

King Saud University  
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# **Basic and Clinical Immunology**

505 PHCL

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1427 - 2006

# IMMUNOLOGY

## Anatomy and Cells of the Immune System

### \* Cells of the immune system:

The bone marrow is the source of the precursor cells "the pluripotent haemopoietic stem cells" which give rise to cell constituents of the immune system as a component of haemopoiesis.

Haemopoiesis is the process by which all cells that circulate in the blood arise and mature.

### • **Granulocytes:**

- They constitute 65% of all white cells.
- They have granular cytoplasm of 3 types:
  - Eosinophils "which red staining granules" 8-5%.
  - Basophils "which blue staining granules" 0.5-1%.
  - Neutrophils "polymorphonuclear" cells 90-95%.
- They mature in the bone marrow & released in the blood. They circulate in the blood and migrate into tissues particularly during inflammatory response.

### \* Mast cells:

- These cells are fixed in tissues.
- Their precursors unidentified possibly in spleen, thymus and lymph nodes.

### \* **Monocytes:**

- 5-10% of circulating white blood cells.
- Large mononuclear cells.
- They have short half life, usually 24 hrs in blood then they become resident in tissues and become macrophages.
- Specialized forms of mature cells exist including alveolar macrophages in the lung, Kupffer cells in the liver, mesangial cells in the kidney, microglial cells in the brain, osteoclasts in bone and other macrophages lining channels in the spleen and lymph nodes.

### \* **Dendritic cells:-**

- They are a small proportion of immune cells in the peripheral blood, lymph nodes, bone marrow and tissues. They are bone marrow derived.
- They have cytoplasmic processes.
- They have specialized function in activation of lymphocytes.
- They are some specialized forms of these cells e.g. follicular dendritic cells in lymph nodes.

### \* **Lymphocytes:**

- 25-35% of white cells.
- They are found in the blood, lymphoid organs, or tissues and at sites of chronic inflammation.
- They are two subtypes:

- B and T lymphocytes present in the blood in a ratio of 1 : 5. They can be differentiated by highly specialized molecules on their surfaces.
- Their precursors arise in the bone marrow and mature in two pathways:
  - B cells: "antibody forming, bursa derived B cells".
    - They mature and differentiate in the bone marrow before being released in circulation.
    - They recognize macromolecules "antigens" through surface receptors "antibodies".
    - They mature into plasma cells and become fixed in tissues to secrete antibodies "immunoglobulin".
  - T lymphocytes: "Thymus dependent".
    - They become differentiated and mature in thymus in early life.
    - They acquire the ability to recognize and distinguish self from non self "foreign tissues and infections agents".
    - They are not capable of antibody production.
    - They can be identified by specialized glycoprotein molecules on their surface "CD = Cluster of differentiation".

**\* Natural Killer Cells "NK Cells":**

- They resemble T cells, but remain distinct.
- They have the ability of killing "lysis" of virus infected cells and tumour cells.
- They have specialized surface glycoprotein molecules.

- They are not thymus dependent "they do not require the thymus for their maturation".

### **\* Organs of the immune system:**

- The total number of lymphocytes in healthy adults about  $10^{12}$ , of this 0.1% are renewed daily.
- They do not reside in a single organ.
- Lymphocytes have the distinctive feature of recirculation between the blood, tissues and lymphoid organs "Lymphocyte traffic".
- Recirculation is not a random process but a highly regulated process of immune surveillance.

### **Organs of the lymphoid system:**

#### **\* Primary Lymphoid organs:**

- Bone marrow and thymus.
- Sites of development and maturation of lymphocytes.

#### **\* Secondary Lymphoid organs:**

They are not essential for generation of lymphocytes but have a key role in the maturation of these cells and development of immunity i.e. they organize the immune response.

- They include spleen and lymph nodes. Mucosal associated lymphoid tissues (MALT) and gut associated lymphoid tissue (GALT) which are specialized non capsulated lymph nodes.

#### **\* Bone marrow:**

They pluripotent hemopoietic stem cells mature into any one of the immune cells in the bone marrow. This occurs under the influence of soluble mediators such as colony stimulating factors.

### **\* The thymus:**

It is a two lobed organ that is located in the mediastinum anterior to and above the heart. It increases in size after both until puberty then undergoes involution in adulthood and old age.

It has an outer cortex and inner medulla. The cortex contains a heavy concentration of thymocytes (Tlymphocytes in the thymus which have arrived from the bone marrow via the blood stream).

- The pre-T lymphocytes mature in the cortex of thymus in close association with thymic epithelial cells and macrophage derived cells.

### **\* Lymph nodes:**

They are capsulated highly specialized lymphoid tissue composed of cortex and medulla.

- The cortex contains follicles which are organized aggregates of lymphoid cells, mainly B lymphocytes, macrophages and follicular dendritic cells.
- Primary follicles are in a resting state and secondary follicles arise following stimulation of a local immune response and contain germinal centers where B lymphocytes undergo proliferation and differentiation.
- The paracortical area of the LN is predominantly composed of T lymphocytes and interdigitating dendritic cells which are accessory cells in T lymphocytes response.
- The medulla is composed of cords of lymphoid cells in which plasma cells predominate during immune reactions.

- Lymphocytes enter the L.N. either through afferent lymphatic or from the blood. Through the afferent lymphatic, lymphocytes enter to the subcapsular sinus then travel into the cortex and then the medulla of the L.N.
- The speed by which the lymphocytes course through the L.N. will depend on the state of activity of the node and whether the cells become involved in an immune response or not.
- They leave via efferent lymphatic, ultimately passing through the thoracic duct then into the venous system.
- Lymphocytes may also, enter the LNs via the blood, via large cuboidal endothelial cells present on specialized structures called high endothelial venules (HEV).
- Lymphocytes require homing receptors on their surfaces to interact with HEVs and such receptors are specific for different lymphocytes and different tissues. So that, lymphocytes that protect against pathogens encountered in the gut will possess receptors specific for HEVs in GALT.

**\* GALT:**

- It is composed of Peyer's patches and isolated lymphoid follicles within the gut submucosa.
- Peyer's patches are lymphoid aggregates with follicles, germinal centers and a surrounding T cell area, but they have no capsule or afferent lymphatic. They are separated from the intestinal lumen by a specialized epithelial cell.

## \* Spleen:

- The spleen is located in the left upper quadrant of the abdomen.
- Blood supply arrives via the splenic artery which divides into progressively smaller branches, then into arterioles which drain into vascular sinusoids which drain into the venous system.
- It contains two types of tissues: red pulp and white pulp.
- The red pulp is composed of reticular tissues and sinuses bathed with blood. It is an important site for removal of old and defective red and white blood cells where they are engulfed and macrophages.
- The white pulp is dense lymphoid tissue arranged around a central arteriole. Which is called periarterial lymphatic sheath (PAL) predominantly T cells zone. Within the PAL, lymphoid follicles with germinal centres (B cell area) are found.
- The spleen is the major site of immune responses to blood born antigens. The slow circulation allows constant monitoring of blood with regard to infectious agents and antigen-antibody complex which signify an active immune response. **N.B.** Splenectomy may be done because of various reasons e.g. blunt trauma with splenic rupture, hypersplenism.
- However, splenectomy is associated with increased risk of infections especially with capsulated organisms e.g. streptococcus pneumoniae.

# Immunity and Immunology

## \* **Definition:**

The Latin "immunis" means free from burden. So, immunity means protection from infection or free from burden of disease.

- The broad definition of the immune system involves the ability to identify self and recognize non self, including pathogens as well as foreign tissue.
- Protection from all these should include a variety of cognitive and destructive processes.
- Understanding these processes forms the basis of Immunology.

## \* **Types of immunity:**

### \* **Innate immunity:**

- Characteristics:

Non specific, is present at birth and does not change in intensity with exposure "i.e. no memory".

- Protects from:

Bacteria (pyogenic organisms), fungi "e.g. Candida" and worms "multicellular parasites e.g. ascaris".

- It is the first line of defence against invading organisms.

- Components:

- a. Physical barriers:

Skin and mucosae, secretions which continuously wash and cleans mucus surfaces "e.g. tears, urine" and cilia which help the removal of debris and foreign matter.

- b. Mechanical removal also by coughing and sneezing.

- c. Colonization resistance:

The presence of normal flora in the skin and gut preventing colonization by pathogenic organisms.

- d. Non specific immune response:

- Immediate acting, directly activated by infectious agents, tissue damage or tumours.
- Its advantage is rapid action but the disadvantage is being non specific, so, may cause host tissue damage.

- \* Cellular:

Granulocytes and natural killer cells.

- \* Humoral:

- The complement.
- Opsonins "which help digestion of bacteria by neutrophils e.g. creative protein".
- Proteolytic enzymes e.g. Lysozyme.

- \* **Acquired immunity:** Adaptive immunity.

\* Characteristics:

- Specific responses acquired from exposure and increased in intensity with exposure "i.e. memory".
- It is the hall mark of the immune system.
- It is not present at birth but acquired with antigen exposure "except that acquired by I baby from the mother".

\* Components:

- Cell "T & B lymphocytes" and secreted products.

\* Protects from:

- Bacteria including intracellular infection, viruses and protozoa.

**N.B.** There is considerable interaction is innate and specific immune response.

# **Innate Immunity:**

## **Cellular Mechanisms**

Several cellular mechanisms are present and functional at birth and constitute the innate cellular immune system.

### **\* Granulocytes:**

- \* Neutrophils, eosinophils and basophils are present in blood and have the capacity to migrate to tissues. The migration into tissues is unidirectional.
- \* Mast cells are resident in tissues particularly at epithelial surfaces.

### **\* Neutrophils:**

Polymorphnuclear cell (PMN) is a highly specialized microbial phagocyte. It is an essential component of the cellular innate system involved in killing bacteria and fungi.

They are abundant in bone marrow and blood. They are released from the bone marrow in large numbers during infections and new cells are produced by the action of granulocyte colony stimulating factor (GM-CSF). [This response causes the characteristic neutrophil leucocytosis found in infectious and inflammatory conditions which may be useful in diagnosis and monitoring of patients].

- Neutrophil's half life in blood is about 6 hours and in tissues 1-2 days.

- **Neutrophil activation:**

- \* Neutrophil migration:

[Margination → Rolling → Sticking → diapedesis & chemotaxis]

- \* A pool of neutrophils continuously rolls along the endothelial surface of blood vessels tethered by weak cell-cell interactions mediated by specific receptors (margination).

- \* In the presence of bacterial or fungal infections, the neutrophil is activated to kill the offending organism.

- \* Neutrophils are activated by numerous stimuli e.g.

