Module 2: Antibodies and Antigens

Lecture 7: Antibodies and Antigens (part I)

Antibodies may be defined as the proteins that recognize and neutralize any microbial toxin or foreign substance such as bacteria and viruses. The only cells that make antibodies are B lymphocytes. Mainly two forms of antibodies exist. One those that are membrane-bound and act as receptor for antigens on the surface of B lymphocytes and the other that are involved in inhibition of entry and spread of pathogens and are found in blood circulation and connective tissues. The substance or molecule identified by antibodies or that can evoke antibody response is called an **antigen**.

Some commonly used terminologies

Serum – Clot formation in the blood leaves the residual fluid that contains antibodies. These antibodies in the residue form the serum.

Antiserum – Serum contains a bunch of antibodies and when these antibodies show specificity to a particular antigen by binding to it, those antibodies are known as antiserum.

Serology- Serology may be defined as the study of blood serum or antibodies and their reactions with particular antigens.

7.1 Antibody structure

Antibodies are also called as **immunoglobulins** and are Y- shaped protein structures. Antibodies consist of two identical light and heavy chains. Amino terminal variable (V) regions are found in both heavy and light chains and they take part in antigen recognition. Effector functions are directed by carboxy – terminal constant (C) regions of the heavy chains but C regions are also found in both the chains. Both the heavy and light chains are composed of Immunoglobulin (Ig) domain. Ig domain is a protein domain that consists of folded repeating units of 110 amino acids in length sandwiched between two layers of β-pleated sheet. The two layers of β–pleated sheet are held together by a disulfide bridge and there are short loops that connect the adjoining strands of each β sheet. Amino acids in some of these loops are most crucial for antigen recognition. Light and heavy chain structure is almost similar. In light chain there is one V region Ig domain and one C region Ig domain whereas in heavy chain V region comprises of one Ig domain

and the C region comprises of three or four Ig domains. Antigen-binding site is formed by the V region of one heavy chain and the adjacent V region of one light chain. Disulfide bonds formed between cysteine residues connect the light and heavy chains in the carboxyl terminus of the light chain and the CH-1 domain of the heavy chain. Association of heavy and light chains occurs partly due to the non-covalent interactions between the VL and VH domains and between the CL and CH1 domains. Two heavy chains of each antibody entity are connected covalently by disulfide bonds. In IgG antibodies disulfide bonds are formed between cysteine residues in the CH2 regions which are near to a region known as **hinge**. This hinge region is more likely to undergo proteolytic cleavage. **Fragment antigen binding** (Fab fragment) is a portion on antibody that has the capability to bind to antigen and consists of one variable and one constant domain of each of the heavy and the light chain. **Fragment crystallizable region** (Fc region) is the distal region of an antibody that is composed of two identical, disulfide linked peptides containing the heavy chain CH2 and CH3 domains. Fc region communicates with some cell surface receptors called Fc receptors and this feature of Fc region helps antibodies to stimulate the immune system.

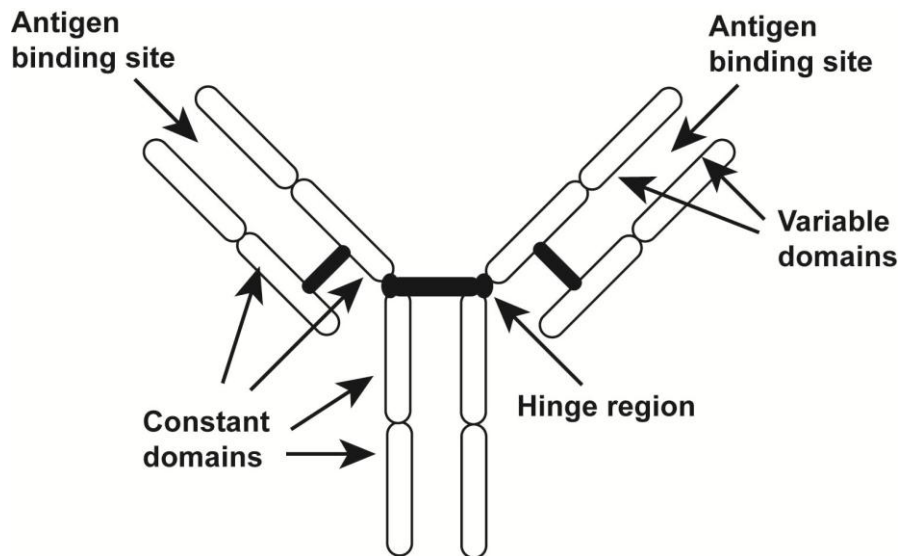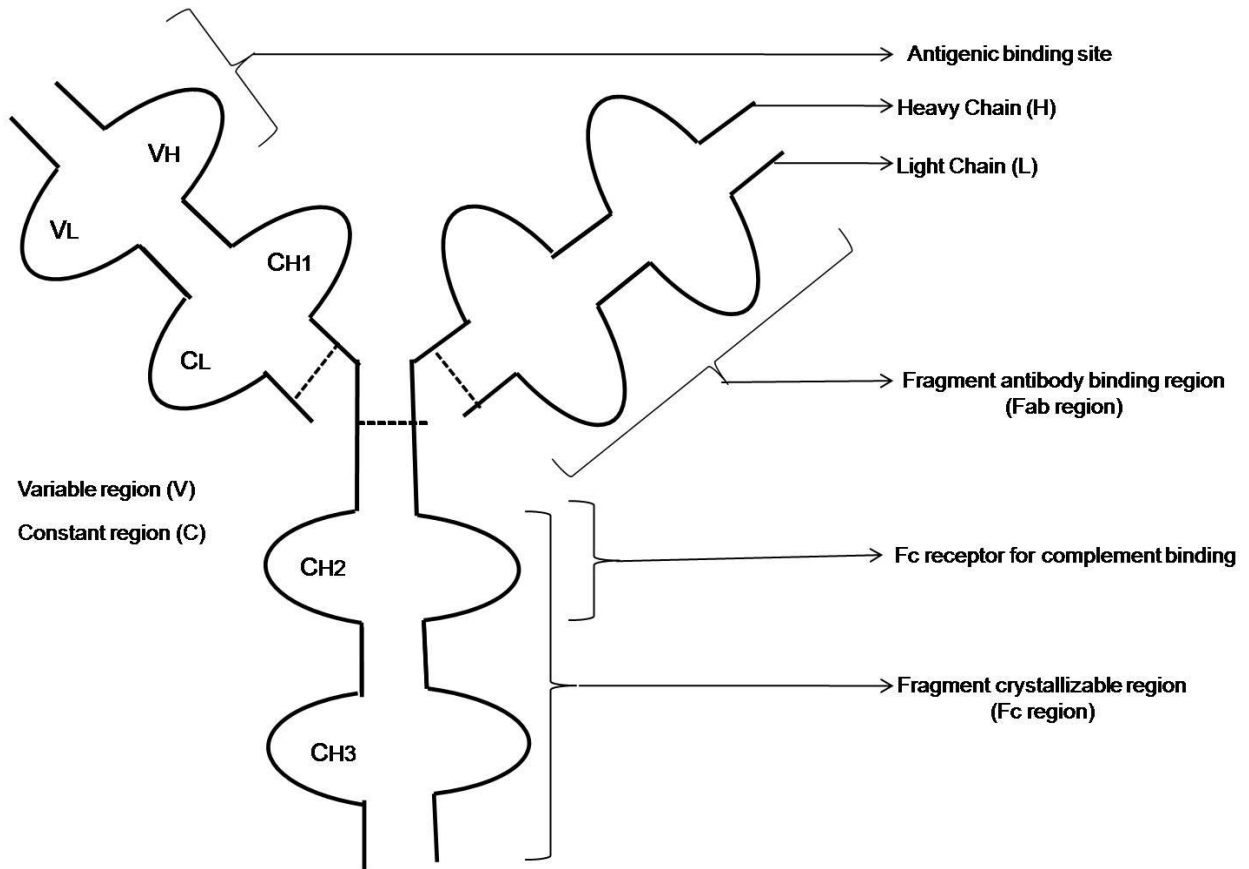**Figure 7.1 Immunoglobulin-G (IgG) molecule:**

Figure 7.2 Schematic representation of immunoglobulin domains:

7.2 Monoclonal antibodies

The concept of monoclonal antibodies was given for the first time by **Georges Kohler** and **Cesar Milstein** in the year 1975. Monoclonal antibodies are the antibodies that are specific to one particular antigen as they are made by identical immune cells that are several copies of a same parent cell e.g. any tumor cell of a specific region say plasma cells, are monoclonal and thus have the ability to produce antibodies of same specificity. The basic technique involved in making of monoclonal antibodies relies on fusion of B cells from an immunized mouse with a myeloma (tumor cell line) cell line and let the cells grow in a condition where unfused normal and tumor cells cannot survive. The cells that are fused and able to grow through this procedure are called as **hybridomas**.

7.2.1 Uses of monoclonal antibodies

1) Monoclonal antibodies help in immunodiagnosis by detection of a particular antigen or antibody.

2) Many tumor-specific antibodies help in tumor detection.

3) Some of the monoclonal antibodies have therapeutic uses. E.g. cytokine tumor necrosis factor (TNF) is used to treat many inflammatory conditions.

4) Monoclonal antibodies help in identification of individual cell populations e.g. lymphocyte and leukocyte differentiation has become possible now.

5) They help in the purification of cells in order to generate the info about their features and functions.

