

## **Module 4 : Mechanism of immune response**

### **Lecture 23: Cytokines (Part I)**

Cytokines are the proteins secreted by the cells of immune system that control the immune responses by interaction between the neighboring cells. In other words Cytokines are the signaling molecules like hormones and are the end products of interaction among immune cells. Cytokines like other signaling molecules tend to bind the specific receptors on the target cells but the structure of cytokines and their receptors is very different. Once the cytokines bind to their receptors, transcription factors are produced as a result of changes in the cell behavior by the process called as signal transduction. Transcription factors stimulate the selected genes for transcription which then secrete new cytokines or signaling molecules.

#### ***23.1 Features of cytokines***

- 1) Cytokines show redundancy i.e. different cytokines may have similar function, e.g. IL-1 and IL-6 both induce fever by acting on brain.
- 2) They are secreted in multiple numbers, e.g. macrophages secrete more than five interleukins and tumor necrosis factor.
- 3) Cytokines produce their effect on many cell types i.e. they are pleiotropic.
- 4) Cytokines need extreme regulation and are toxic in high doses.

#### ***23.2 Cytokine nomenclature***

Most of the cytokines are named according to the Interleukin nomenclature subcommittee of the international union of immunological societies. Although the definition of cytokines is quite broad, but together they can be classified as **lymphokines, interleukins, interferons, chemokines** etc. depending on their function, cell of secretion, or target of action. The name interleukin was coined initially for those cytokines that mediate signaling between lymphocytes and leukocytes. In other words leukocytes were thought to be the principal target for interleukins but now this definition is no longer in function and the name interleukin is given to any new cytokine discovered routinely. Cytokines are produced mainly in response to viral infection or in response to immune attack. Interferons are the cytokines that interfere with viral RNA and protein synthesis to prevent viral replication.

Types of interferon – Interferons are mainly divided into two types.

Type I Interferons --- Interferon- $\alpha$  (IFN-  $\alpha$ ) and interferon- $\beta$  (IFN-  $\beta$ ).

Type II interferon --- Interferon- $\gamma$

Tumor necrosis factors (TNFs) can kill tumor cells and colony stimulating factors assist in regulation of stem cell activities. Chemokines play role in leukocyte recruitment and circulation.

Table 23.1 Different cytokines and their examples:

Cytokine types	Examples
<b>Lymphokines</b>	MAF (Macrophage activating factor)
<b>Interleukins</b>	IL-7, IL-13, IL1-7
<b>Interferons</b>	IFN- $\alpha$ , IFN - $\beta$ , IFN- $\gamma$ , IFN- $\omega$ , IFN -
<b>Tumor necrosis factors</b>	TNF- $\alpha$ (Cachectin), TNF - $\beta$ (lymphotoxin)
<b>Colony stimulating factors</b>	GSF (Granulocyte colony stimulating factor)
<b>Polypeptide growth factors</b>	EGF (Epidermal growth factor)
<b><math>\alpha</math>-Chemokines</b>	PF-4 (Platelet factor 4)
<b><math>\beta</math>-Chemokines</b>	MCP-1 (Monocyte chemoattractant protein 1)
<b>Transforming growth factors</b>	TGF- $\alpha$ , TGF - $\beta$
<b>Stress proteins</b>	Heat shock proteins
<b>RANTES</b>	

### 23.3 Functions of cytokines

Cytokines are crucial regulators of cells and hence control their growth, movement, evolution and differentiation. Being responsible for so many factors they are generated in response to varied stimuli. The most critical of these stimuli are antigen-antibody complexes acting through antibody receptors (FcRs), **pathogen-associated molecular patterns** (PAMPs) such as lipopolysaccharides acting through **toll-like receptors** (TLRs) and antigens acting through the B cell or T cell antigen receptors. Cytokines acts on different cells and in different ways. Cytokines may have an **autocrine** effect (Cytokines bind to receptors that produced them), **paracrine** effect (Cytokines bind to receptors on

neighboring or cells which are very close) or **endocrine** effect (Cytokines flow throughout the body) while acting on the target cells. Cytokines also play role in apoptosis and control various stages of replication. They have the capability for being sensitive markers of chemically induced perturbations in function, but this claim is not accepted from the toxicological point of view. The reasons behind this being that cytokines are released locally with plasma measures being unreliable and they have short half-lives which need precise timing to find.

### ***23.4 Cytokine structure***

Cytokines have distinct structures but can be broadly divided into following.

**Group I cytokines** are involved in immune regulation --- includes four  $\alpha$ -helices in bundles, Interferon subfamily and the IL-10 subfamily.

**Group II cytokines** are engaged in growth and regulation of cells, cell death and inflammation --- IL-1 family, TGF- $\beta$

**Group III cytokines** are involved in inflammation--- Chemokines

**Group IV cytokines** --- IL-12

**Table 23.2 Different cytokines and their source:**

<b>Cytokine</b>	<b>Principal Cell Source</b>
Interleukin-1 (IL -1)	Macrophages, endothelial cells, some epithelial cells
Interleukin-6 (IL -6)	Macrophages, endothelial cells, T cells
Interleukin-10 (IL-10)	Macrophages, T cells (mainly regulatory T cells)
Interleukin-12 (IL-12)	Macrophages, dendritic cells
Interleukin-15 (IL-15)	Macrophages, others
Interleukin-18 (IL-18)	Macrophages
Interleukin-23 (IL-23)	Macrophages and dendritic cells
Interleukin-27 (IL-27)	Macrophages and dendritic cells
Chemokines	Macrophages, endothelial cells, T cells, fibroblasts, platelets
<b>Type I interferons (IFN -<math>\alpha</math>, IFN-<math>\beta</math>)</b>	IFN- $\alpha$ : macrophages; IFN- $\beta$ : fibroblasts
<b>Tumor necrosis factor (TNF)</b>	Macrophages, T cells

## **Lecture 24: Cytokines (Part II)**

### ***24.1 Cytokine receptors***

Cytokine receptors can be distinguished based on their structure and consist basically of two units, one for the ligand binding and the other for signal transduction. Broad classification of cytokine receptors can be listed as under –

- Proteins that acts as Tyrosine kinases and function as growth factors.
- One class of receptors is membrane-bound Guanosine triphosphate – binding proteins called as G proteins. In active state these bind GTP.
- Channel linked receptors.
- This class of receptor stimulates a neutral sphingomyelinase.