- Complement components C<sub>50</sub>.
- Leukotrien B<sub>4</sub> (LTB<sub>4</sub>) which are macrophage derived products of the lipoxygenase pathway of arachidonic acid metabolism.
- FMLP (a peptide which is a bacterial product).
- Cytokines e.g. interleukin 8 (neutrophil activator protein. NAP-1).

- \* These factors are released at sites of infection and inflammation. These activators cause up regulation of adhesion molecules on the surface of neutrophils and endothelial cells.

- \* Following activation of both neutrophil and endothelium, the specialized adhesion molecules halt neutrophil rolling and facilitate their entry into tissues "diapedesis".

- \* **N.B.** Adhesion molecules:

These are surface proteins which facilitates intercellular adhesion. Their functions include cell activation, cytokine release, capture and

rolling of leucocytes along the endothelial cells lining of blood vessels and extravasation. There are 3 main families: selection, integrin and ICAM.

\* Chemotaxis:

The directed movement of a cell along a gradient of increased concentration of the attracting molecule (i.e. the chemoattractant). The most potent chemoattractant are  $C_{5a}$ , FMLP,  $IL_8$  and  $LTB_4$ .

\* Phagocytosis:

The ability to ingest and kill microorganisms is a key component in host defence. Neutrophils have the capacity to ingest more than one bacterium or fungus at once. When large numbers of phagocytes are involved in an infective process, an abscess filled with pus (dead or dying neutrophils) may form.

\* Opsonization:

Opsonins are factors that coat microorganisms and enhance neutrophil's ability to engulf them. The best opsonins are complementing component  $C_3b$ , C-reactive protein and antibody (i.e. interaction between innate and acquired immunity).

\* Phagocytosis is achieved using pseudopodia which are extended to surround the organism or particle and then fuse to form an enclosed vacuole called "phagosome". Then the intracellular phagosome becomes fused to neutrophil granules which releases its contents.

\* Neutrophil killing of pathogens:

Two major mechanisms of killing occur in neutrophils:

- O<sub>2</sub> dependent mechanism "the respiratory burst", in which there is production of reactive oxygen metabolites such as hydrogen peroxide, hydroxyl radicals, singlet oxygen and superoxide anion.
- \* O<sub>2</sub> independent mechanisms: by microbicidal enzymes present in the granules such as lysozyme and cathepsins.

### **N.B.**

Recurrent bacterial and fungal infections occur in conditions associated with failure of neutrophil's action e.g. in patients having deficiency in neutrophil granules or respiratory burst causing defective intracellular killing of organisms.

- \* Also, in cases of deficiency of adhesion molecules either congenital or acquired (due to corticosteroid therapy), there is also repeated infections.

### **\* Eosinophils:**

These comprise 3-5% of granulocytes in the circulation. They are more abundant in tissues where they survive for several weeks.

- \* The main roles of eosinophils in host defence is in protection against multicellular parasites such as worms (helminthes) which is afforded by release of toxic cationic proteins on the surface of the worms.

The eosinophil cationic proteins are: major basic protein (MBP), eosinophil cationic protein (ECP) and eosinophil neurotoxin.

- \* Eosinophils are involved in allergic reactions. They are a feature of infiltrate in tissues involved in allergic responses e.g. asthma. Other

mediators released in those conditions include leucotriens and platelet activating factor (PAF).

**\* Mast cells and basophils:**

They share many features in common. They have histamine containing granules and high affinity receptors for IgE.

The major granule products are histamine and leucotriens which have profound effects on blood vessels and bronchial smooth muscles.

The effects of these granule release products depend on the site and the stimulus, ranging from localized wheel to anaphylactic shock. They are activated in allergic and inflammatory conditions by IgE.

Also, the anaphylatoxins C3a, C4a and C5a activate basophils and may activate mast cells.

## **"Complement"**

### **Definition**

A protein cascade composed of more than 40 proteins including regulatory factors.

The term complement was proposed by Paul Ehrlich to describe the ability of these proteins to complete or augment the actions of immunoglobulins during bacterial destruction.

The complement proteins are formed mainly in the liver. The majorities are soluble but some are membrane bound. The soluble proteins circulate in an inactive state and each must be activated sequentially for the reaction to proceed.

\* **Terminology of the complement system:**

(Proposed by WHO).

- Precursor molecules:
  - Capital C followed by the number for the classic and common pathway e.g. C<sub>1</sub>, C<sub>2</sub>, C<sub>3</sub> . . .
  - Capital letter followed by the number for the alternative pathway e.g. B<sub>1</sub>.
- Fragments "which are derived from enzymatic cleavage of the parent molecules": small letter suffix e.g. C<sub>3a</sub>, B<sub>b</sub>.
- Inactivated components: The letter I prefix e.g. iC<sub>3b</sub>.
- The active state of isolated or integrated complement components: Bar over symbols e.g. C<sub>4b</sub> 2<sub>a</sub>.

\* **Complement activation:**

The final common pathway must be activated via the classic or alternative pathway.

The alternative pathway is relatively primitive and a part of the innate system while the classic pathway combines with antibody to initiate activation and therefore it is associated with acquired (adaptive) immunity.

**The classical pathway:**

- Activation of the classic pathway is usually initiated by antigen-antibody complexes. Other activators include: aggregates of immunoglobulins e.g. IgM, DNA and C reactive protein (CRP).

- The activation of the classical pathway is initiated by activation of  $C_1$  through binding of  $C_{1q}$  (a subcomponent of the  $C_{1qrs}$  molecule) by antigen-antibody complexes.

Activated  $C_1$  causes cleavage of  $C_4$  to  $C_{4b}$  which continues the reaction process and  $C_{4a}$  which has other biological activities.

$C_2$  is then cleaved by activated  $C_1$  into  $C_{2b}$  and  $C_{2a}$  which combines with  $C_{4b}$  to form the classic pathway  $C_3$  convertase  $C_{4b} 2_a$ .

The cleavage of  $C_3$  by  $C_{4b} 2_a$  forms two fragments  $C_{3a}$  which has powerful biological properties and  $C_{3b}$  which becomes bound to the membrane and to  $C_{4b} 2_a$ , leading to the formation of the classic pathway  $C_5$  convertase  $C_{4b} 2_a 3_b$ , which cleaves  $C_5$  which is a component of the membrane attack pathway.

The alternative pathway.

Activators of the alternative pathway include endotoxin found in gram negative bacterial cell walls, as well as fungal cell walls and insoluble polysaccharides.

The main components of the alternative pathway are factor B, factor D and properdin (factor P) as well as  $C_3b$ .

- $C_{3b}$  is formed as a result of low grade hydrolysis of  $C_3$ . Free  $C_{3b}$  binds factor B and the  $C_{3b} B$  complex is acted upon by a circulating enzyme (factor D) which cleaves factor B, removing  $B_a$  fragment and generating  $C_{2b} B_b$  complex which is the alternative pathway  $C_3$  convertase which can cleave  $C_3$ , when stabilized on bacterial surfaces especially in the presence of properdin.  $C_{3b} B_b$  then cleaves more  $C_3$  (positive feedback loop) and binds  $C_{4b}$  to form  $C_{3b} B_b 4_b$  which is the

alternative pathway  $C_5$  convertase which initiate the membrane attack pathway.

The membrane attack pathway.

This starts by cleavage of  $C_5$  by either the classic or the alternative pathway leading to the formation of the small fragment  $C_{5a}$  which is biologically active and the larger fragment  $C_{5b}$  which continues the reaction by binding  $C_6$  which then binds  $C_7$ . Then the complex  $C_{5b67}$  becomes bound to the membranes.

**\* Biological activities generated by complement activation:**

1. Opsonization:

It means coating the walls of pathogens with protein that can attract & bind to phagocytic cells.

The  $C_{3b}$  fragment accounts for the most opsonic activity of the complement. It coats the bacterial wall and becomes bound to complement receptors on neutrophils leading to more efficient engulfment of the pathogen by neutrophils.

2. Cell recruitment to inflammatory sites (chemotaxis):  $C_{5a}$  & to less extent  $C_{3a}$  can attract neutrophils.

3. Cell activation:

Anaphylatoxins as  $C_{3a}$ ,  $C_{5a}$  and  $C_{4a}$  can directly activate basophils and mast cells (through specific receptors) leading to their degranulation and release of inflammatory mediators as histamine.

## 5. Removal of immune complexes:

The binding of  $C_{3b}$  to antibody in a complex inhibits lattice formation. This maintains the solubility of antigen antibody complexes. The antibody coated  $C_{3b}$  can attach to complement receptors found on many cells including R.B.Cs. These complexes are transported on red cells to the liver & spleen where they are released and taken by resident macrophages.

### \* **Regulation of complement activation:**

- Control mechanisms are important because complement activation may cause extensive tissue damage through the mediators of inflammation produced, if this activation mechanism is not controlled.
- Control is achieved by the lability of active molecules, dilution in tissue fluids and regulatory proteins.
- Regulatory proteins include circulating or membrane bound inhibitors.

### \* Circulating inhibitors:

- $C_1$  esterase ( $C_1$  inhibitor) which inactivates activated  $C_1$  by combining with it irreversibly. It is also responsible for regulation of other plasma enzyme systems e.g. kinin system.
- Factors H & I:
  - Factor H binds  $C_{3b}$  and accelerates its destruction by factor H.
  - C4 binding protein:
    - Bind  $C_{4b}$  and accelerates its destruction by factor I.
  - Carboxypeptidase N: Cleaves  $C_{3a}$ ,  $C_{4a}$ , and  $C_{5a}$  leading to its inactivation.

\* Membrane bound inhibitors:

- Membrane attack complex inhibiting factor (CD<sub>59</sub>): (CD = Cluster of differentiation), which is found on most blood cells and interferes with MAC accidental insertion on human cells and preventing cell lysis.

\* **Complement deficiencies:**

- \* The main functions of complement are enhancing neutrophil mediated lysis of the bacteria and direct lysis of bacteria especially neisseria and solubilization and removal of immune complexes.
- \* Failure of these functions lead to impaired non specific immunity with ↑ tendency to bacterial infections and a tendency to immune complex diseases such as SLE.
- \* Complement regulatory protein deficiency e.g. C<sub>1</sub> esterase deficiency → Hereditary angioneurotic oedema which is characterized by recurrent attacks of angioedema in the face, trunk and airway due to uncontrolled activation of complement and kinin system leading to tissue inflammation.

Laryngeal oedema may be fatal.

**Treatment of acute attack:** Fresh frozen plasma.

\* **Other factors in non specific immunity:**

- **Acute phase proteins:**

Acute phase reactants are proteins that are synthesized in response to infection, necrosis, tumours and other inflammatory events.

They are secreted by the liver under the effect of certain cytokines e.g. IL<sub>1</sub> and IL<sub>6</sub> and TNF.