7.3 Genesis of immunoglobulin (Ig) molecules

Like most of the proteins, immunoglobulin heavy and light chains are formed in the rough endoplasmic reticulum. **Chaperones** are the proteins that are required for proper folding or unfolding of Ig heavy chains and also are needed during the assembly of heavy chain with light chain. Assembly process includes stabilizing of both the heavy and light chains by disulfide linkage and mutual association of heavy and light chains and the whole process occurs in endoplasmic reticulum. This is followed by carbohydrate modification which is required at the end of assembly process. At the end of this process Ig molecules get separated from chaperones and are shifted to cisternae of Golgi complex for carbohydrate modification, and finally find the way into the plasma membrane in vesicles. Membrane bound Ig molecules lie within the plasma membrane and the secreted form find its way out of the cell.

Membrane form of the μ heavy chain is synthesized by a prototype called the pre-B cell, which synthesizes the Ig polypeptides. Pre- B cell receptor expression on cell surface requires the association of μ heavy chain with surrogate light chains. Further maturation of B- cells is associated with modification in Ig gene expression leading to the generation of Ig molecules in different forms. The mature B lymphocytes differentiate into the antibody- secreting cells only when stimulated by foreign object or any antigen.

7.4 Half- life of antibodies

Half-life of antibodies varies in circulation. IgG molecules have a half life of 21 to 28 days while IgE has the shortest half-life of about 2 days. Long half-life of IgG is assigned to its ability to bind to Fc receptor called the neonatal Fc receptor. It is neonatal Fc receptor that is responsible for transfer of maternal IgG across the placental barrier.

Table 7.1 Biological properties of different Ig molecules:

Immunoglobulin class/Property	Molecular weight (Daltons)	Subunits	Constant heavy region (CH)	Heavy chain	Synthesis area
IgM	900,000	5	4	μ	Spleen and lymph nodes
IgG	180,000	1	3	γ	Spleen and lymph nodes
IgA	360,000	2	4	α	Intestinal and respiratory tracts
IgE	200,000	1	4	ε	Intestinal and respiratory tracts
IgD	180,000	1	3	δ	Spleen and lymph nodes

Figure 7.3 Different classes of immunoglobulins:
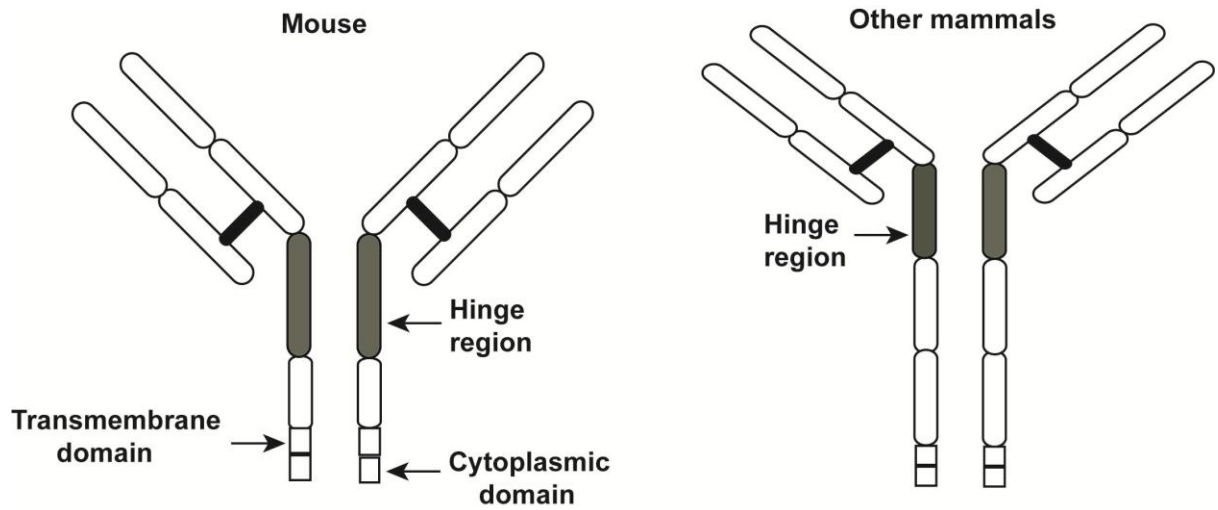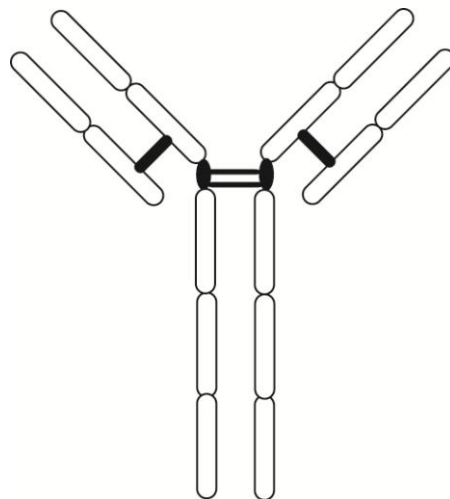
IgM
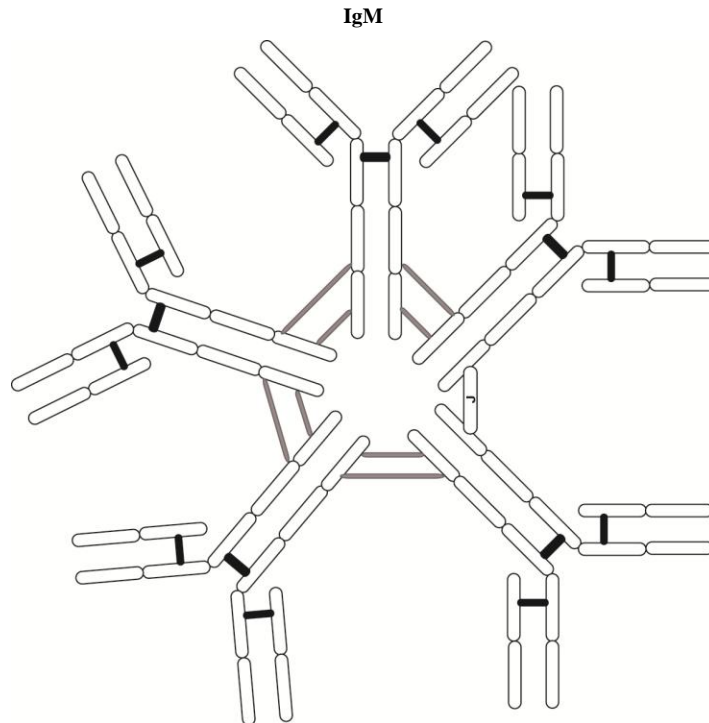
IgA
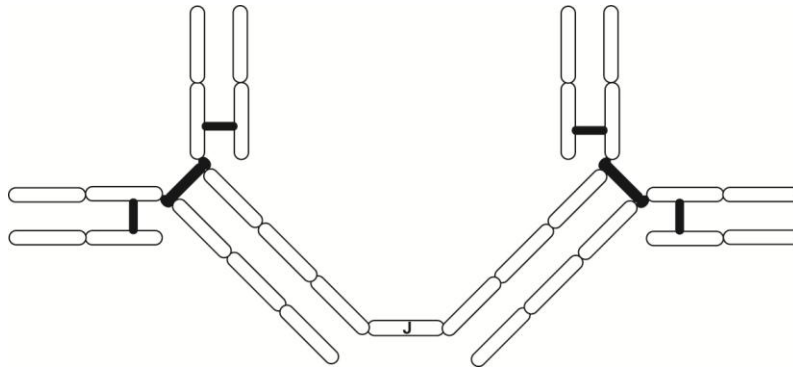
Joining chain= J

IgD

IgE

Lecture 8: Antibodies and Antigens (part II)

8.1 Characteristics of biologic antigens

1) One of the most important characters of antigen is to bind specifically to an antibody.

2) Almost all the antigens are identified by specific antibodies but very few have the ability to stimulate the antibodies. Sometimes in order to provoke an immune response, immunologists adjoin several copies of small molecules called **hapten** to a protein prior to immunization and the protein to which it is attached is known as **carrier**.

3) Foreign antigens are usually much bigger than the region where actual binding occurs between the antigen and the antibody and this region is known as antigen binding region. An antibody prefers to bind to this small region of the antigen known as **epitope**. Epitopes are hence also called as **antigenic determinants**.

4) Random structure on the antigenic molecule that is identified by the antibody as an antigenic binding site forms the epitope of that antigen.

5) Different epitopes are so organized on a single protein molecule that their spacing may affect the binding of antibody molecules in various ways.

8.2 Chemistry of antigen binding

The interaction of an antigen antibody is a reversible binding process that requires several non-covalent interactions like hydrogen bonds, electrostatic forces and hydrophobic interactions. **Affinity** and **Avidity** between the antigen antibodies also play a major role in their interaction. The potency of the reaction between a specific antigenic determinant and its single combining site on the antibody determines its **affinity**. The overall potential of binding of an antigen with many antigenic determinants to its multivalent antibody determines its **avidity**. Normally antigen-antibody binding site on antibodies are more or less flat and hence spacious so that they can attach large complexes or structures.

8.3 Antigen recognition

8.3.1 Specificity- Antibodies are very specific to an antigen and can even understand the minute difference between almost similar antigens. It may however happen that an antibody may bind to different but structurally similar antigen and this phenomenon is termed as a **cross-reaction**.

8.3.2 Diversity – Diversity determines the ability of antibody to bind specifically to a large number of different antigens. The pool of antibodies with different specificities describes the **antibody repertoire**.

8.3.3 Affinity maturation- The efficiency of antibody bonding to antigen is measured in terms of affinity and avidity. Some modification is required in structure of V region of antibodies during T cell dependent humoral immune response to antigens so that the antibodies having high affinity can be generated. B-cells that are responsible for generating high affinity antibodies preferentially bind to the antigen due to selection and become the prominent cells with each antigen antibody reaction. This mechanism is termed as affinity maturation, and it leads to an increase in binding affinity of antigen and antibody as antibody mediated response develops further.

