Goodpasture's syndrome may include fatigue, nausea and vomiting, difficulty breathing, When Goodpasture's syndrome affects the kidneys, symptoms may include, blood in the urine, foamy urine, swelling in the legs, High blood pressure, Burning or difficulty when urinating, Back pain below the ribs

c) Insulin-dependent diabetes mellitus (IDDM):

It is caused by an autoimmune attack on the pancreas. The attack is directed against specialized insulin-producing cells (beta cells) that are located in spherical clusters, called the Islets of Langerhans, scattered throughout the pancreas. The autoimmune attack destroys beta cells, resulting in decreased production of insulin and consequently increased levels of blood glucose. Several factors are important in the destruction of beta cells. First, activated CTLs (Cytotoxic T Cells) migrate into an Islet and begin to attack the insulin producing cells. Auto-antibodies to beta cells may contribute to cell destruction by facilitating either antibody-plus-complement lysis or antibody-dependent 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 in turn cause gangrene of the extremities due to impeded vascular flow, renal failure, and blindness. If it is left untreated, death can also be result.

Symptoms: Frequent urination, excessive thirst, excessive hunger weakness and fatigue, drowsiness, irritability, blurred vision or any change in sight, nausea and vomiting, skin diseases and also sudden unexplained weight loss.

- **Examples of autoimmune disease which are mediated by stimulating or blocking auto-antibodies**

a) 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, thyroxine and tri-iodothyronine. A patient with Graves' disease produces auto-antibodies that bind the receptor for TSH and mimic 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-antibodies are called long-acting thyroidstimulating (LATS) antibodies.

Symptoms: over sweating, weight loss, nervousness, hand tremors, anxiety, an irregular or rapid heartbeat, enlargement of the thyroid gland (goiter).

b) Myasthenia gravis

Myasthenia gravis is the prototype autoimmune disease mediated by blocking antibodies. A patient with this disease produces auto-antibodies that bind the acetylcholine receptors on the motor end-plates of muscles, blocking the normal binding of acetylcholine and also inducing complement mediated lysis of the cells. The result is a progressive weakening of the skeletal muscles. Ultimately, the antibodies destroy the cells bearing the receptors.

Symptoms: The early signs of this disease include drooping eyelids and inability to retract the corners of the mouth, which gives the appearance of snarling. Without treatment, progressive weakening of the muscles can lead to severe impairment of eating as well as problems with movement.

- ## Systemic autoimmune diseases type

In systemic autoimmune diseases, the response is directed toward 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 responses and from direct cellular damage caused by auto-antibodies or by accumulation of immune complexes. These diseases are associated with auto antibodies to antigens which are not tissue specific. One example can be **polymyositis,** here the tissue involved are muscles, however the auto antibodies are found against the auto antigens which are often ubiquitous "t-RNA synthetases". Another example is rheumatoid arthritis (RA). There is symmetric poly arthritis with muscle wasting and may be associated with myositis, and vasculitis, etc. The specific marker (auto antibody) found in blood in these patients is Rheumatoid Factor (RF) which is usually 19s IgM. RF is an antibody against Fc fragment of immunoglobulins. Other systemic autoimmune diseases are polyarteritis nodosa, systemic lupus erythematosus and Sjogren's syndrome.

a) Multiple Sclerosis

Multiple sclerosis (MS) is the most common cause of neurologic disability. The symptoms may be mild, such as numbness in the limbs, or severe, such as paralysis or loss of vision. Individuals with this disease produce autoreactive T cells that participate in the formation of inflammatory lesions along the myelin sheath of nerve fibers. 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 fibers, a breakdown in the myelin sheath leads to numerous neurologic dysfunctions.

Symptoms: The symptoms may be mild, such as numbness in the limbs, or severe, such as paralysis or loss of vision.

b) Rheumatoid arthritis

It is a common autoimmune disorder; 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 auto-antibodies bind to normal circulating IgG, forming IgM-IgG complexes that are deposited in the joints. These immune complexes can activate the complement cascade, resulting in a type III hypersensitive reaction,which leads to chronic inflammation of the joints.

Symptom: It is characterised by chronic inflammation of the joints, although the hematologic, cardiovascular, and respiratory systems are also frequently affected.

❖ Treatment of the autoimmune diseases

Since the cause of the diseases are not quite well understood, the general principle is to somehow stop the immune response to self-antigens and this can be achieved by the following methods:

i. Removal of thymus (some Myasthenia Gravis patients);

ii. Often, in organ-specific autoimmune disorders, the symptoms can be controlled by administration of thyroxine, and thyrotoxicosis by antithyroid drugs.

iii. In pernicious anaemia, metabolic correction is achieved by injection of vitamin B 12, and in myasthenia gravis by administration of cholinesterase inhibitors.

iv. Conventional immunosuppressive therapy with antimitotic drugs at high doses can be used to dam down the immune response but, because of the dangers involved, tends to be used only in life-threatening disorders such as SLE.

v. The potential of cyclosporine and related drugs such as rapamycin has yet to be fully realized, but quite dramatic results have been reported in the treatment of type 1 diabetes mellitus.

vi. Anti-inflammatory drugs are prescribed for rheumatoid diseases with the introduction of selective cyclo-oxygenase-2 (COX-2) inhibitors representing a good development.