Examples:

- C reactive protein (CRP)
- Serum amyloid P protein (diagnosis and monitoring of secondary amyloidosis).
- Complement components (C<sub>9</sub>, factor B, C<sub>3</sub>).
- Fibrinogen (coagulation)
- Haptoglobin (non binding)
- Ceruloplasmin

\* C-reactive proteins:

A protein which has the property of binding of the C-polysaccharide of pneumococci. CRP is produced in the liver and binds phosphorylcholine moieties which constitute a major component of bacterial cell wall constitute a major component of bacterial cell wall teichoic acid. When CRP is bound, it can activate complement through the classical pathway independently of the antibody → deposition of C<sub>3b</sub> on the surface of the microbe → its opsonization.

Its blood levels raise 10-100 folds within hours of the starts of infective or inflammatory process. Since it has a relatively short half life, so its level in blood can be used to monitor infective or inflammatory process and particularly their response to treatment.

\* Lysozyme:

Is a bactericidal enzyme secreted in saliva, tears and other body fluids as well as in neutrophil granules. It cleaves bacterial cell wall at a precise point.

## **Acquired Immunity**

The cardinal features of acquired immune response are specificity, memory and a variable response.

In general, the acquired and innate immune responses are not independent e.g. antibodies generated through acquired immunity are capable of directing components of the innate immune system e.g. complement, neutrophils and mast cells into relevant targets.

The molecular target of the acquired immune response is the antigen.

## **Antigens, antigen recognition and antigen receptors**

A cardinal feature of the specificity of the immune response is its ability to recognize and respond to molecules that are foreign or non self and avoid making a response to those molecules that are self.

Most biological materials serve as antigens which function as immunogens i.e. they induce an immune response against this antigenic substance after its recognition and binding by lymphocytes. Some antigens act as tolegens i.e. they induce immunological tolerance.

**\* Factors affecting immunogenicity:**

Whether exposure to an antigen results in an immune response or not is dependent on a variety of factors:-

(A) Nature of the antigen:-

(1) Molecular weight:

- Macromolecular structures are usually potent immunogenic (> 10,000 daltons), e.g. tetanus toxins, albumin.
- Small molecular weight compounds (< 1000 daltons) are usually not immunogenic.

(2) The degree of foreignness of the molecule: The animals usually do not respond to self antigens (except in pathological states e.g. autoimmune disease).

(3) Chemical complexity:

The more complex the molecule, the greater the likelihood of being immunogenic in most individuals. (Large homopolymers are not immunogenic).

\* So, for a substance to be immunogenic it must be foreign, of large molecular weight and complex structure.

\* Other factor is the susceptibility of this substance to enzymatic degradation.

## **N.B.**

- \* Proteins: Virtually all proteins are immunogenic. In general they are multideterminant antigens.
- \* Carbohydrates: Polysaccharides are potentially not always immunogenic. Glycoproteins are immunogenic.
- \* Lipids and nucleic acids: are usually non immunogenic unless conjugated with protein moieties (carrier), they induce antibodies reactive with nucleic acids.

### (B) Exposure to the antigen:

- Each antigen has an optimum dose for immunogenicity.
- Intermittent exposure for antigen gives greater response.
- Presence of adjuvant (in experimental animals) → greater immune response.

### (C) Nature of recipient:

- Very young animals and elderly have less efficient immune system.
- Genetic make up and state of nutrition of the individual and associated conditions or diseases.

## **N.B. Haptens:** (Haptens to grasp)

Low molecular weight antigens which are not capable of inducing an immune response by themselves (i.e. not immunogenic by themselves) but when they are coupled with larger compounds such as proteins (carriers) they become immunogenic.

- \* Examples: antibiotics, drugs e.g.  $\alpha$ -methyldopa.

**\* Antigen-receptor interactions:**

The immune response elicited by antigen generates antibodies or lymphocytes that react specifically with that antigen.

The smallest portion of the antigen that binds specifically with the binding site of an antibody or a receptor on a lymphocyte is called antigenic determinant or "epitope".

Compounds may have one or more epitopes capable of reacting with immune components.

**\* Antigen receptors are:**

\* Antibodies

\* T-cell receptors

\* MHC molecules.

**\* Antibodies:**

- Large glycoprotein structures with recognition sites for antigenic determinants or epitopes. The part of the antibody which contacts the antigen is called paratope.
- They are present on surface of B lymphocytes or produced by plasma cells.
- They can react with soluble antigens or fixed antigens on surface of cells or tissues.

**\* T-cell receptors:**

- Large glycoprotein receptor on the surface of T lymphocytes. They cannot interact directly with soluble antigens (i.e. native antigens in extra cellular fluid). The antigen must be degraded, held and presented to the T-cells by other glycoprotein molecules (MHC molecules). The T-cell recognizes both MHC and the peptide antigen.

**\* MHC molecules:**

Major histocompatibility complex hold peptide antigens enclosed within a groove. The T-cell receptor recognizes a combination of the shapes formed by the peptide antigen and the wall of the groove in the MHC molecule.

**\* Antigen-Antibody binding:**

- Antigen antibody binding does not involve covalent bonds. The binding may involve electrostatic interactions, hydrogen bonds and Van der Waals forces.
- Antigen antibody binding is reversible.

\* **Affinity:** The strength of binding of antibody to a single combining site (antigen determinant site).

\* **Avidity:** The strength of attachment between the antigen and its receptor. It is the functional affinity, e.g. the reaction of antiserum to a multivalent antigen.

# Antibodies

## Definition

They are soluble glycoprotein molecules that exhibit antigen binding ability. They are termed immunoglobulins (Ig) because they belong to the  $\gamma$ -globulin class of proteins and have an immune function.

\* The structure of immunoglobulin incorporates essential features for participation in the immune response. The most important features are specificity and biologic activity.

## \* Structure of immunoglobulins:

\* Heavy and light chains:

The typical Ig molecule has a molecular mass of 150-200 KDs. and is made up of 4 polypeptide chains two heavy (H) and two light (L) chains.

- The two heavy chains and the two light chains are identical to each other.
- There are alternative types of light chains (K and  $\lambda$ ). An individual Ig molecule either has two K chains (2/3 of cases) or two  $\lambda$  chains and never K $\lambda$  in the same molecule.
- There are five major heavy chain types termed  $\alpha$ ,  $\delta$ ,  $\epsilon$ ,  $\gamma$  and  $\mu$ .
- In an antibody molecule the two heavy chains are linked together by interchain disulphide bonds. Each heavy chain is attached to a light chain by interchain disulphide bonds.
- In an antibody molecule V regions are the variable regions (antigen binding sites). The variability is critical for generating the potential to

bind to more than  $10^7$  different antigen structures. The C regions are relatively constant in each molecule and hold the effector functions of the molecules e.g. complement activation.

**\* Domains:**

Within the heavy and light chains, there are interchain disulphide bonds. These bonds bend segments back into themselves creating regions called domains, within the heavy and light chains. This domain structure is very distinctive. Similar structure was found in MHC molecules, adhesion molecules, T-cell receptors, and cellular co-receptors. So, these were given the term immunoglobulin supergene family.

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**\* Hinge region:**

There is a hinge region in the middle of Ig molecule which allows flexibility of the two arms of the Y shaped molecule bearing the antigen binding sites. This increases the chances of binding two antigens at one time.

- \* Enzymatic cleavage of the antibody molecule by papain at the hinge point produces 3 fragments:

- \* Two identical Fab fragments (fragment antigen binding) and a larger fragment which is crystallizable (Fc fragment), which holds to effector function of the molecule.

**\* Functions of immunoglobulins:**

(A) In host defence:

(1) Elimination of the infective organisms by:

a- Binding to prevent adhesion and invasion of organisms.

b- Opsonization of particles for phagocytosis.

c- Lysis (in combination with complement).

(2) Antitoxin activity (toxin neutralization) e.g. prevention of tetanus.

(3) Sensitization of cells for antibody dependent cell cytotoxicity (ADCC).

(4) Immune regulation: Surface Igs on B cells act as antigen receptors to present antigens to T lymphocytes.

(B) In clinical medicine:

- \* Specific antipathogen antibody levels used in diagnosis and monitoring of diseases.

- \* Pooled antibodies administered passively for host therapy/protection.

(C) In Lab. Sciences:

Antibodies are used in a vast range of diagnostic and research studies.

### \* Immunoglobulin Classes "Variants":

There are 5 major classes of Igs according to the heavy chain type: IgA ( $\alpha$  chain), IgM ( $\mu$  chain), IgG ( $\gamma$  chain), IgE ( $\epsilon$  chain) and IgD ( $\delta$  chain). IgG is further divided into 4 subclasses (IgG<sub>1-4</sub>). IgA is divided into IgA<sub>1</sub> and IgA<sub>2</sub>.

- **IgM:**

It is a large pentamer composed of 5 IgM monomers, joined by J chain. This antibody is confined mainly to the intravascular space.

It is the major antibody formed in primary immune response i.e. the first to be synthesized in an antibody response to an antigen. Elevated levels indicate recent infection or recent immunization.

It does not cross the placenta. Elevated levels of IgM in a newborn infant, indicates intrauterine infection.

It has multiple functional domains; therefore it is the most potent Ig activator for complement.

It has potential binding sites. So, it is an efficient agglutinating antibody!

IgM antibodies include "natural isohaemagglutinins" which are naturally occurring antibodies against red cell antigens of the ABO blood groups. They are responsible for transfusion reactions which arise as a result of ABO incompatibility in which the recipient haemagglutinins react with the donor's R.B.Cs.

### **\* IgG:**

This is the most abundant immunoglobulin in serum. It is present as a monomer and has a high antigen affinity. It is the major antibody in secondary immune response.

- It is the only antibody that crosses the placenta in significant amounts. This gives some specific protection on the new born during the period when its own immune system is immature.

This placental transfer is responsible for the hemolytic disease of the newborn (erythroblastosis foetalis). This is caused by maternal antibodies to foetal red blood cells. The maternal IgG antibodies produced by Rh -ve mother to Rh antigen, pass across the placenta and attack the foetal R.B.Cs. that carry Rh antigen (Rh +ve).

\* IgG has 4 subclasses (Ig G<sub>1-4</sub>).

IgG<sub>1</sub> and IgG<sub>3</sub> are produced mainly in response to protein antigens such as tetanus toxin and many viruses. They are good opsonins, binding Fc receptors on neutrophils and activating complement.

IgG<sub>2</sub> and IgG<sub>4</sub> are produced in response to polysaccharide antigens (e.g. the capsule of the bacteria such as pneumococci and H. influenzae) and they are the major opsonins for such organisms.

### **\* IgA:**

This is the next most abundant Ig molecule. It can occur as monomer (in serum) and as a dimer where two molecules are joined together by a short peptide (J chain). It is the major Ig secreted into external surfaces (saliva, bronchial fluid, GIT secretions, tears, milk)

where it is secretory IgA. It is transported by a secretory component to the mucosal surface.