8.4 Effector functions of antigen antibody reaction

1) As two or more Fc portions are required to stimulate effector functions so effector functions are carried out only by molecules with bound antigens and not with free Ig.

2) Fc region of the antibody molecules play a critical role in effector stimulation, so antibody isotypes varying in Fc region can be easily distinguished on the basis of interactions they carry.

3) Distribution of antibody molecules through different tissues is decided solely by the constant region in the heavy chain of an antibody molecule. This directed distribution through constant region of heavy chain is the reason behind IgA presence in mucosal secretions or recruitment of other antibodies to a particular tissue.

4) In antibody mediated immune response, variation in the isotypes of antibodies decides the ways to eliminate antigen from the body. In addition, isotype switching or class switching also has some role in it. e.g. antibody response to bacteria and viruses is carried

out by IgG antibodies but switching to IgG isotype can also lengthen the humoral response because it has the longest half life period among all the antibodies.

*Isotype- The presence of variations in the constant regions of the immunoglobulin heavy and light chains are called isotypes. Five heavy chain isotypes and two light chain isotypes are present in humans.

# Lecture 9: Major histocompatibility complex (Part I)

The major histocompatibility complex (MHC) was discovered from the studies conducted on transplant immunology. It was discovered from the fact that tissues exchanges between non-identical animal are rejected while from identical twins are accepted. **George Snell** and colleagues identified the single genetic region responsible for this rejection in chromosome 17 of mice and named it major histocompatibility complex. Similarly the gene responsible for graft rejection in humans was identified as **human leukocyte antigen** (HLA).

9.1 Major histocompatibility complex (MHC) gene

The MHC locus contains two types of MHC genes, class I and class II. MHC genes are codominantly expressed in an individual that means the alleles of the gene are inherited from both the parents. MHC class I molecules display peptides to the CD8+ lymphocytes to activate cell mediated immune response, and MHC class II molecules display the peptides to CD4+ lymphocytes to activate humoral mediated immune response. The diversity of the immune system has made MHC class I and II genes to be the most polymorphic genes present in the human genome. In humans, the gene responsible for encoding MHC molecule is located in the chromosome 6 (chromosome 17 in mice). The human MHC class I is encoded by three class of genes namely, HLA-A, HLA-B, and HLA-C. Similarly MHC class II is encoded by genes HLA-DP, HLA-DQ, and HLA-DR. In mice nomenclature for MHC changed to H-2K, H-2D, and H-2L for class I and I-A and I-E for class II (only 2 genes in mice). The set of MHC alleles present on each chromosome are called MHC **haplotype**.

Figure 9.1 Map of human and mice MHC gene loci:
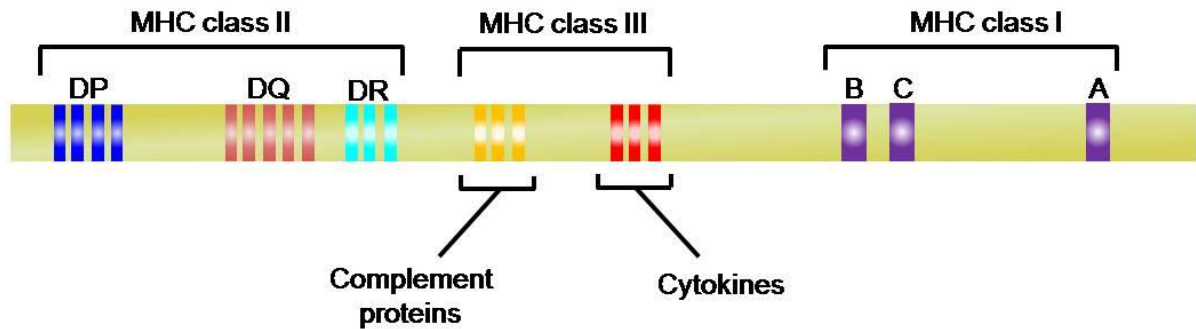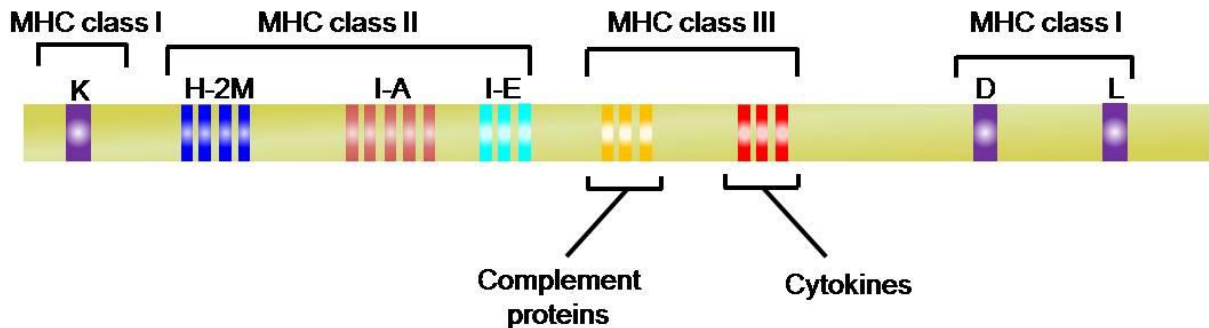
9.2 MHC expression

MHC class I molecules are expressed on all the nucleated cells, while class II are expressed only in dendritic cells, B cells, macrophages and few other cells. Class I restricted CD8+ cells kill the virus infected cells, the cells containing intracellular antigens and tumor antigens. Class II restricted CD4+ cells kill the extracellular antigen presented by mostly dendritic cells. The expressions of MHC molecules are stimulated by the cytokines such as interferons (type-I and II). Interferon-γ secreted by natural killer cells during the early innate immune response is the major cytokine responsible for activating the expression of MHC class II molecules in dendritic cell and macrophages. The rate of transcription of MHC gene is the major determinant for the expression of

MHC molecules. Any mutation in the transcription factor leads to many immunodeficiency diseases such as **bare lymphocyte syndrome**.

9.3 Properties of MHC molecules

1. MHC molecule consists of peptide binding groove, an immunoglobulin like domain, transmembrane domain, and a cytoplasmic domain. MHC class I molecule is made up of one MHC encoded and one non-MHC encoded chain. MHC class II molecule is made up of two MHC encoded chains.

2. The peptide binding groove is located at the adjacent to polymorphic amino acid residue. Because of the variability in the region, different MHC molecule binds and displays different peptides and are recognized by different T cells.

3. An immunoglobulin like domains contains the binding site for CD4 and CD8 cells.

Table 9.1 Features of MHC class I and II molecules:

Characters	MHC class I	MHC class II
Polypeptide chains	α1, α2, α3 and β2 microglobulin	α1, α2, β1 and β2
Size of peptide	8-11 amino acid long	10-30 or more amino acid long
Peptide binding site	Between α1 and α2	Between α1 and β1
Binding site for T cell coreceptor	α3 region (CD8+ binding)	β2 region (CD4+ binding)

Lecture 10: Major histocompatibility complex (Part II)

10.1 Peptide-MHC interaction

There are some characteristic features of peptide-MHC interaction.

I.   MHC class I and II molecules have a single peptide binding cleft that accommodates one peptide at a time but can bind to different peptides.

II. The processed peptide that binds to MHC shares structural compatibility that promotes their interaction.

III. MHC acquires the peptide over their cleft during the processing of the antigen inside the cell.

IV. Only small populations of peptide loaded over the MHC molecules are capable of eliciting the immune responses.

V.   MHC molecules present both the self and non-self peptide to the T cells. Remarkably it is the T lymphocyte that decides to which the body should produce an immune response. Majority of the MHC present in the body are loaded with the self peptide and T cells activated against the self peptides are either killed or inactivated by the host immune surveillance system. Hence, T cells normally do not respond to a self antigen.

10.2 MHC class I

MHC class I molecules are made up of two polypeptide chains, α and β2-microglobulin. The "α" chain is around 44 kD and "β2-microglobulin" chain is around 12kD in size. Each α chain is divided into three parts to accommodate extracellular α1, α2, and α3 domains, transmembrane domain and a cytoplasmic tail. The α1 and α2 is around 90 amino acids long and binds to only 8-11 amino acid long peptides (peptide binding cleft). The ends of MHC class I peptide binding cleft is closed and the larger peptide cannot be accommodated in the designated space. The accommodation length of peptide is highly conformational and globular proteins need to process into 8-11 amino acid length in order to load over the MHC class I cleft. The α1 and α2 contains the polymorphic residues which are responsible for the variation among the MHC I allele and their recognition by a specific T cell. The α3 segment of α chain contains the binding site for CD8+ cells. The α3 segment extends to 25 amino acids residue towards its carboxy terminal covering the

lipid bilayer and more 30 amino acids as a cytoplasmic tail. The β2-microglobulin non-covalently interacts with α3 chain. Binding of the peptide in the cleft between α1 and α2 strengthens the interaction between α and β2- microglobulin chain. The fully formed MHC class I molecule is a heterotrimer consists of α1, α2, α3 and β2-microglobulin chain.