### ***24.2 Cytokine regulation***

The three major ways for regulating the cytokine signaling are –

- By specific binding proteins
- By changes in receptor expression and
- By cytokines that exert opposite effects.

Concept behind understanding the cytokine regulation is that as the single cell receives signals from numerous cytokine receptors, a unification of multiple signals is required to generate a coherent response.

### ***24.3 Signal transduction and pathways***

During the signal transduction the first step is the binding of cytokines to its receptors. Once the binding occurs, the receptor transmits a signal to the cell to alter its behavior. This phenomenon involving conversion of an extracellular signal into a series of intracellular events is called signal transduction. As Cell signaling needs to be precise and quick, enzyme cascade serve this function by the amplification of the responses rapidly. The important features of signal transduction include binding of cytokine to a cell surface receptor, stimulation of a transducer protein by the receptor, secondary activation of various enzymes, production of new transcription factors and ultimately gene stimulation leading to changed cell behavior.

Although there are many pathways of signal transduction but three pathways are of utmost significance to the immune system.

- The NF- $\kappa$ B pathway
- The NF-AT pathway
- The JAK/STAT pathway
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### 24.3.1 The NF- $\kappa$ B pathway

NF- $\kappa$ B has its origins in the family of five transcription factors that play a crucial role in inflammation and immunity. There are more than 150 genes that get expressed on NF- $\kappa$ B stimulation. There are three major pathways for NF- $\kappa$ B activation. These are the **classical pathway**, **alternative pathway** and the **third pathway** that is stimulated by DNA damaging drugs and Ultraviolet light. The classical pathway involves proinflammatory signaling and gets activated by inflammatory cytokines, TLRs and antigen receptors (figure 24.1). IKK (I $\kappa$ B) complex is the central regulator of NF- $\kappa$ B and all the signals induced by various stimuli focus on the same complex IKK. IKK complex is rich with kinase activity and on activation IKK complex phosphorylates I $\kappa$ B. Consequently I $\kappa$ B dissociates from the NF- $\kappa$ B and the newly formed I $\kappa$ B becomes ubiquitinated and is destroyed by proteasomes. Dissociation between I $\kappa$ B and NF- $\kappa$ B liberates NF- $\kappa$ B and it enters the nucleus to activate  $\kappa$ B motif containing promoters on DNA. This stimulates many genes and cytokines IL-1 $\beta$ , IL-6, IL-18, IL-33, TNF- $\alpha$ , GM-CSF, and IL-4. The second pathway or alternative pathway is required for lymphocyte development and is stimulated by a subset of TNF receptors. The third pathway does not require any IKK activation.

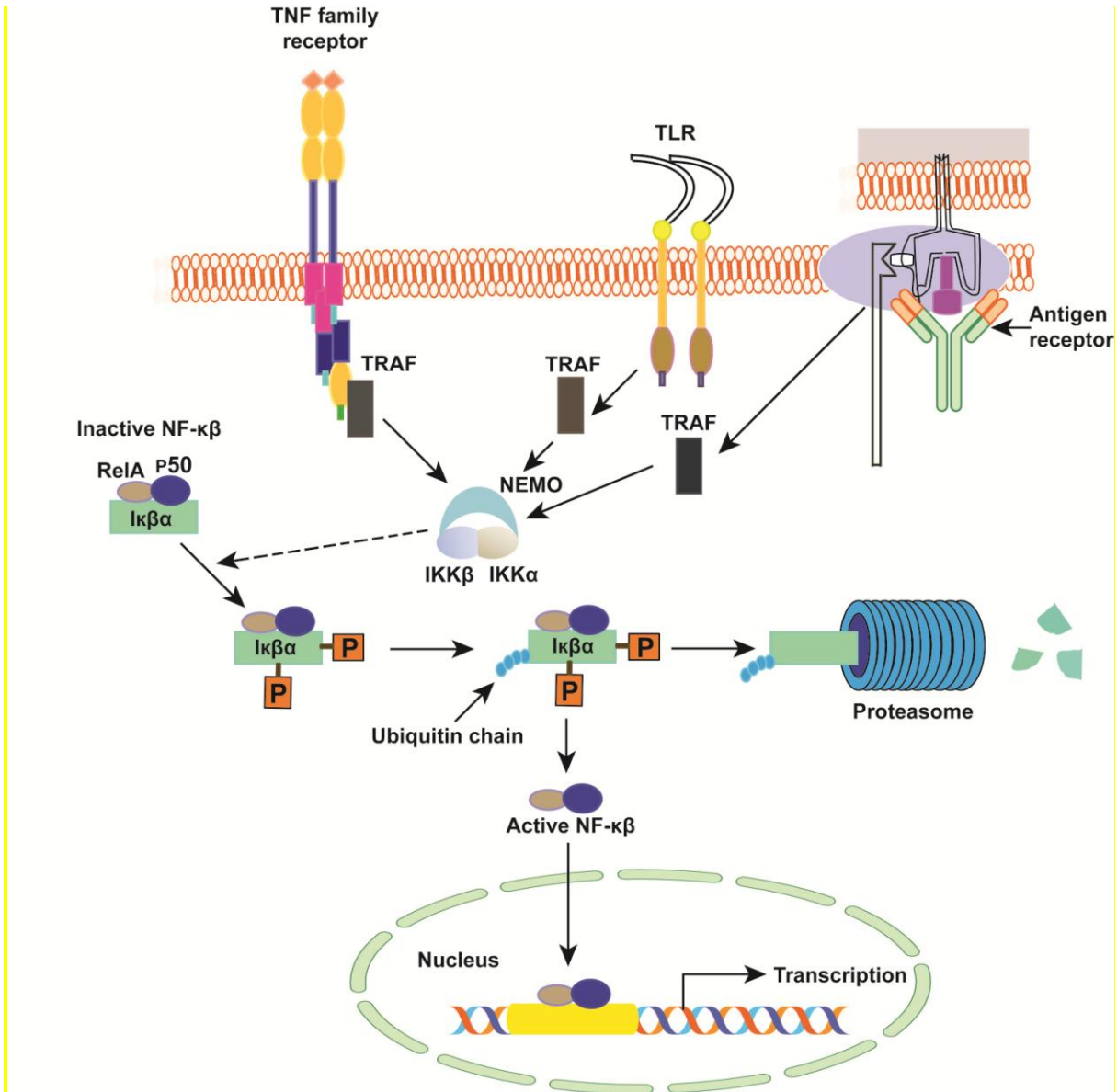
IKK- I $\kappa$ B kinase

I $\kappa$ B- Inhibitor of NF- $\kappa$ B

NEMO- NF- $\kappa$ B essential modulator

TRAF- Tumor necrosis factor receptor-associated factor

Figure 24.1 NF- $\kappa$ B signaling pathway:



### 24.3.2 The NF-AT pathway

The response generated to a T- cell is transmitted from the T cell receptor to a signal transducing complex called CD3 which is followed by clustering of CD3 chains in lipid rafts. Immunoreceptor tyrosine-based, activation motifs (ITAMs) are the specific amino acid sequences in the cytoplasmic domains of CD3 proteins. ITAMs later on become binding sites for several tyrosine kinases on activation. Zeta associated protein – 70 (ZAP-70) is one of the TKs and on phosphorylation can stimulate three signaling pathways. In the first pathway activation of calcineurin, a phosphatase is required and it ends up by generating NF- $\kappa$ B and NF-AT. In the second pathway, ZAP-70 stimulates a

protein kinase C which is required for activation of NF- $\kappa$ B. The third pathway activates ras, a GTP-binding protein, fos and jun.

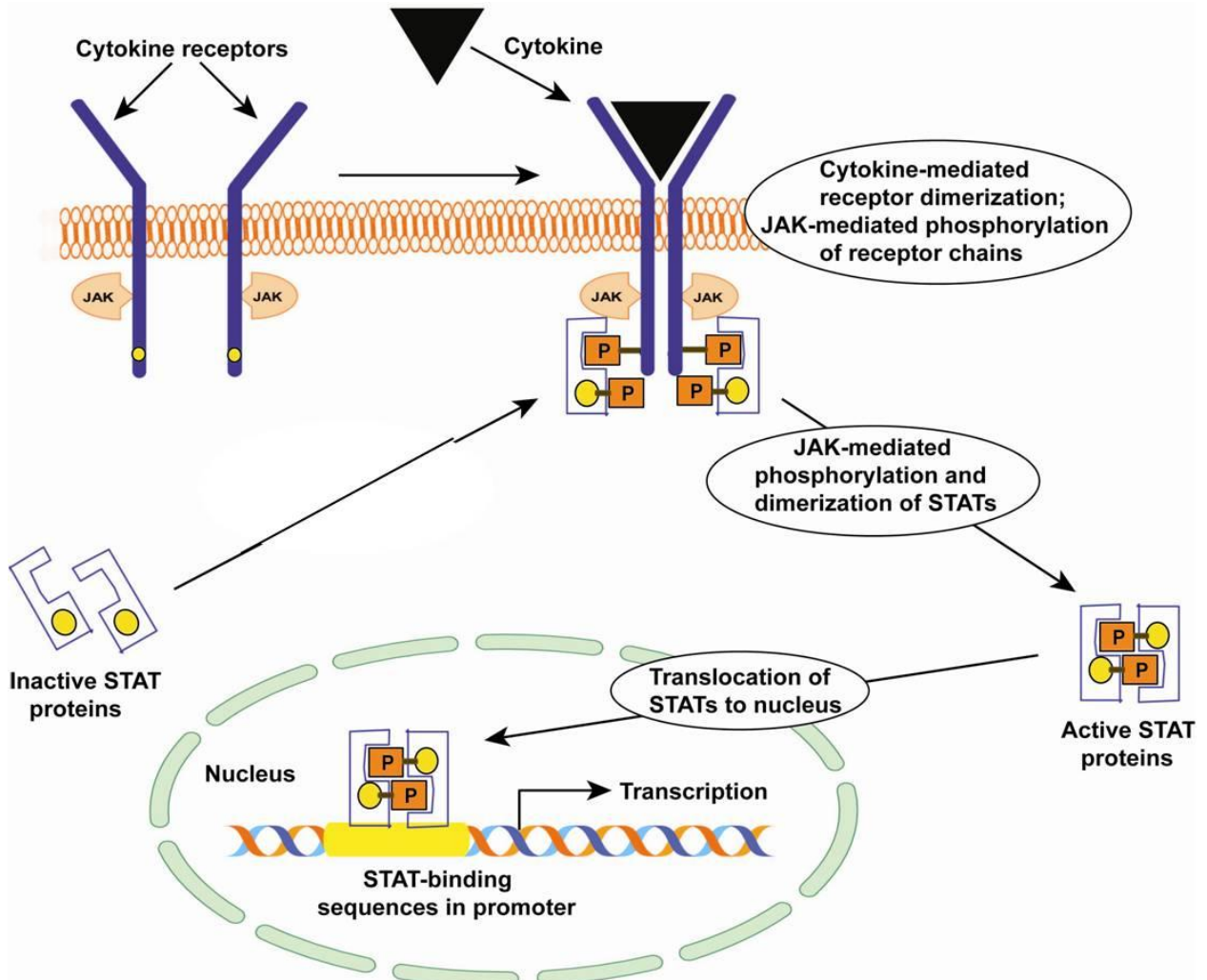
### **24.3.3 The JAK-STAT pathway**

More than 40 cytokines use the JAK-STAT pathway. The stimulated JAK (Janus kinase) molecules phosphorylate tyrosine residues on one of several STAT proteins (signal transducers and activators of transcription). Phosphorylated STAT proteins then dimerize and dissociate from JAK and get shifted to the nucleus where they regulate the expression of target genes.



Figure 24.2 JAK-STAT signaling pathway:

Cytokine ligation to cytokine receptor leads to activation of JAK tyrosine kinase, phosphorylation of its cytoplasmic domain, and recruitment of STAT. Recruitment of STAT and its phosphorylation dimerizes and transport it to nucleus. This event activates the transcription of cytokine gene.



## Lecture 25: Mechanism of cell mediated immune response (Part I)

The recruitment of the leukocyte and its activation is largely governed by the subset of CD4<sup>+</sup> cells. Each leukocyte is specified for certain job and the cooperation of leukocyte to the lymphocytes is an important connection between innate and adaptive immune response.