Immunodeficiency

Immunodeficiency, also known as immunocompromisation, is a condition in which the immune system's ability to fight infectious diseases and cancer is impaired or absent. Most cases are acquired ("secondary") due to external factors affecting the patient's immune system. Examples of these external factors include HIV infection and environmental factors such as nutrition. Impaired immunity can also be associated with genetic diseases/ deficiencies such as SCID. In the clinical setting, immunosuppression by

some drugs, such as steroids, can be either a side effect or a putative goal of treatment. Examples of such uses are organ transplants as an anti-rejection measure and in patients with overactive immune systems, such as autoimmune disease.

A person who has an immunodeficiency of any kind is called immunodeficient. An immunocompromised person may be particularly vulnerable to opportunistic infections in addition to the common infections that can affect anyone.

• Primary Immunodeficiency

Primary immunodeficiencies, which number more than 250, are caused by inherited defects of either nonspecific innate or specific adaptive immune defenses. In general, patients born with primary immunodeficiency (PI) commonly have an increased susceptibility to infection. This susceptibility can become apparent shortly after birth or in early childhood for some individuals, whereas other patients develop symptoms later in life. Some primary immunodeficiencies are due to a defect of a single cellular or humoral component of the immune system; others may result from defects of more than one component. Examples of primary immunodeficiencies include chronic granulomatous disease, X-linked agammaglobulinemia, selective IgA deficiency, and severe combined immunodeficiency disease.

I. Chronic Granulomatous Disease

The causes of chronic granulomatous disease (CGD) are defects in the NADPH oxidase system of phagocytic cells, including neutrophils and macrophages, that prevent the production of superoxide radicals in phagolysosomes. The inability to produce superoxide radicals impairs the antibacterial activity of phagocytes. As a result, infections in patients with CGD persist longer, leading to a chronic local inflammation called a granuloma. Microorganisms that are the most common causes of infections in patients with CGD include Aspergillus spp., Staphylococcus aureus, Chromobacterium violaceum, Serratia marcescens, and Salmonella typhimurium.

II. X-Linked Agammaglobulinemia

Deficiencies in B cells due to defective differentiation lead to a lack of specific antibody production known as X-linked agammaglobulinemia. In 1952, Ogden C. Bruton (1908–2003) described the first immunodeficiency in a boy whose immune system failed to produce antibodies. This defect is inherited on the X chromosome and is characterized by the absence of immunoglobulin in the serum; it is called Bruton X-linked agammaglobulinemia (XLA). The defective gene, BTK, in XLA is now known to encode a tyrosine kinase called Bruton tyrosine kinase (Btk). In patients whose B cells are unable to produce sufficient amounts of Btk, the B-cell maturation and differentiation halts at the pre-B-cell stage of growth. B-cell maturation and differentiation beyond the pre-B-cell stage of growth is required for immunoglobulin production. Patients who lack antibody

production suffer from recurrent infections almost exclusively due to extracellular pathogens that cause pyogenic infections: Haemophilus influenzae, Streptococcus pneumoniae, S. pyogenes, and S. aureus. Because cell-mediated immunity is not impaired, these patients are not particularly vulnerable to infections caused by viruses or intracellular pathogens.

III. Selective IgA Deficiency

The most common inherited form of immunoglobulin deficiency is selective IgA deficiency, affecting about one in 800 people. Individuals with selective IgA deficiency produce normal levels of IgG and IgM, but are not able to produce secretory IgA. IgA deficiency predisposes these individuals to lung and gastrointestinal infections for which secretory IgA is normally an important defense mechanism. Infections in the lungs and gastrointestinal tract can involve a variety of pathogens, including H. influenzae, S. pneumoniae, Moraxella catarrhalis, S. aureus, Giardia lamblia, or pathogenic strains of Escherichia coli.

IV. Severe Combined Immunodeficiency

Patients who suffer from severe combined immunodeficiency (SCID) have B-cell and T-cell defects that impair T-cell dependent antibody responses as well as cell-mediated immune responses. Patients with SCID also cannot develop immunological memory, so vaccines provide them no protection, and live attenuated vaccines (e.g., for varicella-zoster, measles virus, rotavirus, poliovirus) can actually cause the infection they are intended to prevent. The most common form is X-linked SCID, which accounts for nearly 50% of all cases and occurs primarily in males. Patients with SCID are typically diagnosed within the first few months of life after developing severe, often life-threatening, opportunistic infection by Candida spp., Pneumocystis jirovecii, or pathogenic strains of E. coli.

Without treatment, babies with SCID do not typically survive infancy. In some cases, a bone marrow transplant may successfully correct the defects in lymphocyte development that lead to the SCID phenotype, by replacing the defective component. However, this treatment approach is not without risks, as demonstrated by the famous case of David Vetter (1971–1984), better known as “Bubble Boy”. Vetter, a patient with SCID who lived in a protective plastic bubble to prevent exposure to opportunistic microbes, received a bone marrow transplant from his sister. Because of a latent Epstein-Barr virus infection in her bone marrow, however, he developed mononucleosis and died of Burkitt lymphoma at the age of 12 years.