Figure 10.1 Schematic representation of a MHC class I molecule:
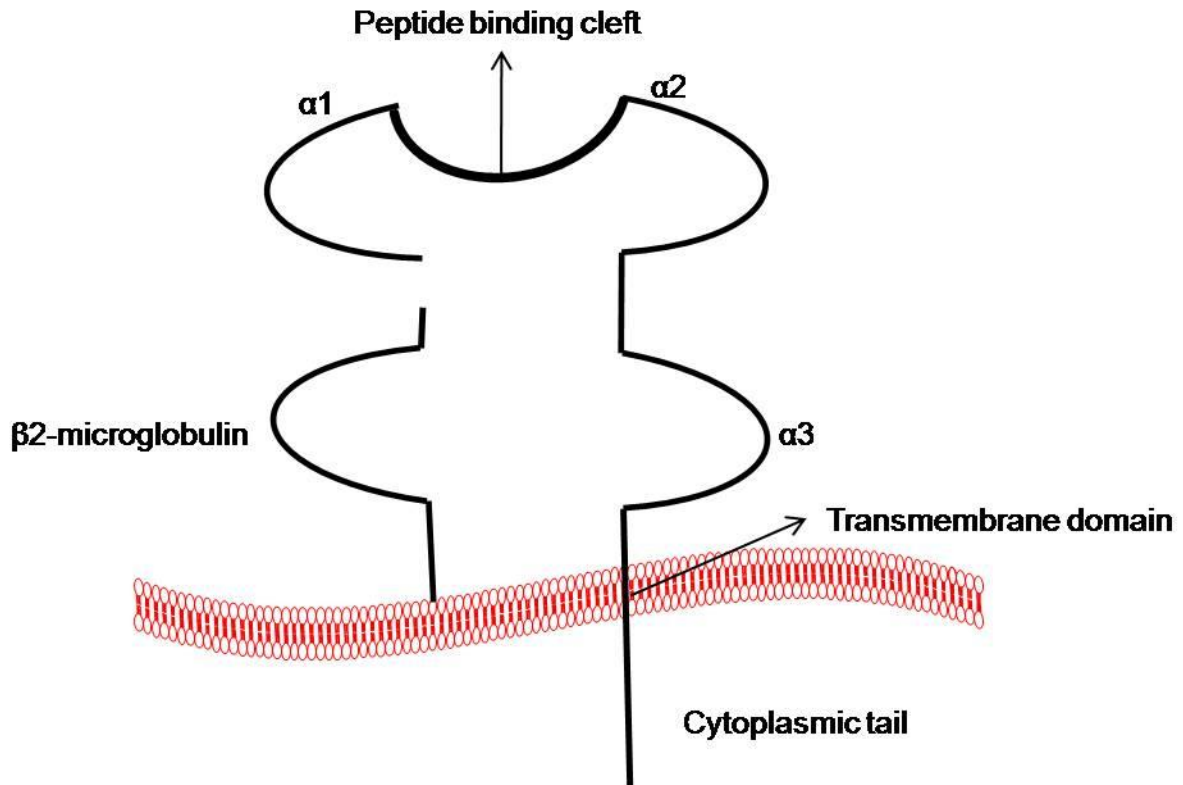
10.3 MHC class II

MHC class II molecules are also made up of two polypeptide chains, α and β. The "α" chain is around 33 kD and "β" chain is around 31kD in size. The α1 and β1 chain interacts with the peptide which is longer than peptide binding to class I molecule. The peptide binding cleft at α1 and β1 are open and so can fit peptides of length 30 or more amino acids. The β2 segment contains the binding site for CD4+ cells. The fully formed MHC class II molecule is a heterotrimer consisting of α1, α2, β1 and β2 microglobulin chain.

Figure 10.2 Schematic representation of a MHC class II molecule:
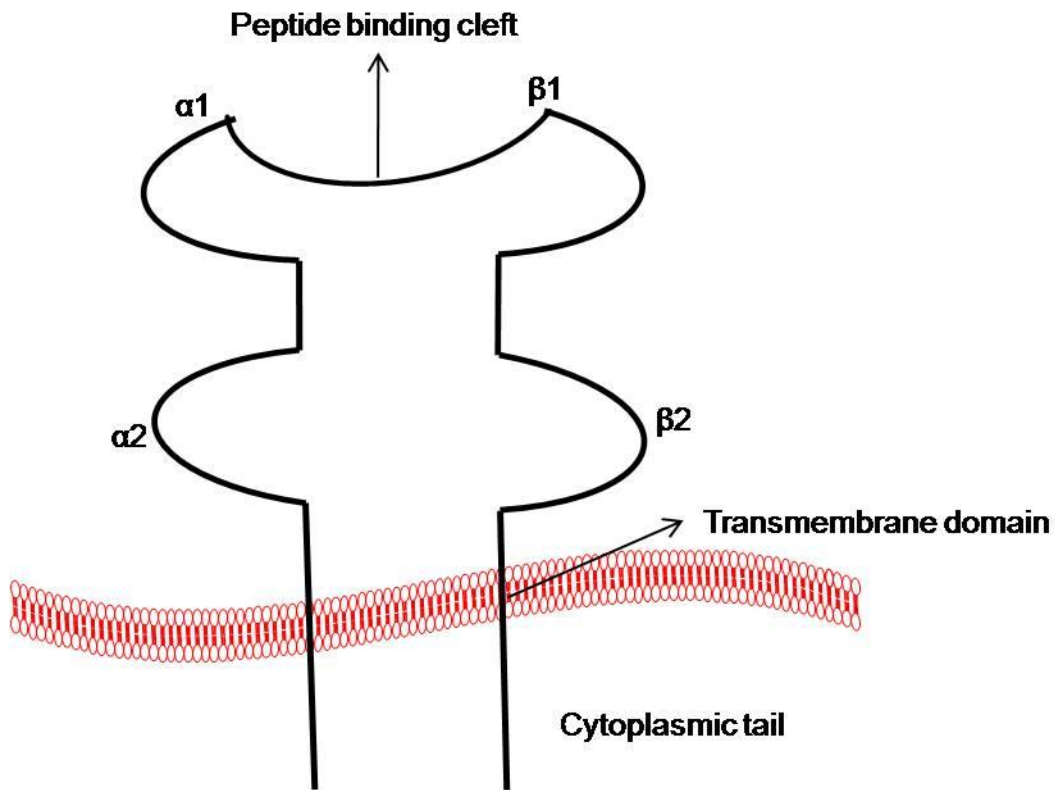
Lecture 11: Antigen processing and presentation to T lymphocyte (Part I)

11.1 Antigen recognition by T lymphocyte

In order to generate an acquired immune response an antigen molecule must be broken inside the cells and presented to the immune cells with the help of major histocompatibility complex (MHC) molecules. These are encoded by the genes of MHC complex and vary between different species. Antigens can trigger an immune response only after bounding to MHC molecules. All vertebrate animals contain the MHC encoding loci in their chromosomes.

Most of the T lymphocytes can recognize the small peptide fragments while the B cells can recognize the peptides, carbohydrates, lipid, nucleic acid and other chemicals. Because of different antigen specificity of T and B lymphocytes, cell mediated immune responses are usually activated by a protein antigen while humoral immune responses are activated by non-protein antigens.

The T cells recognize only protein antigens displayed by MHC molecules because the MHC cannot bind to any other molecules. An individual T cell can recognize only one specific MHC molecules loaded with the peptide, the property is called as **MHC restricted**.

T cells can recognize only the linear peptides and not the conformational epitopes of an antigen because the conformations of the proteins are lost during the processing and loading into the peptide binding cleft of MHC molecules.

T cells can recognize only the antigens that are associated with the antigen presenting cells and not to the soluble protein.

11.2 Antigen presenting cells

Many cell types function as antigen presenting cells to activate the naïve and effector T cells. Dendritic cells are the most common and effective antigen presenting cells in the body. Macrophages and B cells also act as an antigen presenting cells, but only to the previously activated T cells. All the above mentioned cells expresses the MHC type II molecule over their surface and hence also called **professional antigen presenting cells**. The antigen presenting cells displays the peptide MHC complex to T cells and also

provides the additional stimuli to T cells for its proper functioning. These stimuli are sometimes called as **costimulatory** molecules because they function together with the antigen presenting cells.

The antigen presenting function of the antigen presenting cells can be enhanced by microbial products. The induction of T cell response against an antigen is usually enhanced by the administration of purified protein products called as **adjuvants**. Adjuvants are derived from microbes such as killed mycobacterium which mimics the microbes and stimulate the production of immune response.