In general Th1 cells activate macrophages, Th2 activates eosinophils, and Th17 activates neutrophils. The Th1 cells activate the macrophage mediated phagocytosis, Th2 stimulates the production of IgE in response to a helminthic infection, and Th17 activates neutrophils to extracellular microbes including bacteria and fungi. Cytotoxic T lymphocytes (CTLs) kill the microbes which escape the phagocytosis and replicate in the cytoplasm.

### *25.1 Migration of lymphocyte at the site of infection*

Majority of the leukocyte and lymphocyte are migrated towards the site of inflammation and infection, respectively in order to nullify the effect. Once the naïve lymphocyte encounters an antigen presented by the MHC molecule over the antigen presenting cells, they differentiate and migrate as an effector cells through the blood vessels to different parts of the body. The migration of lymphocyte is an interesting phenomenon orchestrated by different cytokines and specific receptors present over the surface of the lymphocytes. Naïve T lymphocytes express **L-selectin** and chemokine receptor **CCR7** which help them to adhere to the lymph node and its surrounding tissues. After antigen stimulation the naïve T lymphocyte decrease the expression of L-selectin and CCR7 and increase the expression of **sphingosine 1-phosphate receptors (S1PR1)**, which help their migration into the blood vessels. Th1 cells produces large amount of ligands that binds to **E and P-selectin** which recognize selectins secreted at the site of bacterial infection and trigger a strong innate immune response. Th2 cells express different **chemokine receptors** such as CCR3, CCR4 and CCR8 which recognize specific Chemokines secreted at the site of helminthic infection. Similarly, Th17 migration to the site of fungal and bacterial infection is modulated by CCR6 and other cytokines. Usually the subset of T lymphocytes is directed to the site of action with the help of different

chemokines and their respective ligands. Similarly memory cells express different integrins that binds to the fibronectins over the extracellular matrices and help in their migration.

## ***25.2 Functions of CD4+ helper cells***

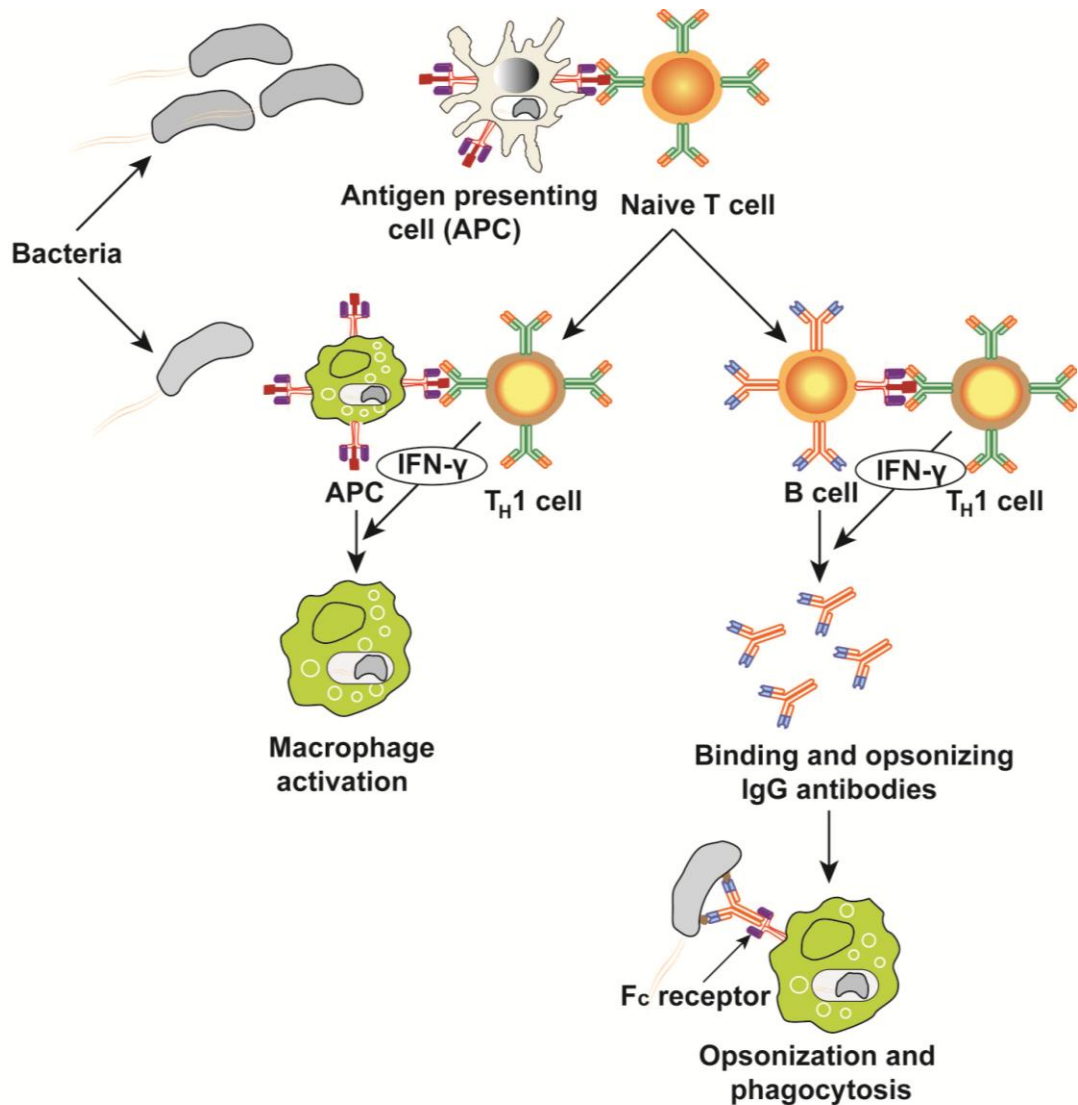
The function of CD4+ cells are divided into four distinct stages.

- **Leukocyte recruitment-** T lymphocytes produces many cytokines that helps in the recruitment of neutrophils, eosinophils and monocytes at the site of infection or injury.
- **Leukocyte activation-** Leukocytes gets activated by the binding of CD40L on lymphocyte to the CD40 express over the antigen presenting cells.
- **Expansion-** The activation response gets expanded by the secretion of cytokines by the T lymphocytes.
- **Control-** The response of the immune system is controlled by some inhibitory cytokines such as IL-10.