- **Secondary Immunodeficiency**

A secondary immunodeficiency occurs as a result an acquired impairment of function of B cells, T cells, or both. Secondary immunodeficiencies can be caused by:

i. Systemic disorders such as diabetes mellitus, malnutrition, hepatitis, or HIV infection

ii. Immunosuppressive treatments such as cytotoxic chemotherapy, bone marrow ablation before transplantation, or radiation therapy

iii. Prolonged critical illness due to infection, surgery, or trauma in the very young, elderly, or hospitalized patients

iv. Unlike primary immunodeficiencies, which have a genetic basis, secondary immunodeficiencies are often reversible if the underlying cause is resolved. Patients with secondary immunodeficiencies develop an increased susceptibility to an otherwise benign infection by opportunistic pathogens such as *Candida* spp., *P. jirovecii*, and *Cryptosporidium*.

v. HIV infection and the associated acquired immunodeficiency syndrome (AIDS) are the best-known secondary immunodeficiencies. AIDS is characterized by profound CD4 T-cell lymphopenia (decrease in lymphocytes). The decrease in CD4 T cells is the result of various mechanisms, including HIV-induced pyroptosis (a type of apoptosis that stimulates an inflammatory response), viral cytopathic effect, and cytotoxicity to HIV-infected cells.

vi. The most common cause of secondary immunodeficiency worldwide is severe malnutrition, which affects both innate and adaptive immunity. More research and information are needed for the more common causes of secondary immunodeficiency; however, the number of new discoveries in AIDS research far exceeds that of any other single cause of secondary immunodeficiency. AIDS research has paid off extremely well in terms of discoveries and treatments; increased research into the most common cause of immunodeficiency, malnutrition, would likely be as beneficial.

Acquired Immunodeficiency Syndrome (AIDS)

AIDS is a viral disease characterized by sever immunosuppressant that led to opportunistic infections, secondary neoplasms and neurologic manifestations.

Transmission:

1. Sexually (homosexual or heterosexual).

2. Blood transfusion (whole blood, plasma, platelets, clotting factors).

3. Intravenous drug abuser.

4. Prenatally from mother to child (utero, during labor, and milk).

5. Virus not spread by insect vectors.

Pathophysiology of AID

• Human infectious Virus belongs retrovirus (RNA virus).

• Human infectious virus infects a limited number of cells including:

1. Lymphocytes "T4"

2. Monocytes/ Macrophages

3. Dendritic cells

• Lymphocyte (T4), monocyte and dendritic cells have receptor which is called CD4.

• Human Infectious virus (HIV) has affinity to binds to the CD4 receptors.

• After HIV binding to the CD4 receptors the virus enters the lymphocyte and sheds its protein coat. Proviral DNA is integrated into DNA of the host cell.

• The infected person remains asymptomatic although serologic can identify antibodies to HIV within 2 weeks to three months

Clinical manifestation

1. Acute stage "associated with primary infection".

2. Asymptomatic stage "prolonged"

3. Advanced stage "AIDS".

Acute stage:

• This stage appears within 3-6 weeks after infection, fever, sweat, myalgia, arthralgia, malasia, sore throat, nausea, headache, vomiting, general lymphadenopathy, hepatic and splenic enlargement, and transient macular erythrematous rashes.

• These symptoms last for one to several weeks and usually subsided as an immune response to HIV develop and the level of plasma viremia disease.

Asymptomatic stage:

• The median time for untreated patients is about 10 years.

• In this stage the number of virus is increased and there is low level decline (unapparent decline) in the count of T4 cell.

• The average rate of T4 cell decline is about 50 cell /ml per years.

Advanced stage (AIDS):

This stage appears when the T4 cell count under 200 cell/ ml, therefore opportunistic infections and tumors can be seen in many organs in the body.

Respiratory infections:

• Pneumonia due to infection with pneumocystis carinii. This microorganism can cause pneumonia in immune-suppressed people, but relatively rare in healthy people.

• Pneumonia occurs due to other infection like: Mycobacterium tuberculosis TB, Streptococcus, Cytomegalovirus.

Digestive system:

1. Esophagitis inflammation of the lower end of the esophagus. Characterized by painful swallowing.

occurs due to:

- fungal infection (candidiasis)
- viral (herpes simplex-1 or cytomegalovirus) infection
- In rare cases, it could be due to Mycobacterium.
- Diarrhea occurs due to bacterial infection like Salmonella, Shigella, Listeria, and Compylobacter, and rarely due to virus infection.

Nervous system:

• The clinical findings arise from the direct effects of the retrovirus on the CNS and from opportunistic infections like toxoplasma encephalitis (caused by Toxoplasma gondii) and cryptococcal meningitis (caused by Cryptococcus neoformans). It can cause fever, headache, fatigue, nausea, vomiting, memory loss, difficulty in concentrating, euphoria, then develop to dementia, ataxia, tremor, paraplasia. Patients may also develop seizure and confusion than death can be occurs.

Probable questions:

1. What is the primary antibody involved in Type I hypersensitivity?
2. What is the mechanism of action of the inflammatory mediators released in Type I hypersensitivity?
3. What are some common clinical manifestations of Type I hypersensitivity?
4. How does repeated exposure to an allergen affect the sensitization process in Type I hypersensitivity?
5. What is anaphylaxis, and how is it related to Type I hypersensitivity?
6. What is Type II Hypersensitivity?