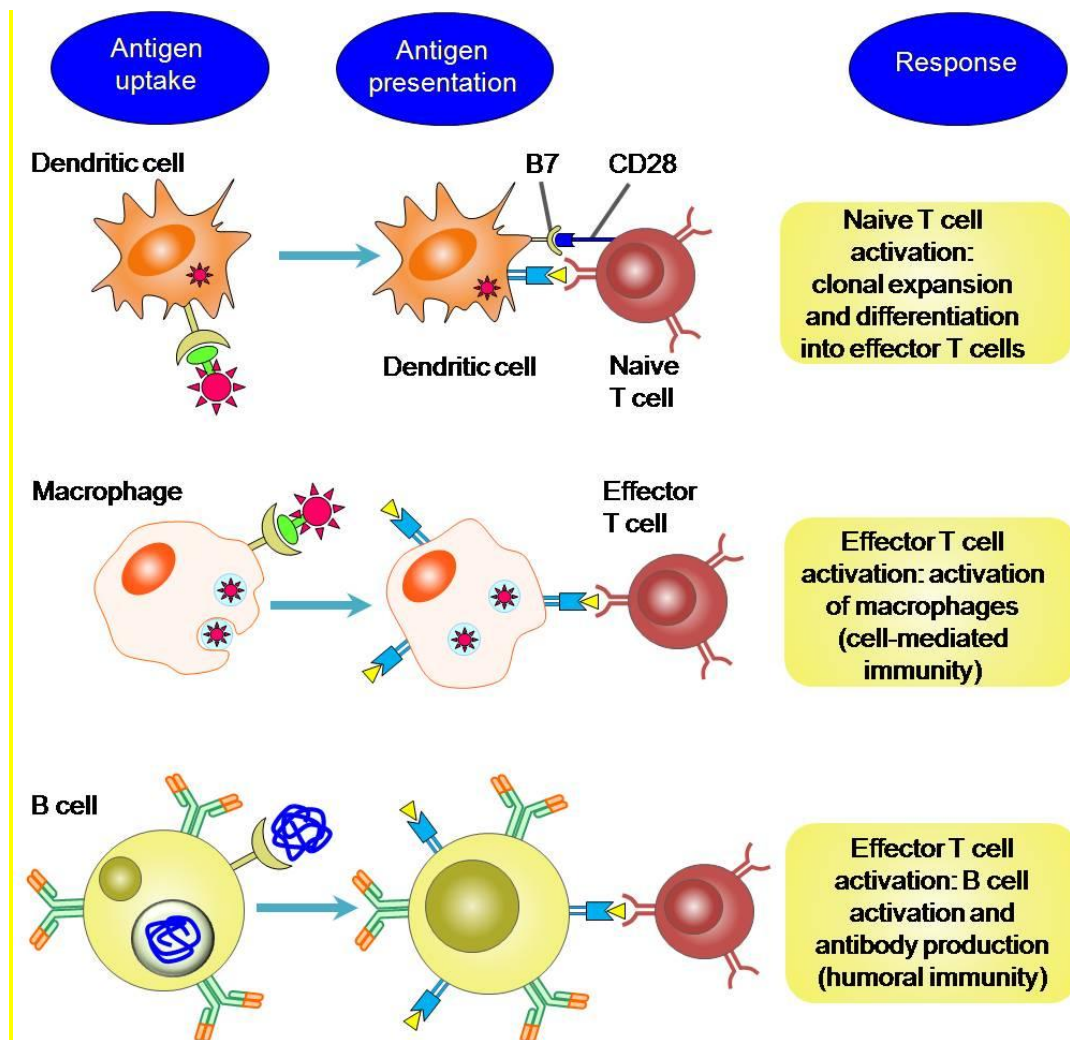**Figure 11.1 Different antigen presenting cells:**

Table11.1 Properties of antigen presenting cells

Cells	MHC class II and costimulation	Function
Dendritic cells	Expression increases with maturation and interferon-γ. CD40 and CD40L interaction acts as costimulator.	Process the protein antigen for T cell response.
Macrophages	Expression increases with interferon-γ. CD40 and CD40L interaction, LPS, and interferon-γ acts as costimulators.	Cell mediated immune response
B lymphocyte	Expression increases with interleukin-4. CD40 and CD40L interaction acts as costimulator.	Humoral immune response

Figure 11.2 Enhancement of class II expression by interferon-γ:
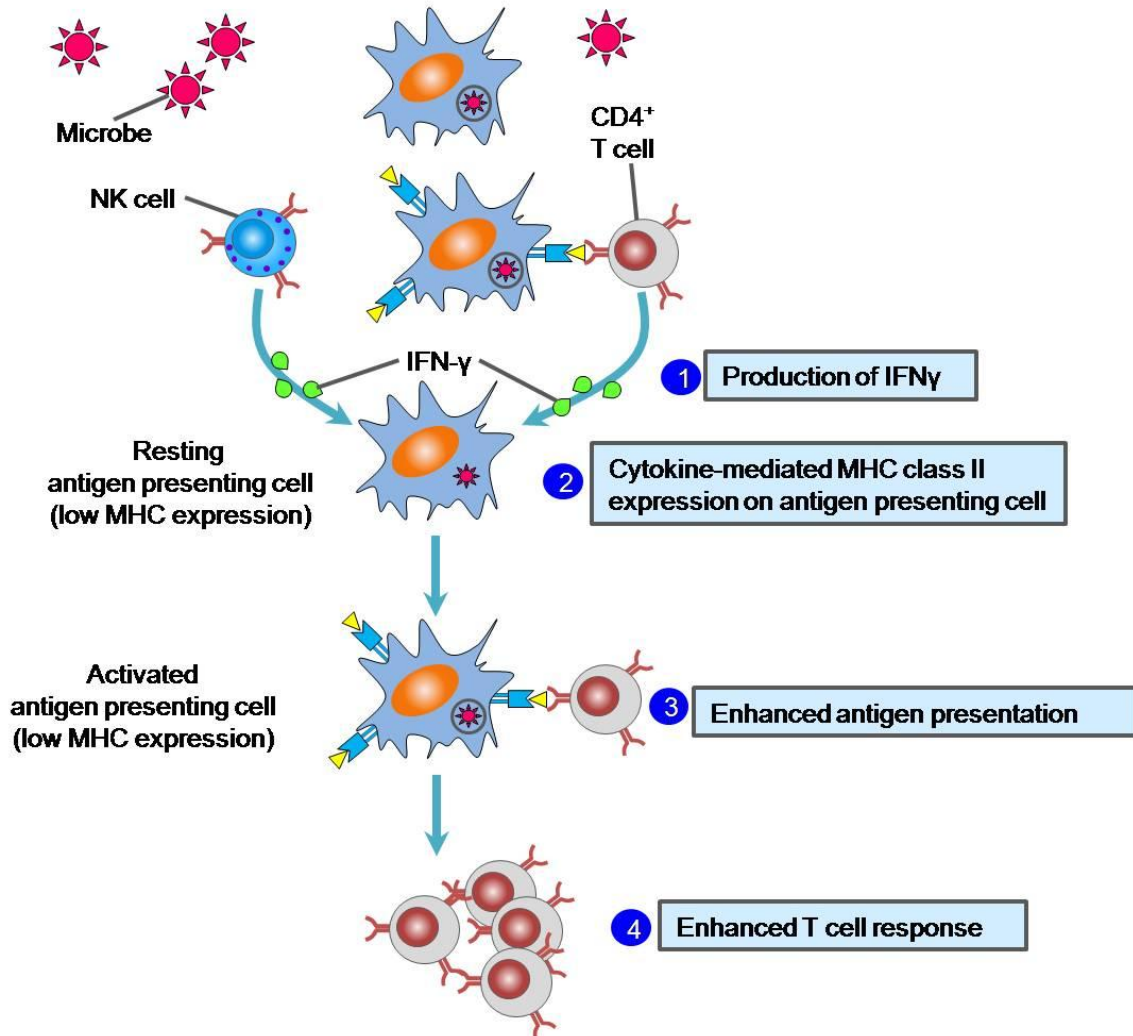
11.3 Dendritic cells

As discussed earlier dendritic cells are the major cells of immune system that act as an antigen presenting cell. Dendritic cells are present in the lymphoid organs and epithelial cells of gastrointestinal tract and respiratory tract. All dendritic cells are derived from the bone marrow precursor mononuclear phagocytic cells. Dendritic cells capture the antigen from the skin and epithelial lining of the tissues and enter the lymphatic vessels. Lymph acts as a reservoir of the cell associated as well as free antigen. There are mainly two subsets of dendritic cells.

11.3.1 Conventional dendritic cells

These are also called myeloid dendritic cells. They are the most abundant dendritic cells in the body and responsible for producing a strong T cell immune response. In tissues, they are called **Langerhans cells** because of its long cytoplasmic process that occupies a large surface area in the epithelial surface which make them highly accessible to the antigens. The surface marker molecules for conventional dendritic cell are CD11c and CD11b. They express high level of Toll like receptor 4, 5, and 8. In addition they secrete high level of tumor necrosis factor and interleukin-6.

11.3.2 Plasmacytoid dendritic cells

They are named so because of their morphology which is similar to plasma cells. The surface marker molecule for plasmacytoid dendritic cell is B220. They express high level of Toll like receptor 7 and 9. They are responsible for the secretion of large amount of type I interferon (α and β) following viral infection.

Lecture 12: Antigen processing and presentation to T lymphocyte (Part II)

12.1 Processing of antigen through MHC class I pathway

Usual antigens that are processed by MHC class I include intracellular bacteria, viruses, and tumor antigens. MHC class I peptides are processed in the cytosol by the proteolytic degradation of the protein. Occasionally the proteins are phagocytized and imported to the cytoplasm in order to load over the MHC class I molecules. The proteins are degraded by the **proteasome** in the cytoplasm which are the complex structure responsible for the degradation of unwanted or improperly folded proteins. After degradation the peptides are transferred to the endoplasmic reticulum with the help of transported proteins called **transporter associated with antigen processing** (TAP). TAP is associated with another protein called **Tapasin**, which acquires specific affinity with the new and empty MHC molecules. Tapasin brings the TAP-antigen complex towards the new MHC molecule. The proper folding and assembly of the MHC class I molecules inside the endoplasmic reticulum is modulated by the chaperons **calnexin** and **calreticulin**. The peptides from TAP-antigen-tapasin complex is processed and loaded over the MHC molecule with the help of **endoplasmic reticulum associated peptidases** (ERAP). The peptide transported to the endoplasmic reticulum are generally presented through the MHC class I pathway. The peptide bound to the MHC class I molecules are then transported to Golgi apparatus and then to cell surface with the help of exocytic vesicles. The MHC class I loaded peptides are recognized by CD8+ T lymphocyte to induce cell mediated immune response.