It has an important function in protection against bacterial, viral and protozoal infections of the mucosae. Its protective effect is by preventing the invading organism from attachment to and penetration of epithelial surfaces.

For IgA responses, localized antigen exposure gives rise to generalized mucosal immunity which is important in vaccination. This is because after encountering antigen, IgA precursor B cells in the mucosal lymphoid follicles journey to regional lymph nodes. After clonal expansion, the cells return to the systemic circulation via the thoracic duct and circulate to settle in the MALT not just the area of antigen exposure.

Selective IgA deficiency is characterized by severe infections of mucosal surfaces (GIT, upper RT & Lower RT).

**\* IgD:**

Its serum levels are very low and its function in the serum is not certain.

IgD is present on the surface of B lymphocytes and may have an immune regulatory role. It has been suggested to have a role in B lymphocyte activation.

**\* IgE:**

It is the largest Ig monomer, having 4 C<sub>H</sub> domains. It is present in the serum of healthy individuals at extremely low levels. Most of IgE is membrane bound on high affinity receptors on mast cells and basophils.

Its levels in the serum rise in response to parasitic infestations especially nematodes e.g. ascaris and in individuals who are a topic or patients with type I (immediate) hypersensitivity reactions.

It has low affinity receptors for on eosinophils & B lymphocytes.

\* **Kinetics of antibody responses:**

- Primary and Secondary antibody responses:

\* Primary response:

The first exposure of an individual to an immunogen (primary immunization) and the measurable response is called the primary response.

After the latent phase (a time before the antibody is detected in serum which is about 1-2 weeks, which includes the time taken for T and B cells to contact the antigen, proliferate and differentiate and the plasma cells secrete antibodies in sufficient amount to be detected). Then there is a rise in concentration of antibodies which becomes steady and then decline. The first class of the antibodies detected is the IgM (sometimes the only class detected). Then IgG production occurs with rapid cessation of IgM production.

\* Secondary response:

After the second exposure to the antigen, there is a short latent phase with greater production of the antibody with higher concentration in serum, which continues for prolonged periods. There is a class shift where the IgG appears in large amounts than IgM, which may be greatly reduced or disappear altogether.

So, the secondary response is therefore antigen specific and demonstrates acquisition of memory and higher in intensity. This is called anamnestic memory response, which may last even years.

**\* Monoclonal and polyclonal antibodies:**

\* Monoclonal antibodies:

Homogenous populations of antibody molecules, derived from a single antibody producing cell, in which all antibodies are identical and of the same precise specificities for a given epitope.

Monoclonal antibodies are produced by the hybridoma technique. Human monoclonal antibodies are produced by genetic engineering (recombinant DNA technology).

\* Polyclonal antibodies:

Most antibody responses to complex macromolecular antigens involve the targeting of multiple epitopes on the antigen. Each epitope may be targeted by more than a single antibody molecule. Thus, many clones of plasma cells and many antibody types are produced in a typical antibody response (Polyclonal antibody).

# Major Histocompatibility Complex "MHC"

- **Definition**

- \* **MHC:**

These are large collection of genes that include those responsible for rejection of transplanted tissues by the immune system. These are present in vertebrate species.

- \* **Human Leukocyte antigens "HLA"**

A term for human MHC.

- \* **Structure:**

- **HLA genes or MHC:**

Each contains 3 classes of genes found on the short arm of chromosome 6.

- \* **Class I:** Include HLA, A, B, C.

- \* **Class II:** Include DR, DP, DQ.

- \* **Class III:** Include genes encoding for complement and TNF.

- **Gene polymorphism:**

- \* The MHC genes (class I & II) display remarkable polymorphism (= the presence of different allelic forms of a gene at a given locus)

DNA). This polymorphism creates a difference in tissue compatibility between different subjects which is a barrier to organ transplantation.

- \* MHC genes and their products i.e. surface molecules are studied by DNA sequence identification and serology respectively.

\* **HLA molecules:**

\* Class I molecules:

- They are expressed on the surface of all nucleated cells.
- They are composed of a three domain & chain glycoprotein molecule and an invariant  $\beta_2$  microglobulin (a product of a gene on chromosome 15).
- They present peptide antigens from endogenous sources (including viral proteins if they are made within the cell presenting antigens) to cytotoxic T-cells ( $CD_8$ ) cells.

**N.B.** Law of MHC restriction:

Antigen specific cytotoxic T-cells responses are restricted to kill only target cells that bear the correct MHC molecule.

\* Class II molecules:

- They are expressed on the surface of B lymphocytes, mononuclear phagocytes, tissue mononuclear phagocytes e.g. Kupffer cells, follicular dendritic cells and activated T-lymphocytes.
- They comprise a pair ( $\alpha$ ,  $\beta$ ) of two chain domains.
- They present exogenous peptides, (plasma proteins, cell surface proteins and bacterial proteins) to T-cell receptor of  $CD_4$  T-lymphocytes.

**N.B.** T-cell receptor makes contact with the lips of the groove within the MHC molecules and the peptide antigen, only T-cells bearing CD<sub>4</sub> surface glycoproteins can bind to class II presented peptides, while CD<sub>8</sub> is required for interaction with those presented by class I.

**\* HLA and disease susceptibility:-**

The association between inheritance of particular genes in the MHC and higher risk of development of certain diseases has been found.

**Examples:**

\* HLA B<sub>27</sub> association with ankylosing spondylitis. (an inflammatory disease associated with stiffness of the vertebral joints of the spine). More than 90% of patients having ankylosing spondylitis have HLA B<sub>27</sub> but not all subjects having HLA B<sub>27</sub> have ankylosing spondylitis.

\* Type I (insulin dependent diabetes):

B<sub>8</sub> DR<sub>3</sub> DR<sub>4</sub> (susceptibility)

\* Grave's dis. B<sub>8</sub> DR<sub>4</sub>.

\* Rheumatoid arthritis DR<sub>4</sub>.

**N.B. ABO System** (blood grouping):

There are 4 blood groups: A, B, AB, O. These are controlled by 3 gene alleles (A, B, and O) where A and B are dominant over O.

\* Group A: Have A antigen on R.B.Cs. and anti B antibodies in serum which agglutinate Group B and AB RBCs.

- \* Group B: Possess B antigen on R.B.Cs and anti A antibodies in serum which agglutinate group A and AB, R.B.Cs.
- \* Group AB: Possess A and B antigens on R.B.Cs but no agglutinins in serum.
- \* Group O: Possess no A and B antigens on R.B.Cs but has both anti A and anti B in serum.
- \* Genotypes are: A (AA or AO), B (BB or BO), AB (AB) and O (OO).

## **Fate of antigens after penetration:**

The reticulo endothelial system (RES) or the mononuclear phagocyte system is designed to trap foreign antigens that have penetrated the body and to subject them to ingestion and degradation by the phagocytic cells of the system.

Also, there is constant movement of the lymphocytes throughout the body, this movement allows deposition of lymphocytes in strategic places along lymphatic channels. The system not only traps antigens but also provides sites (the secondary lymphoid organs) where antigens, macrophages, T-cells, and B cells interact within very small area to initiate an immune response.

Fate of antigen after penetration of skin or mucosal barriers:

- (1) If the antigen is entering through the blood stream, it is carried to the spleen, where it interacts with antigen presenting cells such as dendritic cells & macrophages, T-cells and B cells to generate an antigen specific immune response. Then the spleen releases the

antibodies directly into the circulation. Lymphocytes also leave the spleen to the circulation.

- (2) The antigen may lodge in the epidermal, dermal, or subcutaneous tissues, where it may cause an inflammatory response. From these tissues, the antigen free or trapped by antigen presenting cells, is transported through the afferent lymphatic channels into the regional draining lymph nodes. In the LNs, the antigen, macrophages, T-cells, & B cells interact to generate the immune response. Eventually, antigen specific T-cells and antibodies which have been synthesized in the lymph node enter the circulation and are transported to the various tissues. Antigen specific T and B cells and antibodies also enter the circulation via the thoracic duct and are thereby redistributed to various tissues.
- (3) The antigen may enter the GIT or respiratory tract, where it lodges into the MALT. There it will interact with macrophages and lymphocytes. Antibodies synthesized in these organs are deposited in the local tissue. In addition, lymphocytes entering the efferent lymphatic are carried through the thoracic duct to the circulation and are thereby redistributed in various tissues.

**\* Cells involved in the immune response Identification and Characterization of Cells:**

**The CD Classification**

CD is the abbreviation for "Cluster of Differentiation".

**Definition:**

These are individual surface molecules which are assigned a cluster of differentiation (CD) numbers defined by a cluster of monoclonal antibodies reacting with that molecule.

**In other words:**

It is a surface molecule found on cells according to their lineage and differentiation and identifiable by one or more monoclonal antibodies.

\* The CD numbers reached about 130.

**\* Function:**

\* Every molecule expressed at the cell surface has some legend that binds to it and thus, this surface molecule play some role in receiving a signal from outside.

**\* Examples:**

\* CD<sub>3</sub>: Present on all mature T-cells, intimately associated with T-cell receptor.

Its function is signal transduction, as a result of antigen recognition by T-cells.

**\* CD<sub>4</sub>:**

- Present on T-helper cells (2/3 of circulating T-cells).
- Function as T-cell receptor co-receptor.
- It is a receptor for class II MHC and HIV.

**\* CD<sub>8</sub>:**

- Present on cytotoxic, suppressor T-cells.
- Function as co-receptor & MHC class I receptor.

**\* CD<sub>19</sub>, CD<sub>20</sub>, CD<sub>21</sub>:**

- Present on all mature B cells.
- Have a role in B cell activation.
- CD<sub>21</sub> is an EBV receptor.

**\* CD<sub>28</sub>:**

- Present on activated T-cells.
- Act as T-cell co-stimulation molecule.

**\* CD<sub>14</sub>:**

- Present on mononuclear phagocytes (specific).
- Unknown function.

**\* CD<sub>34</sub>:**

- A marker for early stem cells.

\* **CD<sub>45</sub>:**

- Cells of hemopoietic origin "leucocyte common antigen".
- Has a role in signal transduction.

\* **CD<sub>56</sub>:**

- NK cell marker mediates cell adhesion.

\* **CD<sub>80/86</sub>: (B7.1 and B7.2):**

- Antigen presenting cells co-stimulatory molecule.

\* **CD<sub>95</sub>:**

- Present on multiple cell types, role in programmed cell death.

## **Mononuclear phagocytes and specialized antigen presenting cells.**

\* Cells of monocyte/macrophage lineage are now frequently referred to as mononuclear phagocytes.