7. What causes Type II Hypersensitivity reactions?
8. What are the clinical outcomes of Type II Hypersensitivity?
9. What is another name for type 2 hypersensitivity?
10. Explain the immunological mechanisms that are responsible for allergic reactions.
11. Discuss the type of reaction triggered by a Mantoux test and why it is used.
12. Explain why Type IV hypersensitivity is considered a delayed-type hypersensitivity reaction.
13. How does the mechanism of Type IV hypersensitivity differ from the other types (I, II, III)?
14. What is the primary cell type involved in Type IV hypersensitivity?
15. What disease is caused by Type 4 hypersensitivity?
16. Describe the mechanism of type 1/ type 2/ type 3/ type 4 hypersensitivity reaction.
17. Define autoimmunity. What are autoimmune diseases?
18. What are the agents that lead to autoimmune disease?
19. Describe the various types of auto immune diseases.
20. Describe the causes of auto immunity/ Enumerate the causes of autoimmune diseases.
21. Describe various immunopathology mechanisms of autoimmune diseases.
22. Write short note on Rheumatoid arthritis/ Graves' disease/ Myasthenia gravis.
23. How rheumatoid arthritis/ Multiple Sclerosis/ Graves' disease/ Myasthenia gravis come to occur?
24. Briefly describe the principles and methods of treatment of autoimmune diseases.
25. What is the fundamental cause of a primary immunodeficiency?
26. What is the most common cause of secondary immunodeficiencies?
27. Explain why secondary immunodeficiencies can sometimes be reversed.
28. Discuss the pathophysiology of AIDS.

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Unit XIV

Structure and function of antibody

Objective: In this unit we will learn about structure and function of antibody.

Introduction:

Immunoglobulins are glycoprotein molecules that are produced by plasma cells in response to an immunogen and which function as antibodies. These are found in the blood plasma, lymph and secretions such as saliva, tears, and gastrointestinal fluid. Most antibodies are found in γ-globulin fraction of the serum. The structure of the antibodies was discovered by Rodney Robert Porter and Gerald M. Edelman.

Basic structure of Immunoglobulins

Immunoglobulins are heterodimers containing four peptide chains as their basic unit. They are composed of two identical light chains (23-25kD) and two identical heavy chains (50-70kD). Each light chain is bound to a heavy chain by a disulfide bond, and by noncovalent interactions as salt linkages, hydrogen bonds, and hydrophobic bonds, to form a heterodimer (H-L). Similar noncovalent interactions and disulfide bridges link the two identical heavy and light (H-L) chain combinations to each other to form the basic four-chain (H-L)2 antibody structure, a dimer of dimers. These interconnections between the chains are resulting in a characteristic Y-shaped structure with a total molecular weight of approximately 150 kDa. When the amino acid sequences of many different heavy chains and light chains were compared, it became clear that both the

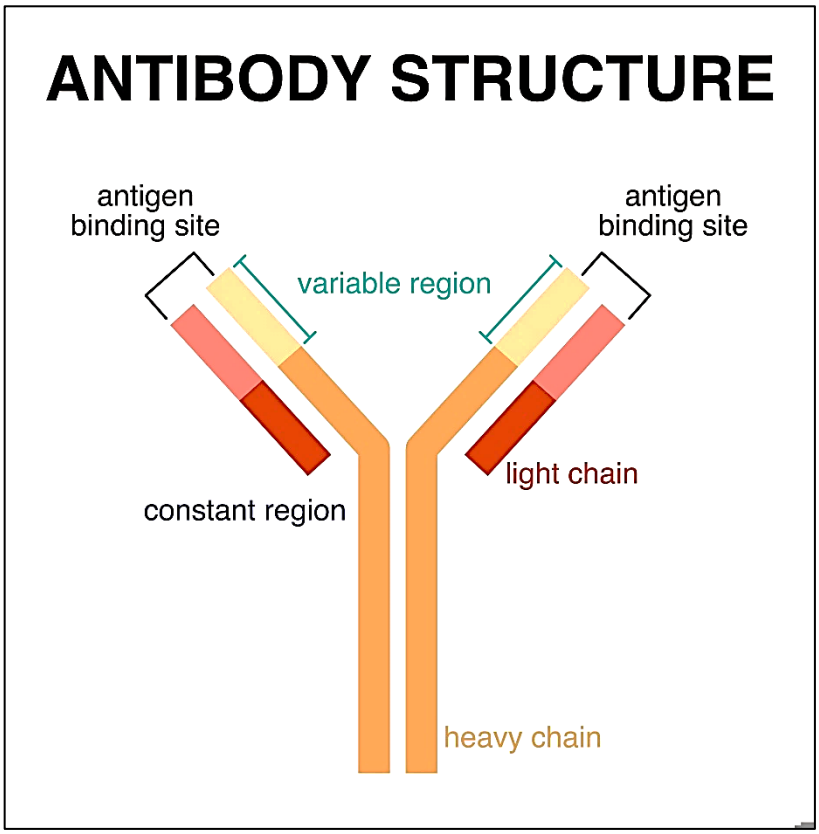


Fig: Structure of antibody

heavy and light chain could be divided into two regions based on variability in the amino acid sequences. Each antibody molecule possesses distinct variable and constant regions.

The variable region confers an antibody's antigen-binding specificity. This region contains two fragment antigen binding (Fab) domains, each capable of binding a specific epitope (the part of an antigen recognized by an antigen). Consequently, a single antibody molecule can simultaneously bind two identical epitopes on the same antigen. The constant region of an antibody houses the fragment crystallizable (Fc) region, which mediates interactions with Fc receptors expressed on the surface of immune cells such as leukocytes (white blood cells), macrophages, and natural killer cells. These interactions are crucial for triggering various effector functions of the immune system.