### **25.2.1 Function of Th1 cells**

Naïve T cells after encountering an MHC loaded peptide over the antigen presenting cells differentiates into Th1 cells. Th1 cells secretes interferon- $\gamma$  (IFN- $\gamma$ ) which is a key cytokine involve in its functioning. IFN- $\gamma$  acts on the activated macrophages and increases its phagocytosis efficiency and microbial killing. In addition IFN- $\gamma$  also stimulates the B cells to secrete the IgG antibody that helps in opsonization and activation of complement pathway. Occasionally they also produce tumor necrosis factor that activate the neutrophils and promotes inflammation.

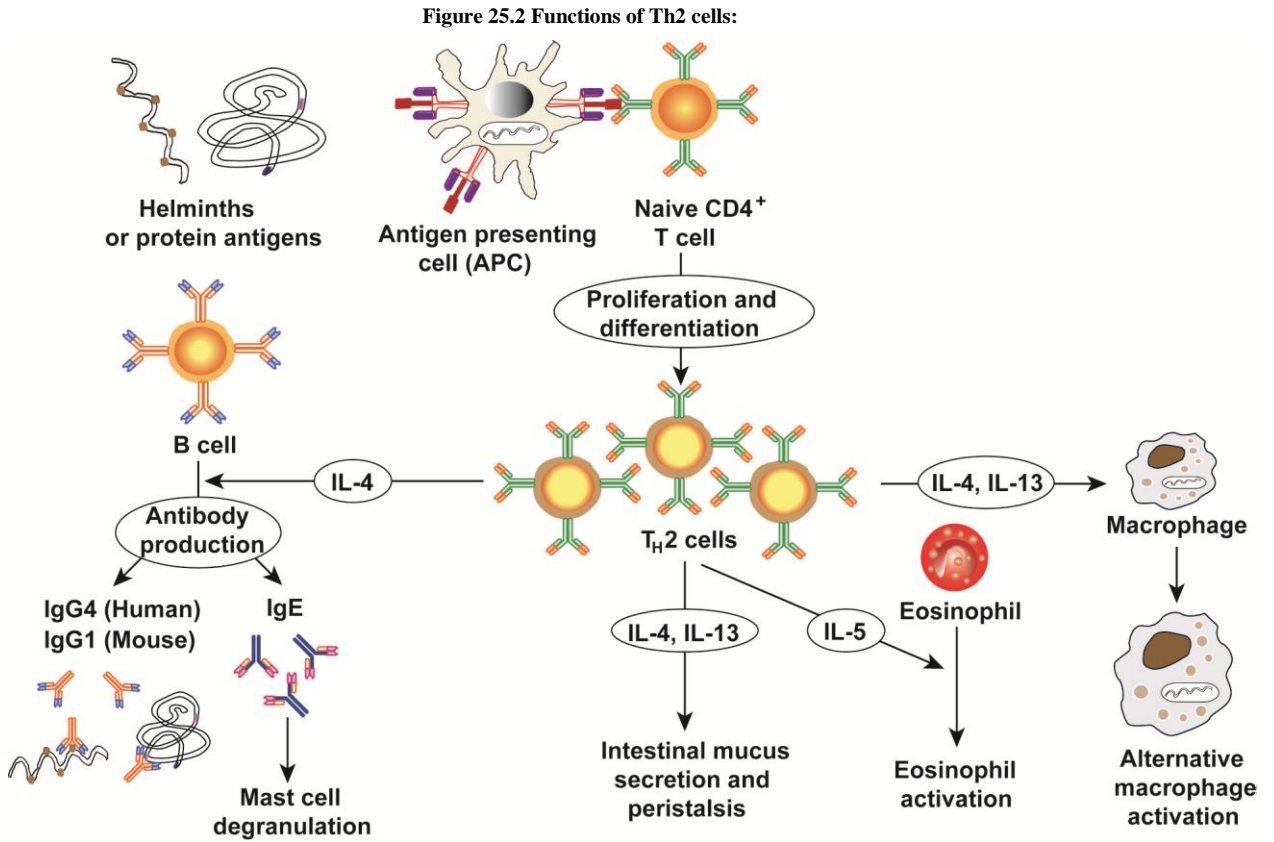
Figure 25.1 Functions of Th1 cells:



### 25.2.2 Function of Th2 cells

Naïve T cells after encountering an MHC loaded peptide (helminths) over the antigen presenting cells differentiates into Th2 cells. Th2 secretes IL-4, IL-13, and IL-5. IL-4 activates the B cells to secrete IgG antibody which can act on the parasites. In addition, IL-4 also stimulates the production of IgE antibody that helps in degranulation of mast cells. In terms of physiological aspect IL-4 along with IL-13 increases the mucus secretion and peristalsis of the intestinal tract. Moreover IL-4 and IL-13 act synergistically to activate macrophages which helps in fibrosis and tissue repair. Increase in the secretion and motion of the intestine is another way to combat the harmful effect of