\* They have none of the cognitive capacities (memory, specificity and amplification) of acquired immune system on their own but they have an integral part in cell mediated acquired immune response.

\* **Distribution and maturation:**

Monocytes are the blood forms. They migrate to various tissues and undergo further differentiation into macrophages which are included in the previously called RES which is not termed mononuclear phagocyte system, which is widely distributed throughout the body.

Monocytes migrate either:

Randomly, into sites of inflammation or in a tissue directed way to become specialized cells.

These include: Kupffer cells in the liver, alveolar macrophages in the lung, splenic macrophages, peritoneal macrophages, microglial cells in CNS, mesangial cells in the kidney and osteoclast cells in bone. Also, multinucleate giant cells in sites of chronic inflammation (granulomas) e.g. T.B.

\* **Surface markers:**

\* CD<sub>14</sub> specific marker, unknown functions.

\* MHC class II molecules.

\* Fc receptors and complement receptors.

\* **Activation of macrophages:**

\* Main activators are: Cytokines e.g.

IL<sub>3</sub> (interleukin 3)

GM-CSF (granulocyte macrophage-colony stimulating factor)

M-CSF (macrophage-colony stimulating factor)

IFN $\gamma$  (interferon gamma)

IFN $\alpha$  (interferon alpha)

\* **Effect of activation:**

- Proliferation – ↑ phagocytic, killing activity, antigen presentation and secretory capacity.

\* **Functions of mononuclear phagocytes:**

(1) Phagocytosis:

The major function is to trap microorganisms and foreign substances that are in the blood stream and various tissues and expose them to phagocytosis. They have many bactericidal activities (as neutrophils). They are important in ingestion and killing of intracellular parasites such as T.B. that is why they are involved in granuloma formation. They also function in the destruction of aged cells such as R.B.Cs.

They have the ability to bind and engulf particulate materials and antigens. Their location along capillaries make them the cells that most likely meet the first contact with invading pathogens and antigens.

- \* During chronic immune responses due to indigestible materials e.g. silicon or chronic infections e.g. T.B., the mononuclear phagocytes differentiate into an end stage form (multinucleated giant cells).

(2) Antigen presentation:

The mononuclear phagocytes have a critical role in activating T-lymphocytes in specific immune responses by processing and presenting antigens to CD<sub>4</sub> T-cells within a groove in class II MHC molecules.

So, they share in innate and acquired immunity.

(3) Secretory function:

Major products of mononuclear phagocytes are:

- Proinflammatory & microbicidal agents e.g. lysozyme.
- Cytokines:

$IL_1$  → T-cell activation & proinflammatory.

$IL_6$  → T-cell activation & proinflammatory.

$TNF_{\alpha,\beta}$  (tumour necrosis factor) → cytotoxic, microbicidal.

$IFN_{\alpha,\beta}$  (antiviral)

### \* Other antigen presenting cells.

Antigen presenting cells include:

Mononuclear phagocytes

Dendritic cells

Langerhans cells of the skin.

B lymphocytes

### \* Dendritic cells:

They are found mainly in the spleen, lymph nodes and in small numbers in blood.

They have an irregular shape and numerous dendritic processes.

They have a major role in antigen presentation to and activation of naïve or virgin T-cells (cells that have not previously encountered an antigen).

- They permanently express class II MHC molecules on their surfaces.
- Specialized forms of dendritic cells are:

Langerhans cells of the skin and follicular dendritic cells in LN follicles which have the capacity to present the antigen to B and T-lymphocytes through less well defined mechanisms.

## **Clonal Selection Theory**

1. B and T lymphocytes of all antigenic specificities exist prior to contact with antigen.
2. Each lymphocyte carries Igs or T-cells receptor molecules of only a single specificity on its surface.
3. Lymphocytes can be stimulated by antigen under appropriate conditions to give rise to progeny with identical antigenic specificity. Then, in case of B cells, the antigen specific receptor Ig is secreted as a consequence of stimulation.
4. Lymphocytes potentially reactive with self are deleted or inactivated. This ensures that no immune response is mounted against self components (otherwise auto immunity will develop).

## **B Lymphocytes**

- \* These are antibody forming cells "B = bursa derived".
- \* The primary lymphoid organ for B lymphocyte development is the bone marrow and the secondary lymphoid organs are lymph nodes, spleen, ...

\* **Identification of B lymphocytes:**

Surface molecules on B lymphocytes are:

- 1- Surface Ig.
- 2- CD<sub>19</sub>, CD<sub>20</sub>, CD<sub>21</sub> [Specific for B cells]
- 3- Class II MHC molecules.
- 4- CD40 (which interacts with CD<sub>40</sub> legend on T cell → Ig class switching).
- 5- CD<sub>23</sub>. Both CD<sub>40</sub> and CD<sub>23</sub> are markers of B cell activation.

\* **B cell receptor complex:**

- Ig as an antigen receptor.
- + a signal transduction complex comprising the disulphide bonded heterodimer ( $\alpha$ ,  $\beta$ ).

\* Functions of B cells:

- 1) Ab. Production with T-cell cooperation.
- 2) Presentation of antigen to T-cells.

There is cognate interaction between T and B cells.

\* **Life cycle of B lymphocytes:**

- Antigen independent development:
  - Progenitor cells (Pro-B cells) migrate to the centre of the Bone marrow.
  - Pre-B cells (in bone marrow): have cytoplasmic  $\mu$  chains. [70% are deleted before leaving the bone marrow].

- Immature B cells (in bone marrow) have surface IgM, but not ready to respond to antigen.
- Virgin B cells (in LN and spleen) have surface IgM & IgD, but not encountered antigen.
- Antigen dependent maturation:
  - Mature B cells (in lymph nodes and spleen): "selection of B cells having highest affinity for antigen occurs "affinity maturation".
  - Effector B cells:
    - \* Memory B cells (resident in lymphoid organs) have surface Ig expressed. Can give rise to a swift, specific, high affinity, class switched secondary response.
    - \* Plasma cells: have characteristic appearance, do not have surface IgS but secrete antibodies of specific class (IgG, IgM, IgA or IgE).

**N.B.** If a plasma cell is committed to the production of a neutralizing antibody to a pathogen e.g. polio virus, immunity is retained for as long as plasma cells continue to secrete the antibody. Repeated stimulation by immunization boosters → appearance of more plasma cells → more antibodies.

\* **B lymphocyte activation:**

- The majority of activation occurs in LN follicles.

\* **Mechanisms of B cell activation:**

(1) Thymus dependent activation: (the dominant pathway)

- B lymphocytes use its surface receptors Ig to interact with antigen, after interaction, the antigen antibody complex is internalized. The antigen is degraded and a peptide fragment is attached to class II MHC molecules & is exported to the surface.
- The T-lymphocytes with a T-cell receptor complementary to that of B lymphocyte i.e. recognizing part of the same antigen, are recruited and activated and then activate the B lymphocyte.
- So, this system results in activation of both B and T-lymphocytes specific for that antigen.
- This generates high affinity, class switched, and specific antibodies.

(2) Thymus independent activation:

a. Polyclonal activation: (type I activation).

This results in activation of all B cells directly e.g. certain bacterial products such as lipopolysaccharides (not dependent on antigen specificity).

b. Type II pathway: "antigen specific".

Repeated linear antigens cross links several antigen specific Ig molecules simultaneously causing sufficient strong activation stimulus that T-lymphocyte help is not required.

**\* Sequence of B cell activation:**

- B cell activation results in clonal expansion with the generation of plasma cells and memory B cells.
- When a B lymphocyte is activated by an antigen → IgM production occurs after 5-10 days then 2-3 days later IgG production occurs (Primary response).

- Rechallenge with the same antigen → activation of primed memory cells → quicker response (3-5 days), IgG produced early and greater in amount (secondary response).

\* **Signals in B lymphocyte activation:**

T cell derived interleukins e.g.

- \* IL<sub>4</sub> (the main B cell activator)

IL<sub>5</sub>, IL<sub>2</sub> → clonal expansion.

IL<sub>6</sub> → B lymphocyte growth factor & enhance IgG class switching.

- \* In vitro activation:

Mitogenesis (polyclonal activation) e.g. Pokaweed mitogen.

\* **Physiological importance of B cell activation:**

- 1- If there is a gamma globulinemia (rare genetic disorder) → there is life threatening infections.
- 2- Vaccination: inactivated pathogen is used to stimulate the primary antibody response. So, when a real pathogen is met, a pre existing immunity and a secondary response can be evoked.

**N.B. Class and subclass switching:**

- \* Class switching means selection during B cell development of different Ig heavy chain.

- \* Effect of cytokines: e.g. IL<sub>4</sub> → IgE

IFN $\gamma$  → IgG

- \* Nature of antigen & site of products e.g. mucosa → IgA.

# T-lymphocytes

\* T-lymphocytes arise in the thymus and carry an antigen specific T-cell receptor.

\* Identification & surface markers:

(1) T-cell receptor (TCR) complex = T-cell marker.

\* This complex is present on 100% of T-cells.

\* T-cell receptor either:

$\alpha\beta$  heterodimer (majority) or  $\gamma\delta$  (minority).

\* CD<sub>3</sub> complex: involved in the transduction of antigen specific activation signals through the T-cell receptor (Pan T-cell marker).

(2) CD<sub>4</sub><sup>+</sup> T-cell (T-helper cells): 66% of T-cells:

- Expressing surface glycoprotein molecules (CD<sub>4</sub><sup>+</sup> T-cells).
- CD<sub>4</sub><sup>+</sup> molecule: stabilizes the interaction between the T-cell and cells presenting the antigen through class II MHC molecule by binding to its  $\beta_2$  domain. It provides an accessory activation signal. It is a cellular + receptor for HIV.

(3) CD<sub>8</sub><sup>+</sup> T-cells (cytotoxic/suppressor T-cells): 33% of T-cells.

- Expressing surface glycoprotein molecules (CD<sub>8</sub>).
- CD<sub>8</sub> glycoprotein molecule interacts with class I MHC molecule binding to  $\alpha_3$  domain stabilizing T-cell interactions with cells presenting the antigens.

\* T-lymphocyte development:

"Thymic education"

\* The bone marrow derived T-cells enter the thymus and mature within it  
"They are termed thymocytes".

\* Thymic education takes about 3 weeks.

\* Only 1% of precursor cells entering will be selected for the periphery and leave as mature cells.

\* The antigen presenting cells in the thymus are thymic epithelial cells and bone marrow derived dendritic cells.

\* The T-cell precursor enters the thymus and become recognizable as potential T-lymphocytes and starts to rearrange  $\beta$ -chain genes of TCR then  $\alpha$ -chain genes become rearranged and expressed. Following this, the CD<sub>3</sub> complex and both CD<sub>4</sub> and CD<sub>8</sub> molecules appear on thymocyte surface.