Immunoglobulins also have hinge region at which the arms of the antibody molecule form a Y. It is called the hinge region because there is some flexibility in the molecule at this point.

- **Immunoglobulin fragments: structure/ Function relationship**

Enzymes such as papain and pepsin, each splits the immunoglobulin molecule into definable fragments.

1. Papain split the monomeric basic unit into three fragments of approximately equal size at the hinge region.

a. Two Fab (antigen-binding) fragments each contain an entire L chain and the amino terminal half of the H chain.

b. One Fc (Crystallizable) fragment contains the carboxy terminal portion of the H chain.

2. Pepsin digests most of the Fc fragment, leaving one large fragment termed the F(ab')2 fragment.

a. The F (ab')2 fragment consists of two Fab fragments joined by covalent bonds.

b. The F (ab')2 fragment has two antigen-binding sites; thus, it is bivalent, possessing the ability to bind and precipitate an antigen

A. Fab

Digestion with papain breaks the immunoglobulin molecule in the hinge region before the H-H inter chain disulfide bond into three fragments. This results in the formation of two identical fragments that contain the light chain and the VH and CH1 domains of the heavy chain. Each of the two fragments has MW = 45000 and had antigen-binding activity and were called Fab fragments. Each Fab fragment is monovalent whereas the original molecule was divalent. The combining site of the antibody is created by both VH and VL.

An antibody is able to bind a particular antigenic determinant because it has a particular combination of VH and VL. Different combinations of a VH and VL result in antibodies that can bind different antigenic determinants

B. Fc fragments

The third fragment had no antigen binding site and had MW = 50000, was called Fc fragments (because it was found to crystallize during cold storage, Fc= fragments crystallizable). Fc contains the remainder of the two heavy chains each containing a CH2 and CH3 domain.

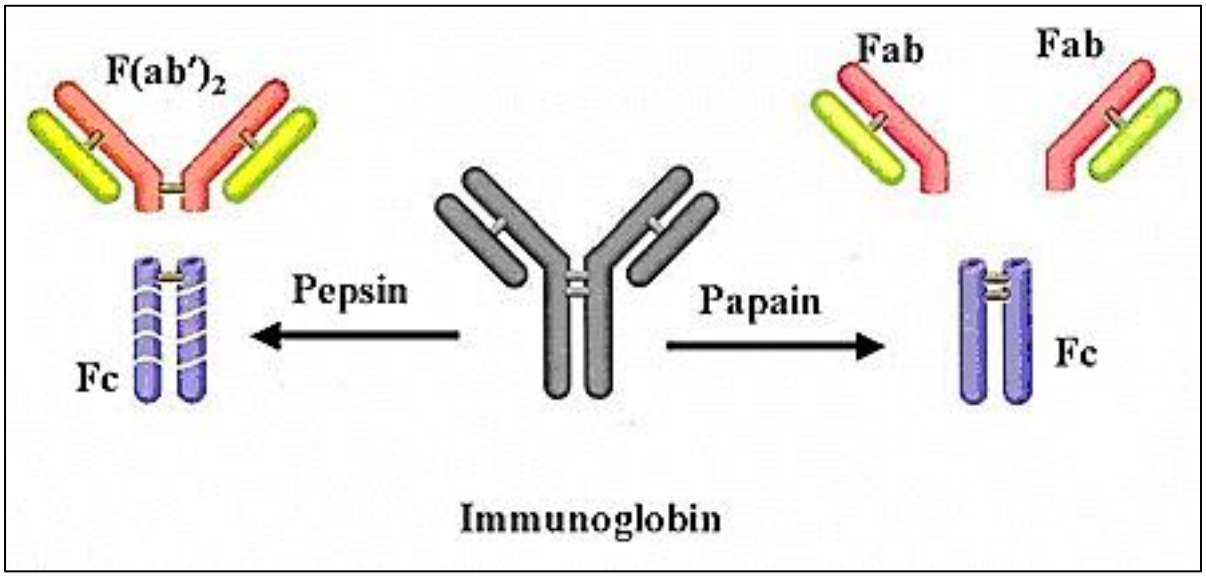


Fig: Different digestion procedures and fragments of immunoglobulin after treatment [Source: https://www.researchgate.net/figure/Different-fragments-of-IgG-obtained-by-pepsin-and-papain-digestion_fig2_285954753]

Paratope: Immunoglobulin-antigen interactions typically take place between the paratope, the site on the Ig at which the antigen binds,

Epitope: It is the site on the antigen that is bound to paratope on antibody

Cross-reactivity: The ability of the same antibody to bind divergent antigens that share equivalent or similar epitopes.

Idiotype: Individual determinant(s), termed idiotype (s), are contained within V domains. An idiotype is a shared characteristic between a group of immunoglobulin or T cell receptor molecules based upon the antigen binding specificity and therefore structure of their variable region.