Figure 12.1 MHC class I antigen processing pathway:
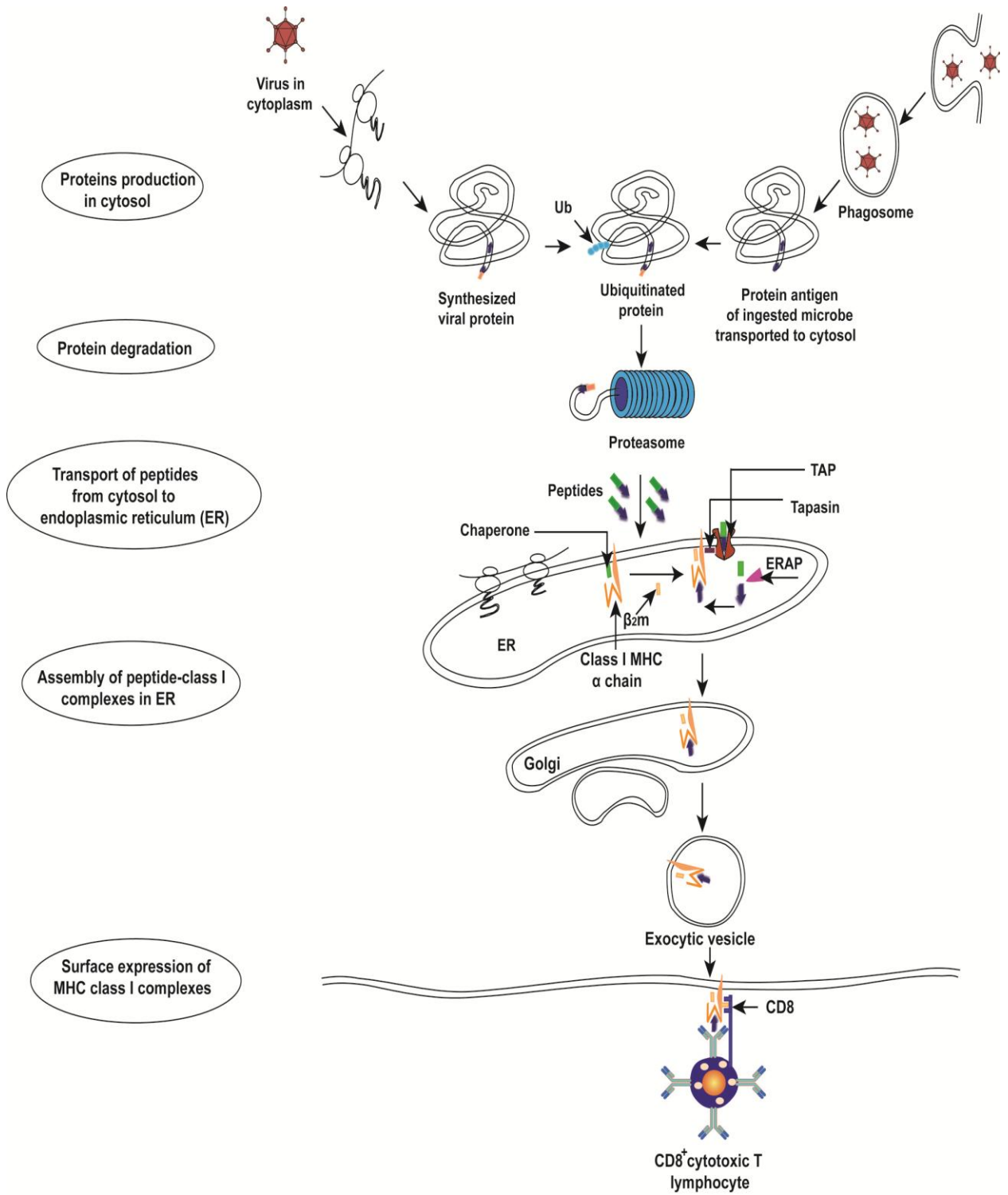
12.2 Processing of antigen through MHC class II pathway

Majority of the peptides associated with the MHC class II are generated from extracellular antigens (protein) that are captured inside endosomes of the antigen presenting cells. The antigen containing endosomes are fused with the lysosome to form endolysosome, the acidic pH of the endolysosome helps in the degradation of the proteins into smaller peptides. MHC class II molecules are synthesized in the endoplasmic reticulum and transported to the endosomes with the help of invariant chain (Ii), which binds to the peptide binding cleft of a newly synthesized MHC class II molecule. Class II molecules with bound invariant chain (CLIP) are transported to endosomes and are degraded by proteolysis to release the invariant chain. The remaining part of CLIP is removed by HLA-DM present in the endosomes in order to create space for peptide. Once CLIP is removed the peptides are loaded over the MHC class II molecule. The MHC class II molecule bound to peptides is delivered to the surface for their recognition by the CD4+ T lymphocytes (humoral immune response).

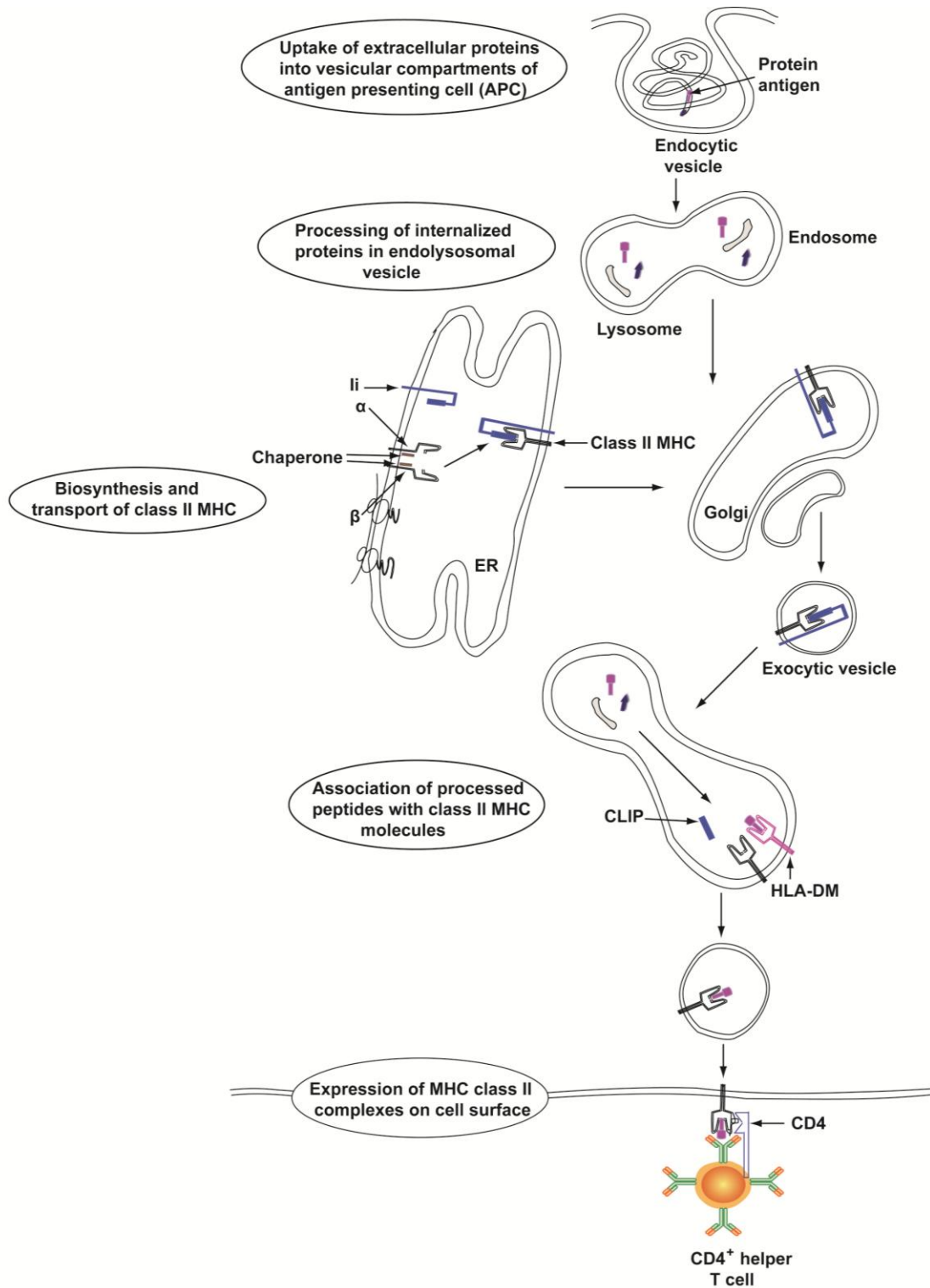**Figure 12.2 MHC class II antigen processing pathway:**

Table 12.1 comparison of MHC class I and II antigenic processing:

Features	MHC class I	MHC class II
Composition of peptide binding cleft	α1, α2, and peptide	α1, β1, and peptide
Antigen presenting cells	All nucleated cells	Dendritic cells, phagocytes, B lymphocyte, macrophages etc
Type of T cells	CD8+ T cell	CD4+ T cell
Source of protein antigen	Cytosolic protein antigens	Endosomal and lysosomal protein antigen
Site of peptide loading	Endoplasmic reticulum	Specialized vesicles

12.3 Cross presentation of peptides

Occasionally dendritic cells capture and ingest the viruses or tumor antigens and present the antigens to the CD8+ T lymphocytes. As mentioned above, antigens captured into vesicles initiate the MHC class II pathway. The deviation of some dendritic cells to present the endocytic degraded peptides to MHC class I molecules and activate the CD8+ mediated immune response is called **cross presentation**.