\* Thymic Selection: is based on affinity for MHC molecules.

● Negative selection:

– Cells with TCR having low or no affinity for peptides presented with class I or class II MHC molecules, have no development signal and undergo apoptosis.

– Cells with high affinity for self peptide-MHC complex are deleted by apoptosis (because of dangerous self reactivity).

- Positive selection:

The optimum characteristics is moderate affinity for a peptide presented by self MHC molecules (The nature of the peptide is unknown).

- \* Cells having moderate affinity for class I molecule-peptide acquire positive selection signal and loss of CD<sub>4</sub> i.e. becoming CD<sub>8</sub><sup>+</sup> T-cell.

- \* Cells interacting effectively with peptide-class II complexes acquire positive selection signal and loss of CD<sub>8</sub> i.e. becoming CD<sub>4</sub><sup>+</sup> T-cell.

- \* Thus, virgin T-cells leaving the thymus, therefore express a TCR selected for binding a particular MHC-peptide complex and either CD<sub>4</sub> or CD<sub>8</sub>.

- \* Functions of T-lymphocytes:

- 1- Signaling for B cell expansion inducing them to produce antibodies and mature into plasma cells or memory cells.

- 2- Recruitment and activation of cells of mononuclear phagocyte lineage.

- 3- Recruitment and activation of specialized T-cells (CD<sub>8</sub> T-cells) in antiviral responses.

- 4- Secretion of cytokines responsible for growth and differentiation of a range of cell types including other T-cells, macrophages and eosinophils.

- 5- Regulation of immune reactions.

\* T-lymphocyte activation:

● **Antigen presentation:**

\* Exogenous pathway:

CD<sub>4</sub> T-cells are activated by peptides that are derived from exogenous proteins which are internalized and processed in the antigen presenting cells and presented bound to MHC class II molecules on the surface of antigen presenting cells.

\* Endogenous pathway:

CD<sub>8</sub> T-cells are activated by endogenously derived peptides presented with class I MHC molecules.

\* Molecules involved in T-cell activation and differentiation:

- Lymphocyte activation requires at least two signals: the antigen + cosignal (from CD<sub>4</sub>, CD<sub>8</sub>, CD<sub>45</sub>, adhesion molecules and CD<sub>28</sub> co-signals).
- To be effective, the signal must be transduced, and amplified within the lymphocyte by a series of reactions.

\* Molecules are:

- TCR CD<sub>3</sub> complex (major key molecules)
- CD<sub>4</sub>, CD<sub>8</sub>
- CD<sub>45</sub>: (Leukocyte common antigen)
- CD<sub>28</sub> (interaction with molecules on antigen presenting cells → up regulation of IL<sub>2</sub> and its receptors).

- Sequence of activation:

(T-cell mediated immune responses).

- \* CD<sub>4</sub> (Thelper lymphocytes): → cytokine production (classified into).

- \* Thelper 1 (T<sub>H1</sub>):

Produce IFN $\gamma$ , IL<sub>2</sub> → activation of macrophages, activation of cytotoxic T-cells and antagonize T<sub>H2</sub>.

- \* Thelper 2 (T<sub>H2</sub>):

Production of IL<sub>2</sub>, IL<sub>4</sub>, IL<sub>5</sub>, IL<sub>6</sub>, IL<sub>10</sub> → activation and maturation of B cells & antibody production and antagonism of T<sub>H1</sub>.

- \* T helper O (T<sub>H0</sub>):

Production of IFN $\gamma$ , IL<sub>2</sub>, IL<sub>4</sub>, IL<sub>5</sub>, IL<sub>6</sub>, IL<sub>10</sub> has variable functions.

- \* CD<sub>8</sub><sup>+</sup> T-cell:

Cytotoxic T lymphocytes (Tc):

- \* Important in defence against virally infected cells, rejection of foreign tissue grafts and possible also in immune responses to certain tumour types.

- \* They kill cells bearing peptide antigens with MHC class I molecules. Target peptides are fragments of virus or tumour antigens.

- \* Recently, Tc (cytotoxic T-cells) are divided into Tc1 and Tc2 on the basis of cytokine production.

\* The induction of cytotoxic T-cell response is dependent upon CD<sub>4</sub> T-lymphocytes which provide activation stimuli.

\* Sequence of activation:

– CD<sub>4</sub>T<sub>H</sub> cells is activated to respond to viral peptides presented by macrophages at site of infection. Then CD<sub>4</sub> T<sub>H</sub> cells activate virus specific T-cells (by cytokines as IL<sub>2</sub>, IFN $\gamma$  and IL<sub>6</sub>).

– Following activation appears in the cytoplasm. The granules contain a protein which causes membrane pores (perforin or cytolysin). Also, they contain proteolytic enzymes and a protein toxin called lymphotoxin.

\* Next, there is cytokine production and release e.g. IFN $\gamma$ , lymphotoxin & IL<sub>2</sub> (has antiviral activity).

\* Mechanisms of killing:-

\* Killing by CD<sub>8</sub> T-cells is class I MHC restricted.

\* Cytotoxic T-cells are not damaged during the process.

\* Steps of lysis:-

1. Cell contact:

TCR interaction with peptide-MHC class I & CD<sub>8</sub> interaction with MHC and other adhesion molecules involve.

2. Activation of cytotoxic T-cells.

### 3. Delivery of lethal hit:

- a. Perforins release → which polymerize and cause membrane pores (as MAC of the complement), if sufficient number of pores → osmotic lysis and death of cells.
- b. Cytotoxic T-cells release lymphotoxin which activate enzymes in the target cell to cleave DNA in the nucleus → clumps of nuclear DNA (i.e. apoptosis: programmed cell death).

4. Dis engagement of cytotoxic T-cells: The cells are protected from the action of its granules by presence of protections on its surface.

5. Osmotic lysis: or programmed cell death of the target cells.

\* Immune suppression by T-lymphocytes: Immune regulation  $CD_8^+$  T-cells with the help of  $CD_4^+$  T-cells.

\* Mechanisms of immune suppression include:

1. Cytokine release (not antigen specific).

- $TGF\beta$  (transforming growth factor  $\beta$ ) → T and B cell proliferation.
- $IL_4, IL_{10}$  → mutual antagonism by  $IFN\gamma$ .

2. Antigen specific soluble inhibitory factors.

3. Cytotoxic suppression & others.

## \* **Immunological tolerance:**

### **Def.**

Immunological tolerance is a specific failure of immune responsiveness resulting from prior exposure to antigen i.e. the controlled inability to respond to antigens to which an individual has the potential for response.

### **Mechanisms:**

- (1) Clonal energy: or unresponsiveness: Autoreactive cells (B mainly) remain present but functionally inactivated and become unresponsive.
- (2) Clonal deletion: The cells are killed (T-lymphocytes mainly).
- (3) Tight control of T-lymphocyte help: by suppressor T-cells effect on unwanted lymphocyte reactions.

### \* **Types:**

\* Central tolerance: in primary lymphoid organs.

\* Peripheral tolerance: in secondary lymphoid organs.

\* **Features of tolerance:** dependent on

\* Dose of antigen, site, delivery signal.

## **Natural Killer Cells**

\* They resemble T lymphocytes morphologically but form a separate lineage from T or B cells. They are able to kill target cells

spontaneously in absence of previous known sensitization to that target.

\* So, it is a cytotoxic cell that has the morphology of large granular lymphocyte but does not express the CD<sub>3</sub> complex or any of the T-cell receptor chains.

\* Function:

- 1- Immune surveillance against tumour cells and virus infected cells.
- 2- Release of cytokines: mainly IFN $\gamma$  early in the infection to activate phagocytic cells and recruit T lymphocytes.

\* Identification:

- Fc receptor for IgG (CD<sub>16</sub>).
  - CD<sub>56</sub> unknown function.
  - They do not carry CD<sub>3</sub> (Pan T-cell marker).
- } Specific markers

\* Activation of NK Cells:

- 1- First stage of activation is mediated by cytokines.

IFN $\gamma$ , IL<sub>2</sub>, I<sub>42</sub>

- 2- Killing mechanisms:

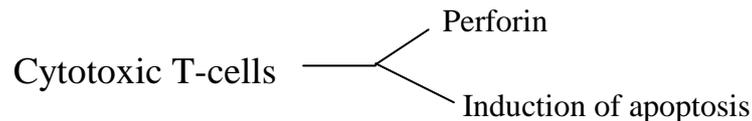
- a. Direct cell-cell contact with target cells:-

Killing is initiated when a lectin type NK surface receptor binds CHO moieties on the target cell surface, in the absence of class I MHC molecule (which gives inhibitory signal to cell lysis). So, NK cells have the ability to identify and lyse cells either.

Having intracellular viral infection (some viruses down regulate MHC molecules and evade recognition by cytotoxic T cells).

Tumour cells (surface proteins of tumour cells are abnormally glycosylated).

\* The mechanism of killing as



b. Antibody dependent cell cytotoxicity (ADCC): Targets are bound by IgG antibody for which the NK cells has a receptor (Fc receptor = CD<sub>16</sub>). This mechanism is shared by other lymphocytes other cell.

## "Apoptosis"

- \* Programmed cell death.
- \* Frequently accompany the process of development of lymphocytes in bone marrow and lymph nodes (in case of B cells) and in thymus (in case of T-cells).
- \* CD<sub>95</sub> (Fas) a key molecule in the process of apoptosis (absence of Fas → no apoptosis).
- \* **N.B.** A mouse model with absent fas, is characterize by massive proliferation of lymphocytes, lymphadenopath and associated autoimmune diseases.

# "Cytokines"

\* **Cell interactions:** Occur by:

- Direct membrane-membrane contact between cell surface proteins.
- Soluble mediators (cytokines) which bind to a specific cell surface receptor.

\* **Def. of Cytokines:**

Small molecular weight soluble factors released by cells (Cyto-) to communicate and influence the function (-kines) of other cells through specific receptors.

- \* Much of the immune system's ability to communicate between different compartments is achieved through these soluble messenger molecules.
- \* They were originally named according to the function e.g. MAF (macrophage activating factor).

MIF (macrophage inhibiting factor)

NAP (neutrophil activating protein).

OR

- \* According to cells producing them e.g. lymphokines (lymphocytes) or monokines (by monocytes or mononuclear phagocytes). Then, the general term (cytokines) became more widely used.

\* Interleukins (ILs):-

Factors released by white cells and act on white blood cells i.e. leukocytes.

ILs from 1-17 have been cloned.

**\* General properties of cytokines:**

1- Pleiotropy:

i.e. they have different effects on different cells.

2- Autocrine:

i.e. it can affect the cell which releases it.

3- Paracrine:

i.e. they have effects on cells immediately around them.