Isotype: Common determinants, termed isotypes, are specific for the constant portion of the antibody and allow grouping of immunoglobulins into recognized classes, with each

class defining an individual type of C domain. It is a mechanism that causes the production of antibodies to change from IgM. or IgD to the other antibody isotypes, IgE, IgA or IgG, that have defined roles in the immune system. In immunology, the "immunoglobulin isotype" refers to the genetic variations or differences in the constant region of the heavy chain of the Ig (immunoglobulins) classes and sub-classes. In humans, there are nine isotypes

Allotype: Determinants common to subsets of individuals within a species, yet differing between other members of that species, are termed allotypes and define inherited polymorphisms that result from gene alleles

- **Location and Formation of antibody:**

The antibodies may be bound to a cell membrane or they may remain free. Antibodies are produced by B lymphocytes and plasma cells. In fact, B-lymphocytes get transformed into plasma cells. The mature plasma cell produces antibodies at an extremely rapid rate—about 2000 molecules per second. Antibodies direct the antibody- mediated immunity (=humoral immunity).

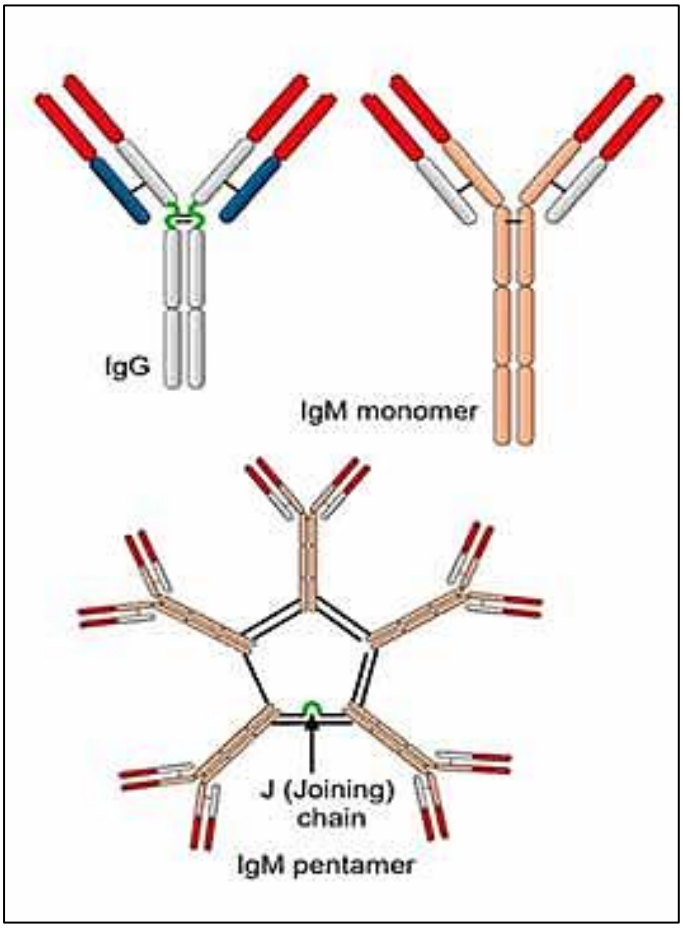
- **Different types of antibodies**

There are five types of antibodies viz:

1. IgA (Ig α);
2. IgD (Ig δ);
3. IgE (Ig δ);
4. IgG (Ig δ) and
5. IgM (Ig μ).

Among the antibodies, IgG forms 80% of the antibodies in the body.

IgG is the most abundant immunoglobin, which accounts for about 80% of the total serum antibodies. The concentration of IgG in the blood is about 10mg/ml.