Lecture 13: Antigen receptors and accessory molecules of T lymphocytes (Part I)

Receptors that initiate the signaling pathways are generally associated with the plasma membrane. The extracellular domain of the receptors recognizes the ligands present over the cell surface and this interaction may lead to conformational changes in the receptor. The conformational changes are associated with the recruitment of the phosphate group at its carboxy terminal on tyrosine, serine, or threonine residue. The enzyme that adds the phosphate group on amino acid residues are called **protein kinases**. The **tyrosine** is the major amino acid residue that takes part in this event (phosphorylation) hence the enzymes are referred as **protein tyrosine kinases**. Alternatively the enzymes which are responsible for the removal of phosphate group from amino acids are called **phosphatase**. **In general the protein kinases can initiate while phosphatase can inhibit the signaling pathways**. Several types of protein modification can also modulate the binding of an antigen to the receptor such as phosphorylation (addition of phosphate group), acetylation (addition of acetyl group), methylation (addition of methyl group), and ubiquitination (addition of ubiquitin). The ubiquitination is the event that takes place during the degradation of proteins through proteasome.

13.1 Types of cellular receptors

There are several types of cell receptors based on their signaling mechanism and biochemical pathways.

13.1.1 Receptor tyrosine kinases

They are associated with the cell membrane and are involved in the phosphorylation of tyrosine residue located in their cytoplasmic tail. The pathway begins after binding with a suitable ligand over the receptor. e.g. Insulin receptor, epidermal growth factor receptor, platelet derived growth factor receptor, and receptor involved in the process of hematopoiesis.

13.1.2 Non-receptor tyrosine kinases

They are associated with the cell membrane and are involved in the phosphorylation of proteins by a non-receptor tyrosine kinases following binding with a ligand. Immune receptors, cytokine receptors, and integrins are known to follow non-receptor tyrosine kinases signaling pathway.

13.1.3 Seven transmembrane receptors

These are the polypeptide receptors that traverse **seven** times in the plasma membrane and hence also named as **serpentine receptor**. The receptor generally binds to GTP hence also called as **G protein-coupled receptors** (GPCR). Binding of the ligand to GPCR activates the hetrotrimeric G protein and initiates the downstream signaling pathway. Inflammatory cytokines and cAMP are activated following binding to GPCR.

13.1.4 Nuclear receptors

The modulation of transcription is usually done at the level of nuclear membrane. The receptors that use lipids as its ligand either increase or decrease the transcription of genes. Vitamin D receptor and glucocorticoid receptor are the examples of nuclear receptors.

13.1.4 Miscellaneous receptors

Notch receptors are involved in the embryonic development and tissue maturation. The binding of the specific ligand with notch receptor leads to the proteolytic cleavage of the cytoplasmic tail of the receptor that can act as a transcription factor for different developmental pathways. A group of ligand called "**Wnt**" can modulate the level of **β-catenin** which contributes to B and T cell development.

Figure 13.1 Signaling pathways for cytosolic and nuclear activation:
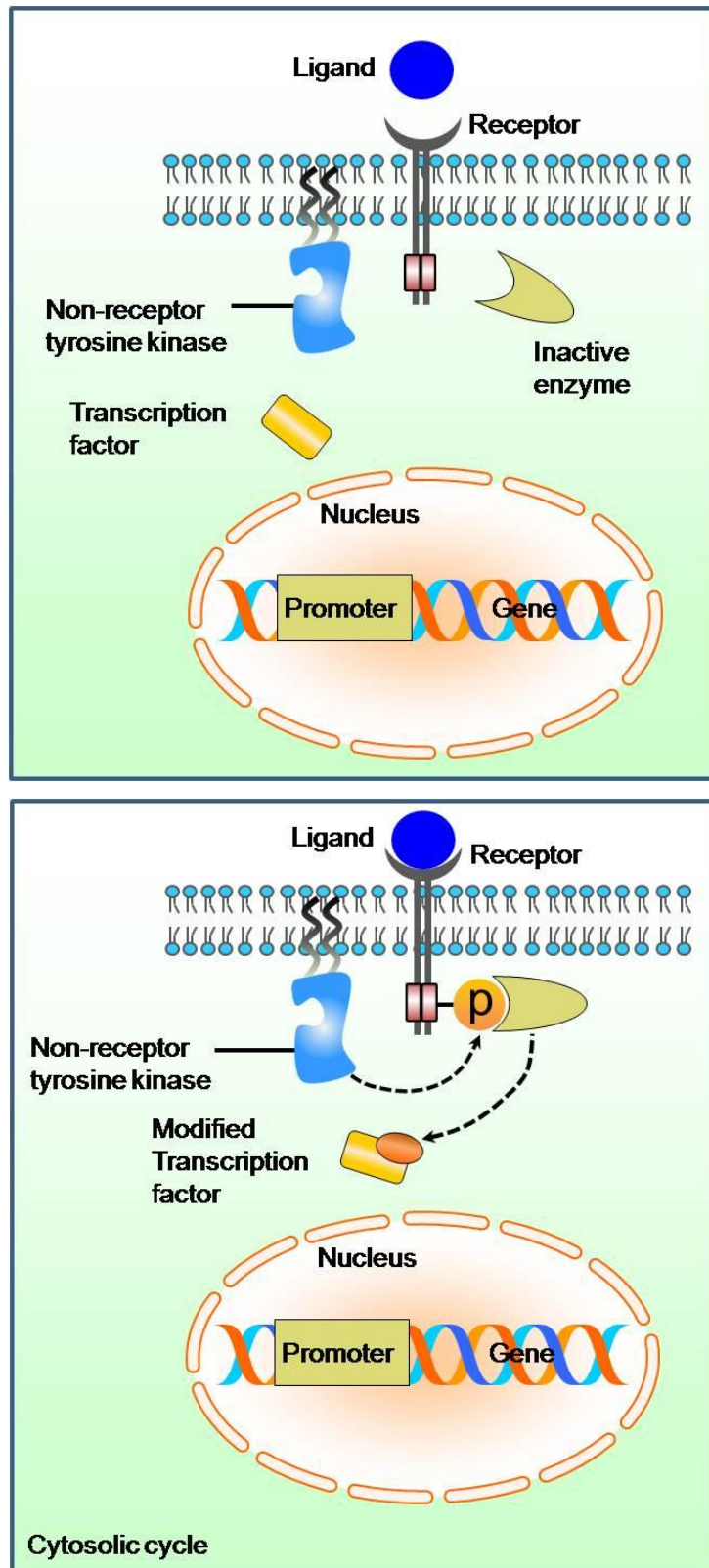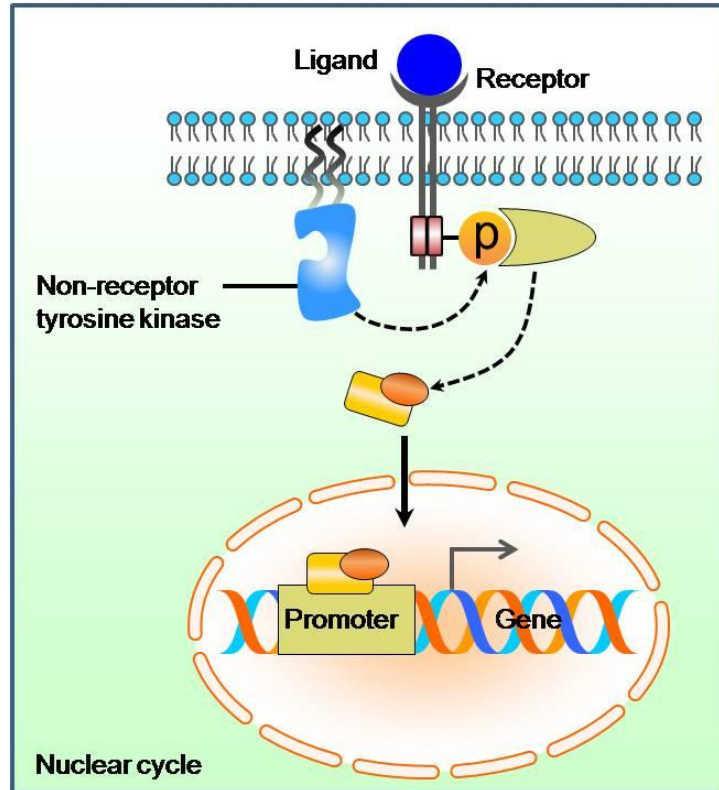
13.2 Immune receptor family

Immune receptors are made up of immunoglobulin superfamily which are involved in ligands recognition and contain tyrosine motif in their cytoplasmic tails. The cytoplasmic tail contains the **immunoreceptor tyrosine-based activating motifs** (ITAM) which are involved in the activation process. Phosphorylation of ITAM recruits the Syk/ZAP-70 tyrosine kinase which activates the immune cells. Contrary to ITAM some immune receptor contains **immunoreceptor tyrosine-based inhibitory motifs** (ITIM) which lead to inhibition of immune signaling. Immune receptors family includes B and T cell receptors, IgE receptor on mast cells, and activating and inhibitory receptor Fc receptors.

13.3 Characteristics of antigen receptor signaling

Signaling event in the T and B cells undergoes similar downstream pathway.

Binding of ligands to the receptor activates the Src family kinase that phosphorylates the ITAM motif in the cytoplasmic tail of the receptor. The phosphorylated tyrosine in ITAM recruits the Syk tyrosine kinases which further activate the downstream signaling cascade.

Lecture 14: Antigen receptors and accessory molecules of T lymphocytes (Part II)

14.1 T cell receptor complex and T cell signaling

The T cell receptors (TCR) are made up of heterodimer of two polypeptide chains, α and β that are covalently linked with each other by a disulfide linkage. The TCR containing these two chains are called as αβ T cells. Another type of TCR called γδ T cells contains γ and δ polypeptide chains. Each polypeptide chain in TCR is made up of amino terminus, variable and carboxy terminal, constant region (similar like immunoglobulin molecules). The variable regions are the complementarity determining regions in the TCR and are responsible for its polymorphism. The α and β chains contain 5-12 amino acids residue at their cytoplasmic tail to take part in signal transduction pathway. The other two structures that are associated with the TCR are CD3 and ζ proteins which are noncovalently associated with the αβ chain. **TCR together with CD3 and ζ proteins forms the TCR complex**.