4- Endocrine:

i.e. they have effects on cells and organs remote from the site of release.

5- They often induce the release of other cytokines.

6- Synergism:

Cytokines act together to produce effects greater than the summation of their individual actions.

\* Cytokines play a crucial role in amplification of immune response.

\* They are non antigen specific glycoproteins, which are generally synthesized and rapidly secreted in response to a stimulus, thus, they

are not stored within the cell that makes them. Most cytokines have very short half lives, consequently, cytokine synthesis, production and function occurs in a burst.

- \* It is worth mentioning that the production of a too high level of cytokines by a powerful stimulus can trigger deleterious systemic effects: one example is the toxic shock syndrome, which may result from staph. enterotoxin stimulation of T-cells. Similarly, macrophage release of high levels of  $\text{TNE}\alpha$  may also lead to septic shock.

\* **N.B. Chemokines:**

Low molecular weight cytokines which are primarily associated with chemoattractant actions. They are released at sites of infection and inflammation. They control the movement of leucocytes. They have a role in certain diseases e.g. asthma, rheumatoid arthritis and atherosclerosis. e.g. MCP, Eotaxin, RANTES,  $\text{IL}_8$  (interleukin 8 which attract neutrophils).

\* **Examples of Cytokines:**

\* Interferons: (IFNs)

They were first noted as a part of the innate immune system as natural antiviral agents. They are two types: type I and type II.

- Type I interferons:

$\text{IFN}\alpha$  and  $\beta$ .

- $\text{IFN}\alpha$  derived from mononuclear phagocytes.
- $\text{IFN}\beta$  derived from fibroblasts.

- They have paracrine protective function on all cell types in the body to inhibit viral replication (i.e.  $\uparrow$  synthesis of viral proteins).

They enhance expression of class I MHC which & renders the cells more susceptible to killing by cytotoxic T-cells. Also, they enhance class II expression on macrophages, enhancing antigen presentation. So, enhancing the possibility of immune recognition of virally infected cells by enhancing and inducing expression of MHC class I and II molecules.

**\* Uses:**

- 1- Recombinant IFN $\alpha$  used in patients who have chronic hepatitis B and C.
- 2- Treatment of some hematological malignancies e.g. Hairy cell leukemia (B cell tumours), Kaposi Sarcoma, Melanoma and renal carcinoma.

- Type II interferons:

IFN $\gamma$

It is released by T lymphocytes and activated NK cells ( $T_{H1}$ ).

- Its function mainly activation of macrophages ( $\uparrow$  phagocytic and killing activity).
- It  $\uparrow$  expression of class I & II MHC molecules  $\rightarrow$  antiviral protection.
- Activation of T lymphocytes ( $\downarrow$   $TH_2$   $CD_4T$ ) and B cells and class selection of Igs.
- Activation of NK cells.

\* Colony stimulating factors:

- Cytokines stimulate growth and differentiation of bone marrow progenitor cells.
- Granulocyte colony stimulating factor: G-CSF: Produced by T-cells, mononuclear phagocytes and endothelial cells → act on granulocyte lineage.
- Granulocyte macrophage colony stimulating factor: GM-CSF: promotes granulocyte and macrophage growth.
- Macrophage colony stimulating factor: M-CSF. Promotes mononuclear cell development.

\* Practical application: G-CSF, GM-CSF is used to restore the white blood cell count in patients receiving antileukemic chemotherapy causing bone marrow depression.

\* Tumour necrosis Factor: TNF.

- TNF $\alpha$ :

Produced by mononuclear phagocytes.

- TNF $\beta$ : (Lymphotoxin)

Produced by T-cells.

\* Actions:

- Local release → up regulation of adhesion molecules on vascular endothelium and neutrophils to enhance cell migration, activation of neutrophils and macrophages to kill microbes and stimulation of

cytokine release from macrophages. So, it is involved in inflammatory responses.

- Systemic effects ( $\text{TNF}\alpha$ ) → induction of fever and acute phase response. → It has a major role in the development of gram negative septicemia and septic shock.

\* **N.B.**

Long term effect of  $\text{TNF}\beta$  → appetite suppression and cachexia (severe weight loss).

\* Transforming growth factor:  $\text{TGF}\beta$

- \* Produced by T cells ( $\text{T}_\text{H}$ ) and mononuclear phagocytes.
- \* Its actions are inhibitory (down regulation) to mononuclear cell and T cells. i.e. anticytokine function
- \* It enhances production of IgA.

\* Interleukin 1:

- \* Produced by mononuclear phagocytes and other cells.
- \* Effect: It is a major mediator of inflammatory response.
- \* Local: ↑ endothelial expression of adhesion molecules to increase leukocyte adhesion and promotes release of  $\text{IL}_6$ .
- \* It activates  $\text{Th}_2$   $\text{CD}_4$ T cells.

\* Systemic effects:

Has endocrine effects, inducing fever and synthesis of proteins of acute phase response in the liver.

\* Practical application:

Trials to ↓ levels of IL<sub>1</sub> can reduce its inflammatory actions e.g. in septic shock and rheumatoid arthritis.

\* Interleukin 2: (T cell growth factor)

- Produced by T-cells.

→ Caused activation of T-cells through autocrine and paracrine function.

→ B lymphocyte development.

\* Practical application:

1-Blockade of IL<sub>2</sub> production → inhibition of T cell activation e.g. in transplant rejection (e.g. cyclosporine). Trials with antibodies blocking IL<sub>2</sub> action in GV HD and renal transplant but are too immunogenic.

2- Promotion of IL<sub>2</sub> → enhancement of immune response against tumours e.g. malign. melanoma, renal cell carcinoma.

\* Interleukin 3 (IL<sub>3</sub>):

- Multilineage colony stimulating factor.

- Produced by T cells and stimulate the growth and differentiation of haemopoietic cells.

\* IL<sub>4</sub>:

- Produced by T-cells (Th<sub>2</sub> CD<sub>4</sub> T-cells) and mast cells.
- Stim. B lymphocyte function and IgE production.
- Macrophage function (↓ Th<sub>1</sub> function).

\* IL<sub>5</sub>:

- Produced by T-cells (Th<sub>2</sub>) and activated mast cells.
- Stim. B cell growth and Ig secretion.
- Stim. growth and differentiation of eosinophils (antiparasitic and allergic responses).

\* IL<sub>6</sub>:

- Produced by T-cells, mononuclear phagocytes and endothelial cells.

\* Effect:

- \* Acute phase response to inflammatory episodes.
- \* ↑ synthesis of acute phase proteins by the liver e.g. CRP, complement fs. And clotting fs.
- \* Promotes growth and differentiation of β cells and IgG production.
- \* T-cell activation \* IL<sub>2</sub> production.

\* IL<sub>7</sub>:

Produced by bone marrow stromal cells → early lymphocyte development and proliferation (B cells).

\* IL<sub>8</sub>:

(Neutrophil activator protein) produced by many cells types including mononuclear phagocytes → neutrophil activation (chemoattractant).

\* IL<sub>9</sub>:

\* From activated T-cell → mast cell activation.

\* IL<sub>10</sub>:

Produced by TH<sub>2</sub> CD<sub>4</sub> T-cells →

\* TH<sub>1</sub> CD<sub>4</sub> T-cells (down regulate of T-cell immune response).

\* ↓ macrophage function (↓ TNF & IL<sub>2</sub> sec.).

\* Stim. B cell differentiation.

\* IL<sub>11</sub>:

\* From fibroblasts → stim. megakaryocytes (platelet precursors) growth.

\* IL<sub>12</sub>:

\* Produced by B cells, macrophages, T cells.

\* Most potent activator of NK cells → release IFN $\gamma$ .

\* IL<sub>12</sub> → as IL<sub>4</sub>

\* IL<sub>15</sub> → as IL<sub>2</sub>

## \* Effect of cytokines:

(1) Activation of cells of immune system:

- $IL_1 \rightarrow$  T cell activation.

$IL_2 \rightarrow$  T-cell activation, NK cells & others.

$IFN\gamma \rightarrow$  macrophage activation.

$IL_{4,2,6} \rightarrow$  activation of B cells  $\rightarrow$  plasma cells.

(2) Promotion of hemopoiesis:

$IL_3 \rightarrow$  multilineage colony stimulating factor.

$IL_7 \rightarrow$  B cells.

G-CSF  $\rightarrow$  stim. granulocyte lineage.

M-CSF  $\rightarrow$  macrophage development.

$IL_5 \rightarrow$  eosinophil differentiation.

(3) Role in inflammation:

$IL_1, TNF\alpha, IFN\gamma \rightarrow$  expression of adhesion molecules on endothelial cells  $\rightarrow$  extravasation of cells to infl. sites.

$IL_8 \rightarrow$  chemoattractant of neutrophils.

$IL_1, IL_6, TNF\alpha \rightarrow$  systemic effect = acute phase response.

- Lymphocyte  $\rightarrow$  activation.

- Bone marrow → neutrophil release and activation.
- Endothelium → activation.
- Fibroblast → proliferation.
- Liver → acute phase proteins products.
- Muscle → protein breakdown.
- Hypothalamus → fever.

(4) Cytostatic and antiviral activities:

TNF → tumour necrosis and regression.

IFNs → interfere with (inhibit) viral replication in infected cells and have a defensive role in the early stages of viral infection.

## **Hypersensitivity reactions and clinical allergy**

- **Def.**

Hypersensitivity reactions are exaggerated or inappropriate immune responses that result in tissue damage.

- **Classification:**

They are classified into 4 types according to Gell & Coombs:

\* Type I:

Immediate hypersensitivity reactions resulting from activation of mast cells by allergen and specific IgE.

\* Type II:

Cytotoxic or cytolytic reactions mediated by interactions of antibodies with cell surface antigens.

\* Type III:

Immune complex reactions occur when complexes of antigen and antibody accumulate in tissues or in the circulation and become deposited in tissues causing activation of complement and attraction of granulocytes.

\* Type IV: Delayed type or cell mediated hypersensitivity mediated by T cells, activated macrophages and cytokines.

\* N.B. Type V hypersensitivity reactions "stimulating or blocking reactions" where antibodies interact with cell surface receptors causing stimulation or blocking effect. This is classified also as type II reactions

## **Type I Hypersensitivity**

- **Def.**

\* Immediate hypersensitivity reactions mediated by interaction of an antigen (allergen) with IgE antibody on the surface of mast cells causing their degranulation and release of vasoactive mediators within minutes of introduction of the allergen.

- **Mechanism:**

\* IgE antibodies formed in response to an antigen (sensitization phase), bind to specific receptors on the surface of mast cells and basophils.