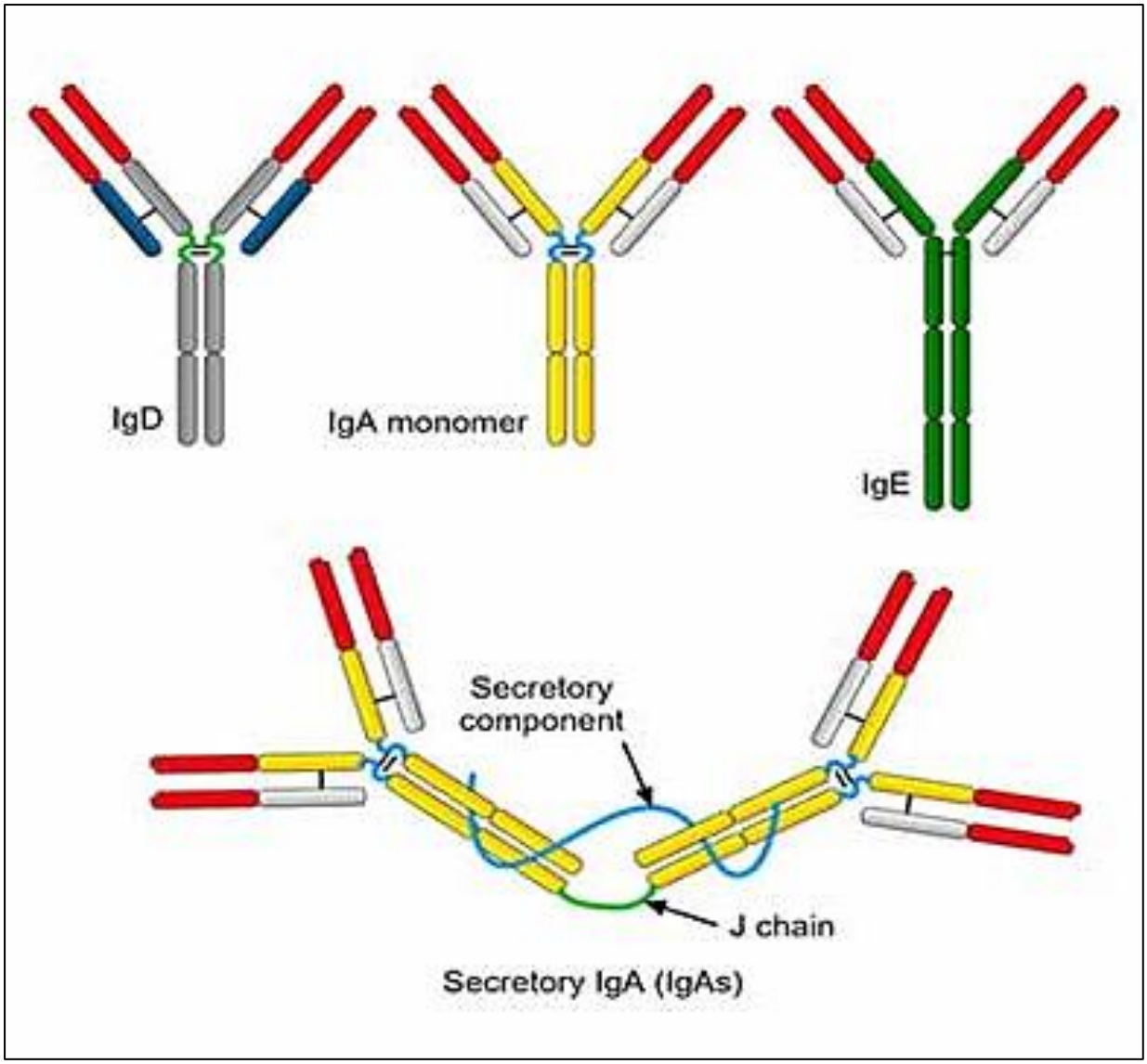


Fig: Different types of antibodies [Source: https://microbenotes.com/properties-and-function-of-different-classes-of-antibodies/]

I. Structure of IgG

- The basic structure of IgG is composed of a Y-shaped protein where the Fab arms are linked to the Fc arms by an extended region of polypeptide chain called the hinge.
- The region is exposed and sensitive to attack by proteases that cleave the molecule into distinct functional units arranged in a four-chain structure.
- An IgG molecule consists of two identical γ heavy chains, usually of the size 50kDa.
- The light chains in IgG exist in two forms; κ and λ, where the κ form is more prevalent than λ, in the case of humans.

- The Fc regions of the molecule have a highly conserved N-glycosylation site in the heavy chain.

Properties of IgG

- The IgG antibodies exist in the serum in the monomeric form, and these can cross the placenta from the mother to the fetus.
- Each IgG antibody has two paratopes that bind to two different epitopes on different antigens.
- IgG has four subclasses classified on the basis of the subclasses of the γ heavy chains.
- IgG antibodies participate predominantly in secondary immune response as these are generated as a result of class switching and maturation of the response.

Functions of IgG

- IgG antibodies provide long-term protection against various agents like bacteria, viruses, and bacterial toxins.
- IgG is one of the most potent complement activators when compared to all other antibodies.
- The binding ability of IgG to antigens is more effective as it enhances phagocytosis.

II. Structure of IgM

- IgM is secreted in a pentameric form with five distinct units, where each are composed of two μ heavy chains and two light chains.
- A J chain might be present in the hexameric form of the molecule, but it isn't always present. The J chain is usually added just before the secretion of the pentamer as it helps in the polymerization of the monomers.
- IgM is the third most abundant immunoglobulin in serum, with a concentration of 1.5 mg/ml. It is the largest antibody and is the first antibody to appear in response to the initial exposure to antigen.
- Each of the monomers has two antigen-binding sites, resulting in 10 binding domains in a single molecule. However, all the domains cannot be occupied at the same time due to limitations in space.
- The pentameric form of IgM has a molecular weight of 900 kDa.

Properties of IgM

- IgM is the largest and the only pentameric antibody in humans. It is also the first antibody to be produced in response to the initial exposure to an antigen.
- IgM is the first immunoglobulin to be synthesized by the fetus, beginning at about 20 weeks of age.

- IgM is a pentameric molecule with 10 antigen-binding sites and 5 Fc portions held together by disulfide linkages.
- The monomeric form of IgM occurs as the major antibody receptor on the surface of B lymphocytes.
- IgM is relatively short-lived and usually disappears earlier than IgG.
- The large size of the molecules does not allow effective diffusion of the antibody, and thus, it is found in very low concentration in the intracellular fluids.

Functions of IgM

- IgM is very effective against viruses as less IgM than IgG is enough to neutralize viral infections.
- IgM is also a better agglutinin as it takes 100 to 1000 more molecules of IgG than that of IgM for the same level of agglutination.
- IgM is involved in activating the classical pathway of complement in the immune system due to the presence of two Fc regions in close proximity.