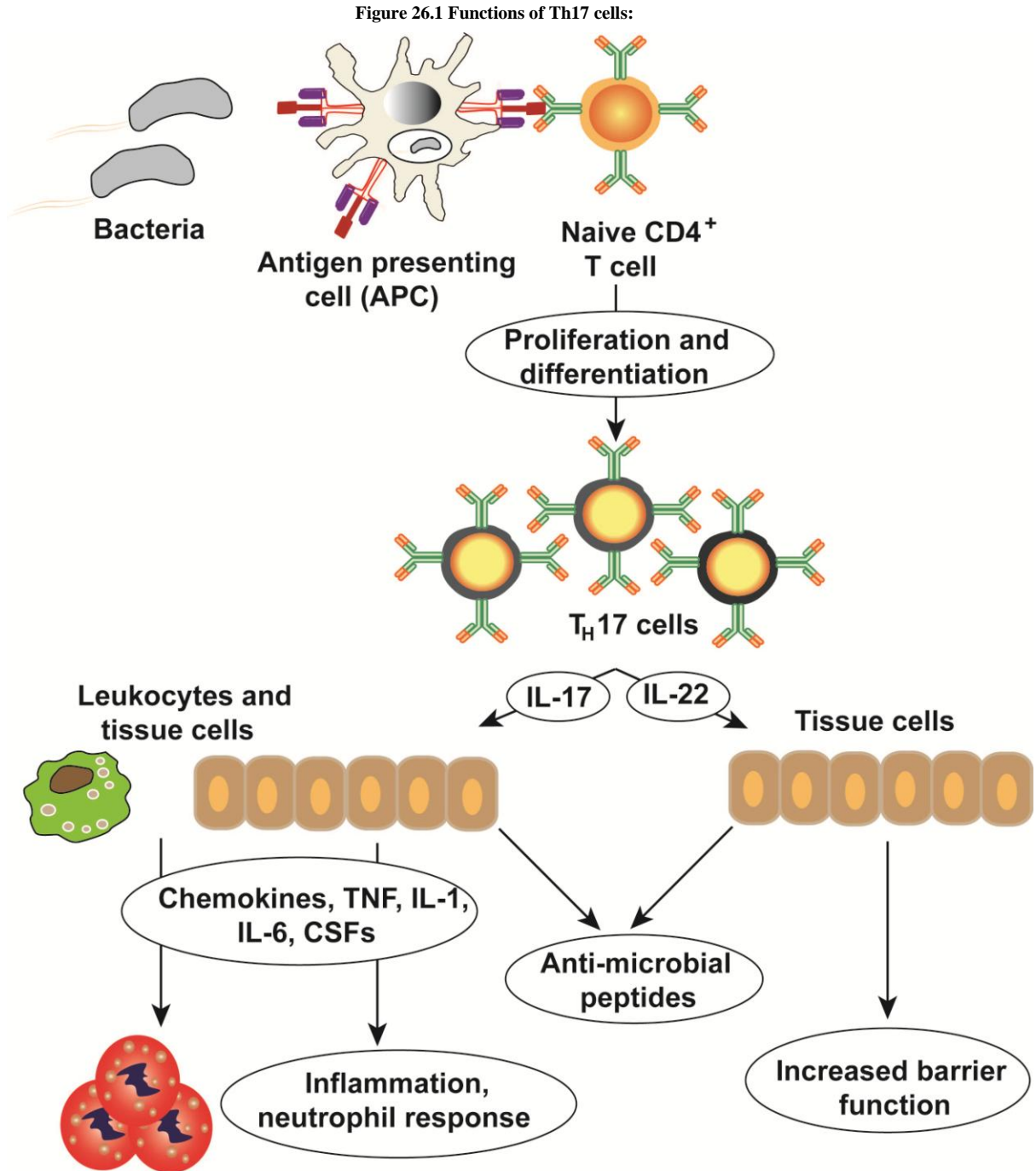
microbes entering into the digestive tract. IL-5 activates the eosinophil that helps in clearing the helminthic infection.



## **Lecture 26: Mechanism of cell mediated immune response (Part II)**

### ***26.1 Function of Th17 cells***

After differentiation of naïve T cells into Th17 effector cells, they start secreting IL-17 and IL-22. IL-17 is a unique cytokine that do not have any homologue in the body. IL-17 stimulates the production of chemokines, TNF, IL-1, IL-6, and granulocyte-colony stimulating factors (G-CSF) which take part in inflammatory reactions. In addition, it is also involved in the production of defensins from the epithelial surface which in turn acts as a barrier for the invading pathogens. IL-22 is a relatively new interleukin and its function is not well established. However IL-22 is known to maintain the epithelial integrity, increase barrier function and involve in the repair activity following inflammation.



## 26.2 Function of CD8<sup>+</sup> cytotoxic T lymphocyte

Cytotoxic T lymphocytes (CTLs) get activated after antigenic recognition over the MHC in the antigen presenting cells. The costimulatory molecules and their close association with other receptors results in the formation of immunological synapse (ICAM-1 to LFA-1). Activated CTLs secrete cytotoxic granules such as **granzyme** and **perforin**. Perforin is homologue to complement protein-9 and is known to produce holes and perforation in

the target cell. Perforin polymerize to form an aqueous pore in the target cells through which granzyme enters the target cells. Granzyme is serine proteases which is responsible for the cleavage and activation of caspase in the cells leading to the apoptosis of the target cells. In addition, activated CTLs also secrete granules such as **serglycin** which are known to assemble the granzyme and perforin over the surface of target cells.

CTLs also kill the target cells in granule independent fashion by **Fas Ligand** mediated pathway. The Fas ligand binds to the death receptors that are expressed in many cell types and activates the caspase mediated apoptosis pathway.

The infection of intracellular microbes to host needs participation of CTLs because it will eliminate the reservoir of infection by killing the infected cell. Hepatitis B and C virus infection to a host activates the CTLs in order to kill the liver cells to eliminate the reservoir.

### ***26.3 $\gamma\delta$ T cells***

These are clonally distinct cells containing heterodimer of  $\gamma$  and  $\delta$  chains, which are homologous to  $\alpha$  and  $\beta$  chains in  $\alpha\beta$  T cells. Usually less than 5% of total circulating lymphocytes are composed of  $\gamma\delta$  T cell receptor. The  $\gamma\delta$  T cells present in the epithelial surface of bowel are called as **intraepithelial lymphocytes**. The  $\gamma\delta$  T cells do not recognize the MHC loaded peptide and are MHC independent. They require protein and non-proteinous antigen that do not require processing by MHC molecules.

### ***26.4 Natural killer cells***

T cells population that express the CD56 marker over their surface are called natural killer or NK cells. NK cells recognizes the lipid bound to CD1 receptor (MHC class I homologue). NK cell secretes IL-4 and interferon- $\gamma$  after activation and help in the production of antibody by B cells. NK cell plays an important role in the protection against mycobacterium infection.