The CD3 and ζ proteins are constant in all T cells regardless to its specificity towards any ligand. CD3 contains ε, γ, and δ polypeptide chains which resembles the immunoglobulin superfamily members. CD3 contains two heterodimer made up of εγ and εδ polypeptide chains. The ε, γ, and δ polypeptide chains of CD3 molecule is made up of 44-81 amino acids and contains one ITAM molecule for modulating the downstream signaling pathway. CD3 polypeptide chain contains a negatively charged aspartic acid residue that interacts with the positive charged residue present in the αβ chain. The ζ polypeptide chain contains a long cytoplasmic tail that contains three ITAM molecules and usually expressed as a homodimer in a TCR complex.
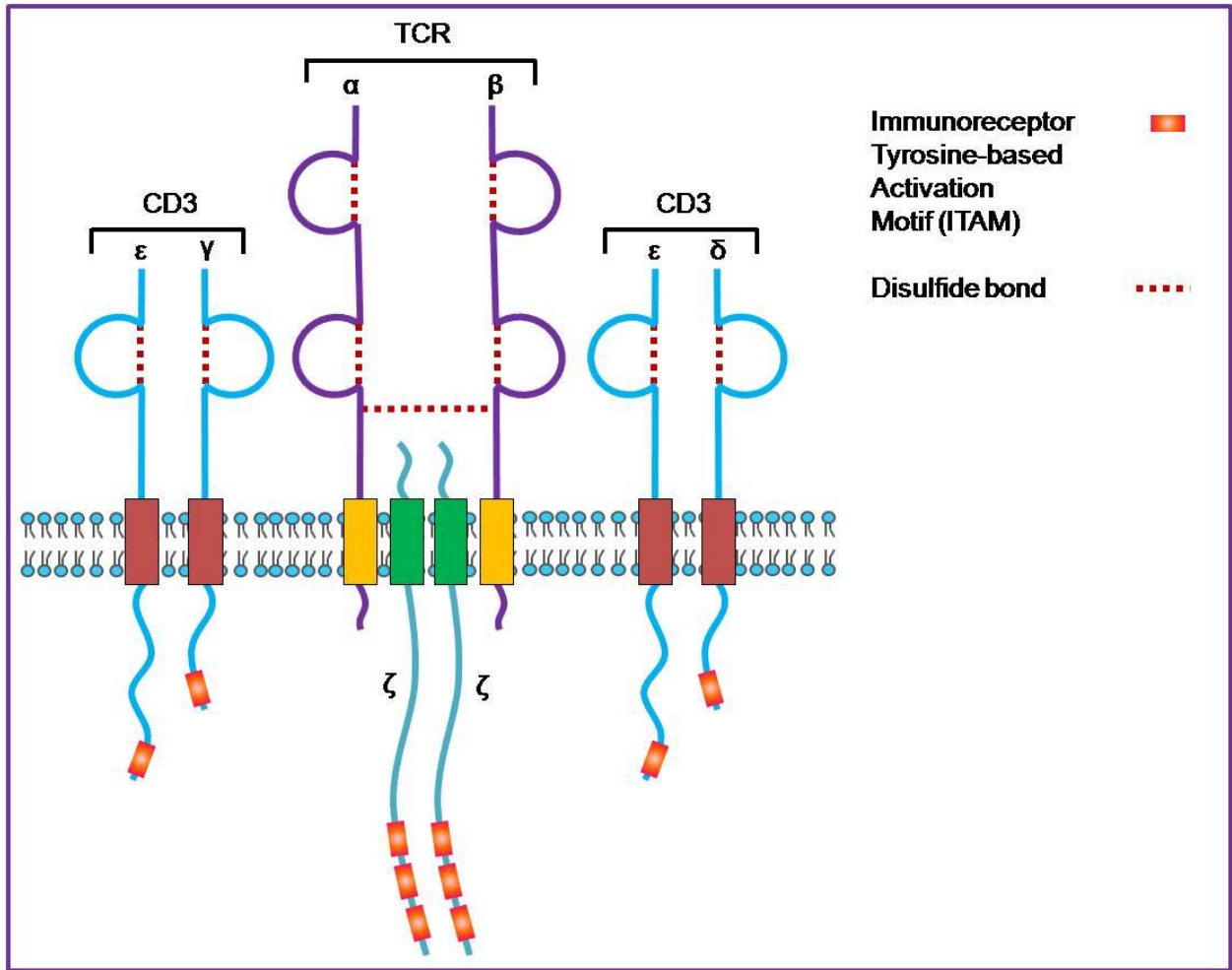
Figure 14.1 T cell receptor complex:

Table 14.1 Difference between T cell receptor and immunoglobulins:

Features	T cell receptor	Immunoglobulins
Polypeptide chain	α and β chains	Heavy and light chains
Associated molecules	CD3 and ζ	Igα and Igβ
Complementarity determining regions	Three	Three
Isotype switching	No	Yes
Somatic mutation	No	Yes
Secretory form	No	Yes (IgA)

14.2 Signaling in T cell receptor

The ligation of TCR with a peptide loaded MHC molecule results in grouping of CD3, ζ proteins and other coreceptors. CD4 and CD8 are T cell coreceptors that bind to the MHC molecule and facilitate the TCR signaling pathway. As we learned in MHC molecule CD4 recognizes class II while CD8 recognizes class I molecule, to maintain this equilibrium, T cells can express either CD4 or CD8 receptor but never both. The interaction of TCR with an MHC-peptide complex results in phosphorylation of the ITAM residue present over the TCR complex. Phosphorylation of the ITAM activates the tyrosine kinase which phosphorylates the tyrosine present over the other coreceptor molecules. The cytoplasmic tails of CD4 and CD8 recruits a Src family kinase **Lck**. Another Src family kinase associated with the TCR complex is **Fyn**. Lck phosphorylates the tyrosine in the ITAM present over the CD3 and ζ chain. The phosphorylated ITAM in the ζ chain recruits the Syk family tyrosine kinase called ζ associated protein of 70kD (ZAP70). ZAP70 in turn phosphorylates the adaptor proteins such as SLP-76 and LAT. Phosphorylated LAT then recruits several components of adaptor proteins including PLCγ1 (key enzyme involved in T cell activation). This entire event activates the Ras and mitogen activated protein kinase (MAPK) pathway which in turn activates the transcription factors and activated T cell response.
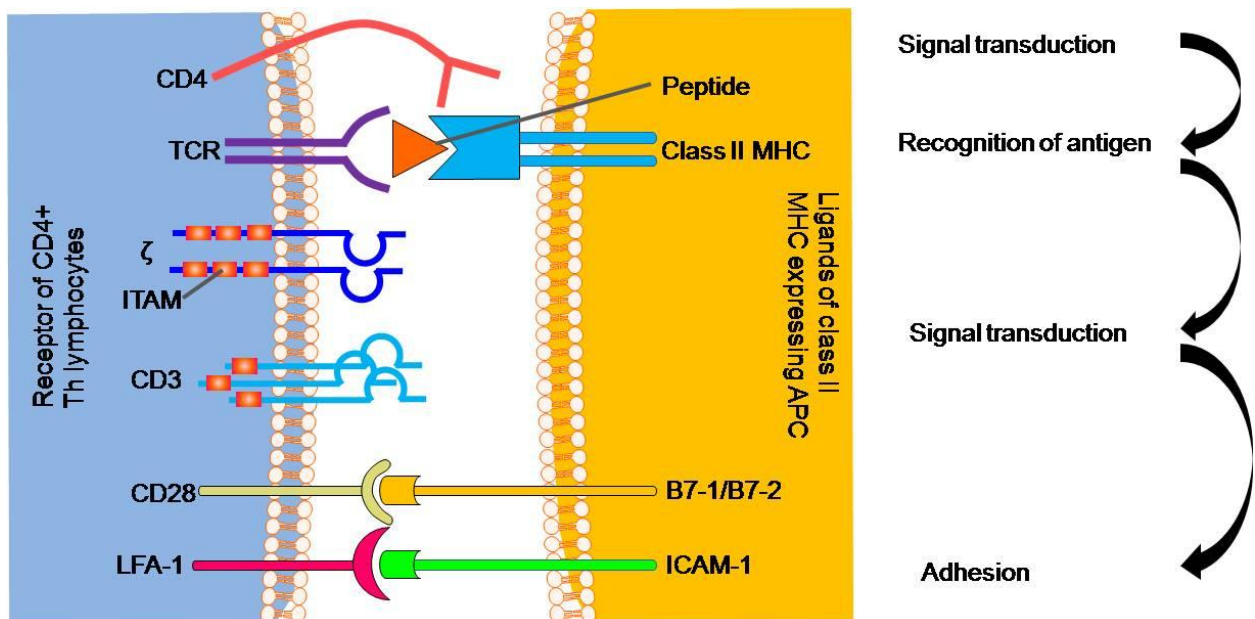
Figure 14.2 Receptor and ligands involve in T cell activation:

Figure 14.3 Schematic representation of T cell signaling:

TCR complex, CD4 and CD8 receptors are cluster over the membrane after binding with an MHC loaded peptide

Lck phosphorylates the tyrosine in the ITAM present over the ζ chain cytoplasmic tail

Phosphorylation of ζ chain leads to activation of ZAP70 and further phosphorylation of the adaptor proteins

Phosphorylation of the adaptor proteins assembles and activates the Ras, MAPK pathway