III. Structure of IgA

- The molecular size of IgA is 160 kDa with a four-chain monomeric structure, however, it can occur in dimeric and trimeric forms.
- The heavy chain of IgA is divisible into three constant domains, CH1, CH2, and CH3, and a variable VH domain.
- The hinge region occurs between the CH1 and CH2 domains held together by disulfide linkages.
- sIgA has a secretory component as an additional component with a polypeptide chain of 75 kDa and extracellular proteolytic fragment.
- The molecule also has a J-chain linked to the chains via disulfide bridges. The secretory and J chain facilitates the transport of IgA across epithelial cells and protects the molecule from proteolytic digestion by enzymes.
- IgA or sIgA is the main immunoglobulin found in the mucous membrane in the form of secretory antibodies. The concentration of IgA is found in small quantities in blood, but it is found in high concentrations in tears, saliva, and sweat

Properties of IgA

- IgA is the second most abundant immunoglobulin in humans, with a concentration of 2-4 mg/ml. It accounts for about 10-15% of the total serum concentration but is the most abundant antibody in external secretions.

- IgA is the first line of defense as it works by inhibiting bacterial and viral adhesion to epithelial cells and by neutralizing viral and bacterial toxins intracellularly.
- The secretory IgA mostly occurs in dimeric form with two monomeric units linked together by a joining peptide.

Functions of IgA

- IgA is the first line of defense as it protects the body from the entry and colonization of mucosal surfaces by different foreign particles.

IV. Structure of IgD

- IgD has a structural diversity throughout evolution in the vertebrates as it is flexible to complement the function of IgM.
- It is a glycoprotein with two identical δ heavy chains and two identical light chains.
- IgD found on the surface of B lymphocytes has some extra amino acids at C-terminal in order to anchor to the membrane.
- The light and heavy chains are linked together by disulfide links, but they have additional intrachain disulfide links that divide the chains into domains.
- The IgD molecule also has an extended hinge region which increases the flexibility of the molecule but decreases its resistance against proteolytic cleavage.
- IgD is a monomeric antibody that occurs on the surface of immature B lymphocytes. It is produced in a secreted form in a small amount in the blood serum.

Properties of IgD

- IgD is found in low concentration in serum, and its exact function in the immune system is not yet clearly understood.
- It represents about 0.25% of the total serum immunoglobulins with a relative molecular mass of 185 kDa and a half-life of 2.8 days.
- It also accounts for about 1% of the proteins present in the plasma membranes of B lymphocytes. Here, it usually coexpressed with another cell surface antibody, IgM.

Functions of IgD

- The most important function of IgD is antigen receptor on B cells. It also regulates B cell function if it encounters an antigen.
- It is also secreted in some amounts in the blood, mucosal secretions, and the surface of innate immune effector cells.

V. Structure of IgE

- IgE has a typical antibody structure with ε heavy chains that have a high carbohydrate content.
- IgE has two identical antigen-binding sites consisting of both light and heavy chains and a valency of 2.
- Like all antibodies, heavy and light chains are further divided into variable and constant regions.
- The heavy chains consist of five domains, out of which one is variable, and four are constant.
- IgE is a type of immunoglobulin found only in mammals and synthesized by plasma cells. It occurs in a monomeric form with two ε heavy chains and two light chains.

Functions of IgE

- IgE is mostly associated with allergic reactions where it binds to reintroduced antigens and triggers the release of pharmacologically active agents.
- It also plays an essential role in response to allergens and antigen preparation used in desensitization immunotherapy.

Function of Antibody

The role of antibodies in immunity however includes:

- To prevent pathogens from entering and damaging the host cells, by binding to the antigens
- By stimulating the removal of pathogens by macrophages and other immune cells. This is enabled by the antibodies coating themselves on the pathogen, which triggers the production of these immune cells.
- By triggering the destruction of pathogens. This is enhanced by stimulating the functions of other immune responses including complement activation pathways.
- By triggering vasoactive amine degranulation which contributes to providing immunity against certain antigens such as allergens, helminths.
- Opsonization: antibody together with other complements, covers antigens and prepare it to phagocytosed by phagocytic cells. IgG is the opsonizing immunoglobulin.
- Toxin/viral neutralization: exotoxins secreted by some bacteria can be neutralized by these antibodies. IgM, IgG and IgA are found to neutralize some viral particles.
- Complement activation: it's a series of enzymatic events that can be initiated by antigens or binding to the antibodies. IgM and IgG are able to start complement system.

Antibody dependent cell mediated toxicity: ADCC: natural killer cells recognize infected cells and malignant cells by antibodies bound on the surface of these cells.

Probable questions:

1. Which type of immune cells produce immunoglobulins?
2. Describe the basic function of antibody.
3. What is Fab and Fc portion?
4. Describe the fragmentation procedure of immunoglobulin and comment on fragmented parts.
5. What is isotype, idiotype, allotypes, epitope, paratope?
6. What is plasma cell?
7. State the structure of IgM/IgA/IgD/IgE/IgG with diagram.
8. Comment on the properties of IgM/IgA/IgD/IgE/IgG.
9. State the function of IgM/IgA/IgD/IgE/IgG
10. What is the main function of the immunoglobulin?
11. What is the name of the hypervariable region of immunoglobin, which is responsible for its diversity?
12. Which amino acid is found in the hinge region?
13. Name the class of immunoglobulin which has a pentameric structure?
14. Name the class of antibody which can cross the placental barrier.
15. What is secretory antibody? Give example.
16. Which antibody is associated with allergic reaction?

Suggested reading:

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The study materials of this book have been collected from books, various e- books, journals and other e-sources.