### ***26.5 Points to remember***

- Cell mediated immunity is a type of adaptive immune response that is stimulated by microbes and can be transferred to naïve animal by T cells.
- Differentiation and migration of lymphocytes are orchestrated by different cytokines.
- Both CD4+ and CD8+ cells are responsible for cell mediated immunity

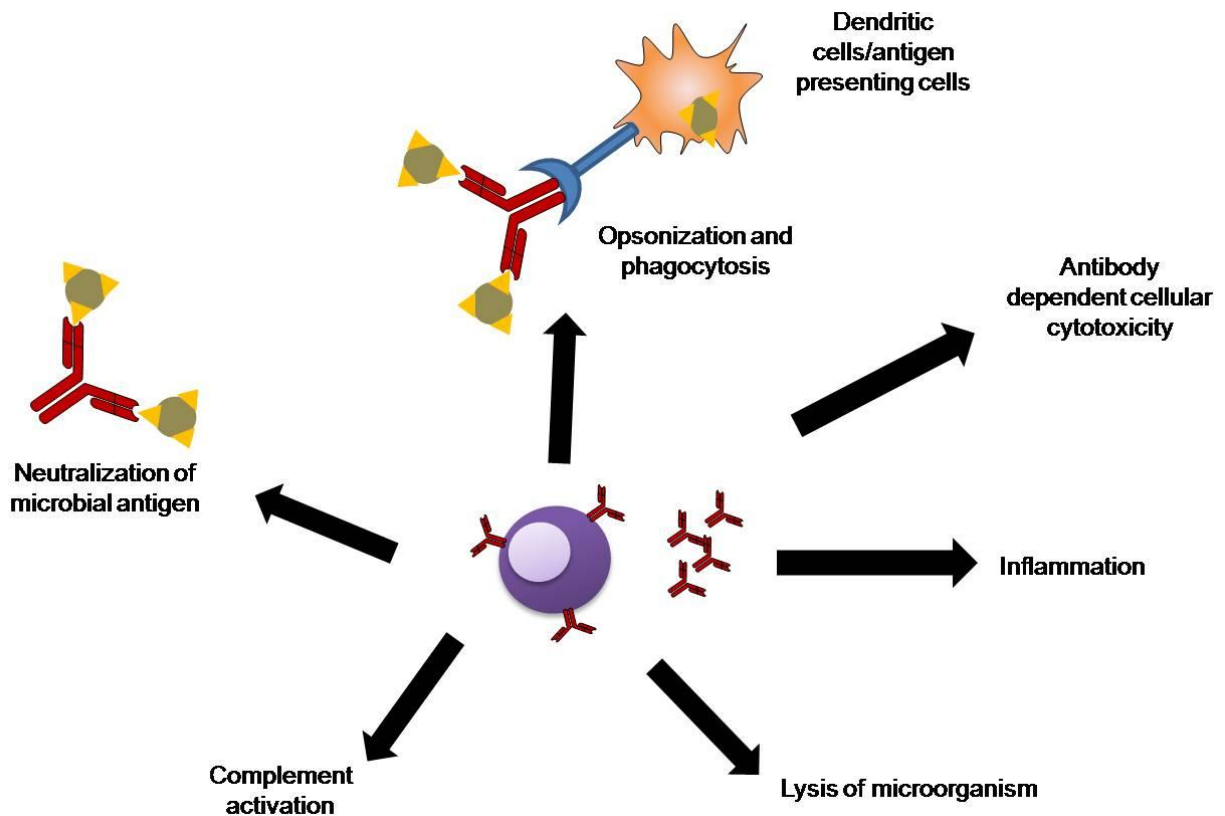


- CD4+ cells are comprised of Th1, Th2, and Th17 subsets and have different effector functions.
- CD8+ cells are converted into cytotoxic T lymphocyte upon differentiation and help in eradication of infection by killing the target cells.

## Lecture 27: Mechanism of antibody mediated immune response (Part I)

The humoral immunity is the other essential arm of adaptive immune response which is mainly governed by the antibodies. The humoral immune response is important against extracellular microbes, microbial toxins, fungi, and viruses. Any defect in the production of antibody leads to severe complications in immune compromised individuals. Current day vaccine strategies are mostly directed to increase the antibody production against specific pathogens.

Figure 27.1 Overview of effector functions of antibody:



### 27.1 Neutralization of microorganisms and their toxins

Most of the microorganisms attack and enter the host body after binding to a specific cell receptor present over the host cell. The specific structures present over the surface of bacteria, viruses, parasite or fungi are responsible for their attachment to the cell receptors. When the microorganism and its toxin enters or are produced inside the body the host immune system produces the antibody specific to bind their surface molecules.

The binding of specific antibodies to these molecules in the microbes and toxin makes it inaccessible to the host cell and hence neutralized. The host cell produces antibodies against the surface glycoprotein hemagglutinin in influenza virus infection in order to inhibit their binding to the sialic acid receptor present on the respiratory epithelium. Similarly, tetanus toxin binds to the cellular receptors over the neurotransmitter junction leading to paralysis and lockjaw condition. The antibody produced in *Clostridium tetani* infection neutralizes the toxins and inhibits its binding to receptors present in the motor end plates. Neutralization of the microbes and their toxins only requires antigen binding region of the antibody, i.e Fab or F (ab)<sub>2</sub> fragments. Most of the neutralizing antibodies in the blood are of IgG and IgA types in the mucosal surface.

### ***27.2 Opsonization and phagocytosis***

Coating of microbes by the IgG type antibody helps in their phagocytosis by macrophages and neutrophils, the phenomenon is called **opsonization**. The Fc receptor of the IgG containing microbes binds to the neutrophils and participates in their intracellular degradation and killing. In addition to IgG molecules, complement protein C3b also helps in the opsonization and phagocytosis of the microbes by binding to leukocytes. The substance that helps in opsonization and phagocytosis including antibodies and complements are called as **opsonins**.

Table 27.1 Functions of different antibody:

<b>Types</b>	<b>Function</b>
<b>IgG</b>	Neutralization of toxins and microbes. Opsonization of antigens Activation of classical pathway of complement system Antibody dependent cell mediated cytotoxicity Transfer through placenta to neonates Negative feedback to B cell activation
<b>IgA</b>	Neutralization over the mucosal surface Activation of lectin and alternate pathway of complement system
<b>IgM</b>	Activation of classical pathway of complement system Antigen receptor on the naïve B cell
<b>IgE</b>	Mast cell degranulation Allergic reaction Immediate type hypersensitivity reaction
<b>IgD</b>	Antigen receptor on the naïve B cell

## Lecture 28: Mechanism of antibody mediated immune response (Part II)

### *28.1 Antibody dependent cell-mediated cytotoxicity*

Many subclasses of IgG antibodies bind to infected cells and are recognized by the Fc receptors of NK cell. NK cell contains **Fc receptor** called **FcγRIII (CD16)** that binds to the antibody coated infected cells. Many therapeutic monoclonal antibodies against tumor cells are working on the same principle by coating the tumor cells which facilitates their killing by NK cells.