When these receptors are cross linked by contact with specific antigen (activation phase), the cell is triggered to respond by releasing its performed mediators within its granules and synthesizing other products. The pharmacological effects of these mediators produce the immediate symptoms typical of this response (the effector phase).

\* Effect of mediators:

\* Histamine:

- Local release → wheal & flare (due to ↑ permeability and vasodilatation).
- Systemic release → wide spread reactions (broncho-constriction, uterine cramps, ↑ vascular permeability → hives and oedema and fall of blood pressure → shock.

\* Arachidonic acid metabolites: Products of cyclo-oxygenase pathway (prostaglandins) and lipoxygenase pathway (leukotriens = previously called SRS – A i.e. slow reacting substance of anaphylaxis).

\* Other mediators include PAF (platelet activating factor), eosinophil chemotactic factors and neutrophil chemotactic factors.

\* Classic reactions occurs within minutes.

\* Late phase response:

These are delayed reactions occurring in some cases 4-8 hours after the acute reactions and may persist for several days, which occurs in response to granule matrix mediators causing recruitment & activations of infl. cells especially eosinophils.

## "Clinical allergy"

\* Allergic diseases characterized by type I hypersensitivity reactions and include:

- Extrinsic asthma
- Allergic rhinitis; hay fever
- Urticaria
- Generalized anaphylaxis

\* Allergen:

A small protein which for unknown reasons in some individuals produces a persistent IgE response e.g. pollens, house dust mites, pets (cats), ...

\* Atopy:

The tendency to type I hypersensitivity reactions in some individuals which has both inherited and environmental components. This tendency is demonstrated by positive skin tests to certain allergens.

\* Diagnosis of allergic diseases:

- Clinical manifestations and seasonal occurrence.
- Skin testing (wheal & flare reactions).
- ↑ IgE levels.
- RAST (radio allergosorbent test).
- Blood eosinophilia.

**\* Asthma:**

Extrinsic asthma is an example of type I hypersensitivity reaction, associated with positive skin tests, atopy, early onset, family history, ↑ IgE levels and seasonal occurrence (sometimes).

**\* Allergens:**

House dust-mites, grass pollens, ...

**\* Mechanism:**

Type I hypersensitivity, usually with late phase response.

\* The disease is characterized by bronchial hyper-responsiveness which is manifested as bronchoconstriction, inflammation and mucous production with airway plugging in response to triggers such as upper, respiratory tract infection, exercise, cold air, smoke and paint fumes.

**\* Diagnosis:-**

\* Clinical picture (cough, wheeze, dyspnea).

\* Lung function tests (airway obstruction).

\* Bronchial hyper-responsiveness.

\* Immunologic tests.

**\* Treatment:**

\* Prevention (avoidance of precipitating fs as possible).

\* Bronchodilators, disodium chromoglycate, corticosteroids.

- Allergic rhinitis:
  - \* Nasal congestion, sneezing (paroxysmal), itching, nasal and postnasal discharge.
  - \* There is also hyper-responsiveness (i.e. smoke, paint fumes, triggers symptoms).
  - \* Allergens: → grass pollens (seasonal).

→ House dust mites, pet furs (perennial).

\* Treatment:

- Avoidance
- Antihistamines
- Local: chromoglycate, steroids

- **Urticaria:** (hives)

Well circumscribed, itchy wheals erupt over different areas of the body caused by localized vasodilatation and oedema in superficial layer of dermis.

\* Angioedema: oedema in deeper layers of skin.

\* Allergens:

- Food (seafood, nuts, berries, eggs, chocolate).
- Insect stings.
- Drug reactions.

\* Clinically:

- Tingling, swelling of lips & tongue.
- Itching wheals in different parts.

\* Treatment: Avoidance, anti-histamines

● **Anaphylaxis:**

Means: ana: away from phylaxis = protection i.e. away from protection.

\* Acute generalized IgE mediated immune reactions involving specific antigen, mast cells and basophils.

\* This reaction requires priming by the allergen following by re-exposure.

\* The allergen must be systemically absorbed (by ingestion or injection).

\* Allergens include:

- Foods: Citrus, fruits, mango, strawberry, nuts, shellfish, chocolate, legumes.
- Venoms: Wasps, bees, ...
- Medications: Hormones e.g. insulin, antisera (antitetanic serum), penicillin.

\* **N.B.** Anaphylactoid reactions:

Non IgE mediated mast cell activation caused by: complement products ( $C_{3a}$ ,  $C_{5a}$ ), radiographic contrast media, morphia, codeine, heat, cold, or pressure.

\* Mechanism of anaphylaxis:

- Allergens are moderate sized proteins or haptens (e.g. penicillin).
- Activation of mast cells and basophils with systemic release and generation of mediators.

\* Manifestations:

- Tingling, warmth and itching.
- Flushing, urticaria & angioedema.
- Hypotension (due to V.D. and fluid loss into tissues).
- Bronchospasm.
- Laryngeal oedema.
- Cardiac arrhythmias.
- Respiratory arrest.
- Death may occur within minutes.

\* Further reactions may occur up to 8 hours later due to release of mediators from recruited cells (Late phase response).

\* Treatment:

\* Adrenaline ( $\rightarrow$  bronchodil, V.C. and  $\downarrow$  mast cell mediator release).

\*  $O_2$ ,  $\beta_2$  agonists.

\* Fluids and inotropes for hypotension.

\* Corticosteroids.

\* Monitoring for 24 hours.

\* Prevention:

\* Avoidance of triggers.

\* Carrying adrenaline.

\* Desensitization: especially if exposure is unavoidable or unpredictable e.g. insect stings.

## • Desensitization

Immunotherapy or hyposensitization.

\* Indications:

\* Disorders in which hypersensitivity is clearly IgE mediated.

\* Usually skin tests and RAST are done before.

\* Examples: insect stings, drug allergy if the drug cannot be avoided, allergic rhinitis (?), pets (avoided).

\* Procedure:

Repeated injection of an identified allergen. S.C. injections twice/week. Maximum dose reached after 6-10 weeks. Then monthly for up to 1 year.

\* Mechanism: Theories:

Blocking antibodies (IgG), anti IgE antibodies, T-cell suppression, changes in effector cells and changes in immune regulatory T-cells ( $\uparrow$   $T_{H1}$ ).

# **Type II Hypersensitivity**

## **"Cytotoxic or Cytolytic Reactions"**

- \* Binding of specific antibody directly to an antigen on the surface of cells → damage of cells through different mechanisms which include:
  - \* Complement activation → cell lysis, mast cell activation and neutrophil recruitment.
  - \* Antigen-antibody binding causes cell recruitment through direct binding through Fc interactions.
  - \* The arrival of cells with cytotoxic capabilities (neutrophils, eosinophils, macrophages, killer cells) may lead to a mechanism of damage described as ADCC (antibody dependent cell cytotoxicity).
  - \* Antireceptor antibody disease: Type V hypersensitivity. Antibody directed against cell surface receptor e.g.

- Grave's disease:

Stimulatory antibodies (IgG) bind to TSH receptor on thyroid gland → activation → hyperthyroidism.

- Myasthenia gravis:

Blocking antibodies bind to the acetylcholine receptors on motor end plates of muscles → muscle weakness of all striated muscle group.

Examples of type II hypersensitivity reactions:

- Auto immune haemolytic anaemia:

Auto antibodies cause ↑ destruction of R.B.Cs.

- Idiopathic thrombocytopenic purpura:

Autoantibodies cause of ↑ destruction of platelets → purpura (bleeding and purpuric spots).

- Transfusion reactions:

Incompatible blood transfusion causes haemolysis of transfused cells by recipients IgM antibodies.

- Haemolytic disease of newborn:

Haemolysis of foetal R.B.Cs by anti-Rh antibodies from the mother.

- Drug induced reactions:

Some drugs act as haptens and combine with cells or circulating blood constituents and induce antibody formation. When antibodies combine with cells coated with the drug, cytotoxic damage results.

Examples of drug reactions:

\* Chloramphenicol (on white cells) → agranulocytosis.

\* Phenoacetin (on red cells) → haemolytic anaemia.

# Type III Hypersensitivity

## "Immune complex disease"

\* Antigen – antibody complexes are deposited in tissues causing complement activation and cell recruitment and activation → inflammation and tissue damage.

\* Types:

1. Arthus reaction: Complexes formed within tissues.
2. Circulating immune complexes which become deposited in tissues (serum sickness like reaction).

**N.B.** Serum sickness is characterized by transient arthritis, skin rash & fever, which occurs after injection of foreign serum.

- Normally formed immune complexes are carried to the liver and spleen and removed by macrophages.
- Factors causing deposition of these complexes (i.e. induction of type III reaction):-
  - ↑ rate complex formation due to persistence of antigenic stimulation e.g. infective endocarditis.
  - Changes in host response: e.g. deficiency of complement (e.g. C<sub>4</sub> def. ⇒ S.L.E.).
  - Sites of deposition where plasma is filtered in certain sites favouring deposition of the complexes at these sites as renal glomeruli and joint synovium.
  - Characteristics of the complexes: i.e. relative proportion of antibody and antigen producing insoluble complexes →

deposition. (if there is antigen or antibody excess → no deposition).

\* Examples of immune complex mediated disorders:

(1) Immune complexes deposited in tissues:

\* Post streptococcal glomerulonephritis.

\* SLE: The antigen is DNA.

- Complexes deposited in kidney glomeruli and serous membranes → nephritis and serositis.

\* Infective endocarditis:

- Bacterial antigens → nephritis.

(2) Complexes deposited in walls of blood vessels: causing vasculitis. e.g. SLE, infective endocarditis.

(3) Complexes form in tissues (resembling arthus reaction) Extrinsic allergic alveolitis e.g.

Farmer's lung (reaction to mouldy hay).

Bagassosis (reaction to mouldy sugar cane).

## Type V Hypersensitivity

- \* Cell mediated or delayed type hypersensitivity.
- \* Tissue damage initiated by activation of  $T_{H1}$   $CD_4$  cells (sometimes called  $T_{DH}$  i.e. T delayed hypersensitivity).
- \* This is usually delayed in onset (at least 24 hours after challenge with provoking stimulus).
- \* Mediated by  $CD_4$  T cells, macrophages and cytokines.

\* Examples include:

(1) Contact dermatitis:

- \* Sensitivity to antigens e.g. nickel in Jewellery or dichromate in leather industry.
- \* → eczematous reaction with erythema, oedema, vesicles and scaling.
- \* Diagnosis: Patch testing.

(2) Granulomatous diseases e.g.

- T.B.
- Bilharziasis

The reaction to chronic persistent infection with granuloma formation with cellular infiltration, angiogenesis and fibrosis.

**N.B.** \* Reaction to mycobacteria is demonstrated by tuberculin testing → redness and induration 48-72 hours after intradermal injection for testing of T.B. immunity.