### *28.2 Antibody dependent killing of helminths*

Helminths are larger in size and cannot be processed by phagocytic cells of the immune system. Antibodies like IgE, IgG, and IgA coats the surface of helminths which can bind to the Fc receptor present over the eosinophils. Eosinophils contain granules in their cytoplasm which are responsible for the secretion of microbicidal **major basic proteins**. Binding of the antibody coated helminths to Fc receptor degranulates the eosinophils and secretion of major basic protein which is responsible for their killing. **Mast cells** also take part in the clearance of helminths from the body. Mast cell releases many cytokines and chemokines which attracts and helps in the degranulation of eosinophils and in turns secretion of major basic protein.

### *28.3 The complement system*

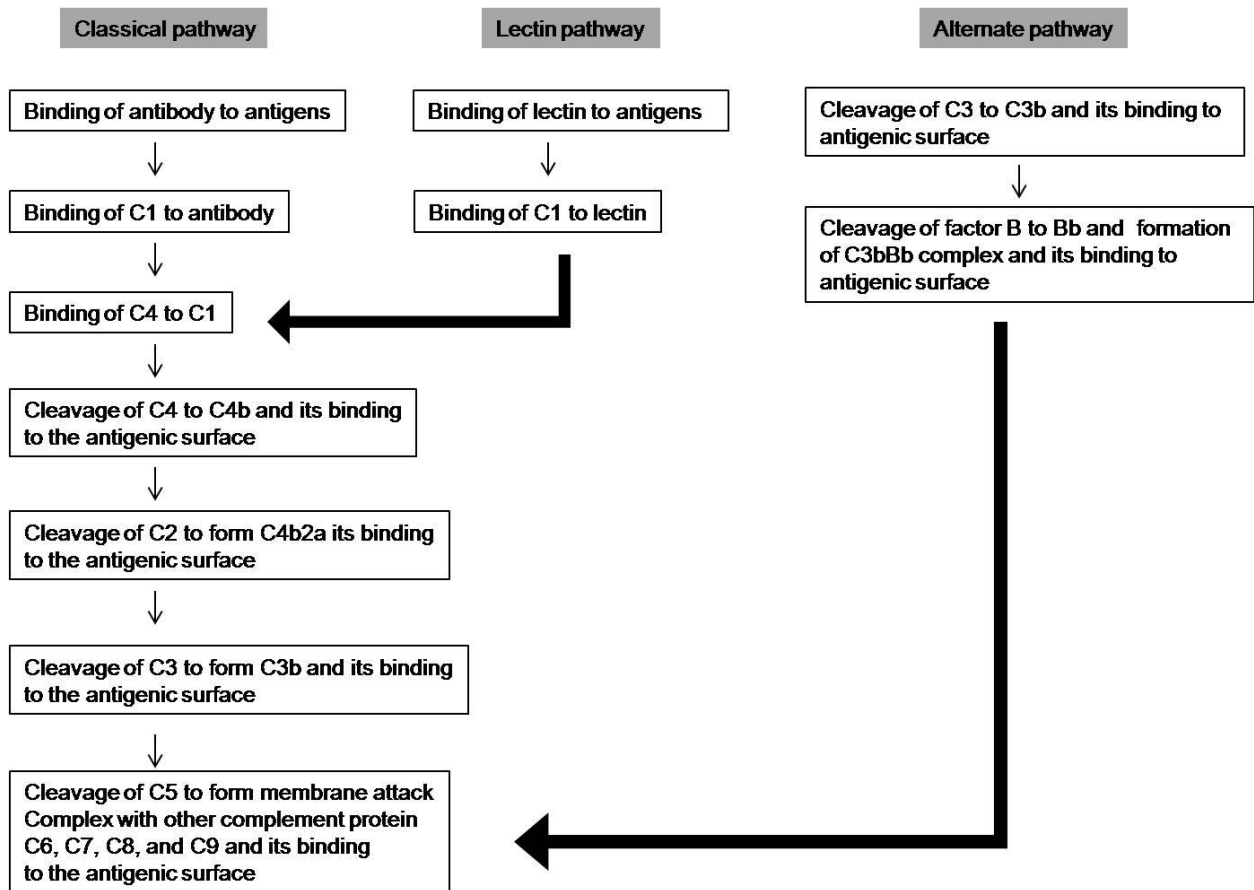
The complement system consists of different serum proteins that works through different pathways and interacts with each other in order to kill the invading microbes. The complement system is activated by the microbial antigen and the antibody produced against them by the host immune system. The proteins of the complement system attaches sequentially over the surface of microbes and help in their lysis. The complement system comprises of **classical, alternate, and lectin pathways**. The classical pathway is activated by the antibody binding with an antigen, alternate pathway activates in absence of antibody, and lectin pathway activates in response of lectin that binds to mannose. The final step in all these three pathways leads to the formation of **membrane attack complex**. These complexes are around 100 Å in diameter and forms, a channel that allows the free flow of water and ion into the microbial cells. The movement

of water and ions inside the cells increases the osmotic pressure of the cells and its rupture. The pore formed by complement proteins are similar to what we observe in case of NK cells, where instead of water and ions, perforin and granzyme enters and kills the cell.

### 28.3.1 Function of complement system

- Promotes phagocytosis of the microbes by opsonization.
- Stimulates inflammation by degranulation of mast cells.
- Lysis of microbes by forming a membrane attack complex.
- Solubilization of those complexes that are unwanted for the body and helps in their clearance.

Figure 28.1 Different complement pathways:



### ***28.4 Neonatal immunity***

The body of neonate is sterile and passive transfer of antibody takes place by placenta before birth and through milk immediately after birth. The passive transfer of antibodies to neonates is important in providing immunity during the early phase of life when the immune system is not fully functional. Transfer of antibody through the gut in early life phase is called **transcytosis**. The maternal IgG is transferred through placenta and IgG and IgA through milk.