Introduction to the Immunogenetics

The field of Immunogenetics is at the core of research aiming at identifying and understanding associations between genetic factors and immunological phenotypes or immunity-related diseases.

Immunogenetics is made from the words, immunity and **genetics** and is concerned with the genetics of the immune system. **Immunity** derives from the Latin word **immunis** which means ”exempt” and ”genetics” derives from the Greek word **genesis** which means **origin**.

Immunogenetics has now become one of the most challenging disciplines in modem Imrnunogenelin biology. In recent times with the advent of modern techniques, like genetic engineering and monoclonal antibodies, it has come to the forefront of modem sciences.
Immunology has its origins in the study of how the body protects itself against infectious diseases caused by microorganisms, such as bacteria, viruses, protozoa, and fungi, and also parasitic organisms, such as helminth worms.

In its most complex forms, the immune system consists of two branches:

- The Innate immune system that provides a rapid, general, response when alerted by certain typical signals of infection (essentially forming a first-line of defence)
- The adaptive immune system that is able to develop highly specific responses (and a persistent ‘immune memory’) to target the infection with extraordinary accuracy.

Both systems work in close cooperation and, to an important extent, the adaptive immune system relies upon the innate immune system to alert it to potential targets, and shape its response to them.

1- Innate immunity

Innate immunity consists of protective mechanisms we are born with, and are the first line of defence against anything recognized as non-self. The produced immune response is not specific to the antigen and no memory of the antigen persists. However, innate immunity is the crucial first step in most adaptive immune responses.

The following are the protective mechanisms of innate immunity(see Table 1):

- Physical and Chemical Mechanisms
- Phagocytosis
- Molecular Response
- Inflammatory Response

Mast cells and basophils are innate cell types that, when activated, secrete histamine, which can be an important inflammatory mediator produced in response to initial tissue damage as a result of infection. Mast cells are tissue resident (e.g. in mucosal tissues) whilst basophils are found in the blood. In particular, they play a key role in the so-called allergic response. Innate immunity comprises both cellular and humoral ('in solution') elements. The cellular elements are represented notably by **phagocytes** (specifically **neutrophils** and **macrophages**) that can respond to signs of infection (i.e. inflammation) in the tissues and home-in on infective bacteria before

neutralising and engulfing them ('phagocytosis'). Recognition of microorganisms by the innate system occurs via characteristic pathogen-associated molecular patterns (PAMPs) on microbial surfaces, and an important family of innate receptors called pattern-recognition receptors (PRRs) are responsible for this (notably including Toll-like receptors [TLRs]). **The natural killer (NK) cell** is another important innate cell that is able to detect and target intracellular infection of body cells by viruses. A further specialised innate cell is the **eosinophil** that plays a particular role in targeting larger infective organisms, such as parasitic worms.

-The complement system represents the humoral arm of innate immunity, and consists of a number of proteins (found in solution in the blood) that can interact directly, or indirectly, with infective bacteria (through different activation pathways). Inflammation, as a result of infection, allows plasma, containing complement proteins, to enter infected tissues. Once activated, the member proteins assemble to form complexes on the surface of microbes that punch holes in the membrane. The complement activation pathways are termed: the classical pathway, the alternative pathway, and the mannose-binding lectin pathway.

Cytokines form an important family of proteins that function as immune mediators and have important roles during immune responses , they can serve to both stimulate or inhibit the differentiation, proliferation or activity of immune cells. A subset of cytokines, chemokines, play an important role in guiding immune cells to sites of infection by forming a chemical 'trail'.

Table 1 - Innate Immunity

Physical and Chemical Mechanisms	Phagocytosis	Molecular Response	Inflammatory Response
Physical barriers: • intact skin • mucous membrane barrier (sneezing, coughing) • cilia **Chemical barriers:** • tears • acid (pH) • saliva • bile	**Macrophages:** • engulf and kill invading organisms **Dendritic cells:** • engulf pathogen • display antigen on cell surface • travel to lymph node to present antigen to T cells • critical link between the innate and adaptive immune responses.	**Cytokines:** Cytokines are small proteins made by a cell that affect the behavior of other cells. Examples: • Cytokines cause vasodilation (heat and redness) • Some types of *interferon* are antiviral cytokines which help healthy cells resist viral infection **Chemokines:** Chemokines are proteins secreted by macrophages that attract cells out of the blood stream and into the infected tissues. **Complement:** The complement system is a group of approximately 20 proteins that coat bacterial surfaces and promote bacterial destruction by macrophages.	The accumulation of fluid and cells at the site of infection causes the redness, swelling, heat, and pain known as inflammation. Inflammation is beneficial because it: • recruits cells out of the blood stream, • increases the flow of lymph to take away microbes and antigen-bearing cells to the lymphoid tissue which will lead to adaptive immunity, and • brings the T cells and B cells back to the site of infection.

2- Adaptive immunity

Adaptive immunity is the second line of defence against anything recognized as non-self and it provides protection against re-exposure to the same pathogen.

- **Characteristics of adaptive immunity:**

1- Specificity: the immune response is specific to the antigen that produced it (e.g. antibody for measles antigen has no effect on rubella antigen).

2- Tolerance: the immune response is able to differentiate between self and non-self so that body tissues are not destroyed

3- Memory: with subsequent exposure to an antigen there is a rapid and strong immune response. This is called an anamnestic response.

-The adaptive immune response consists of two branches: : (See Figure 1)

- Cellular adaptive response (effected by cytotoxic T cells)
- Humoral adaptive response (effected by B cells).

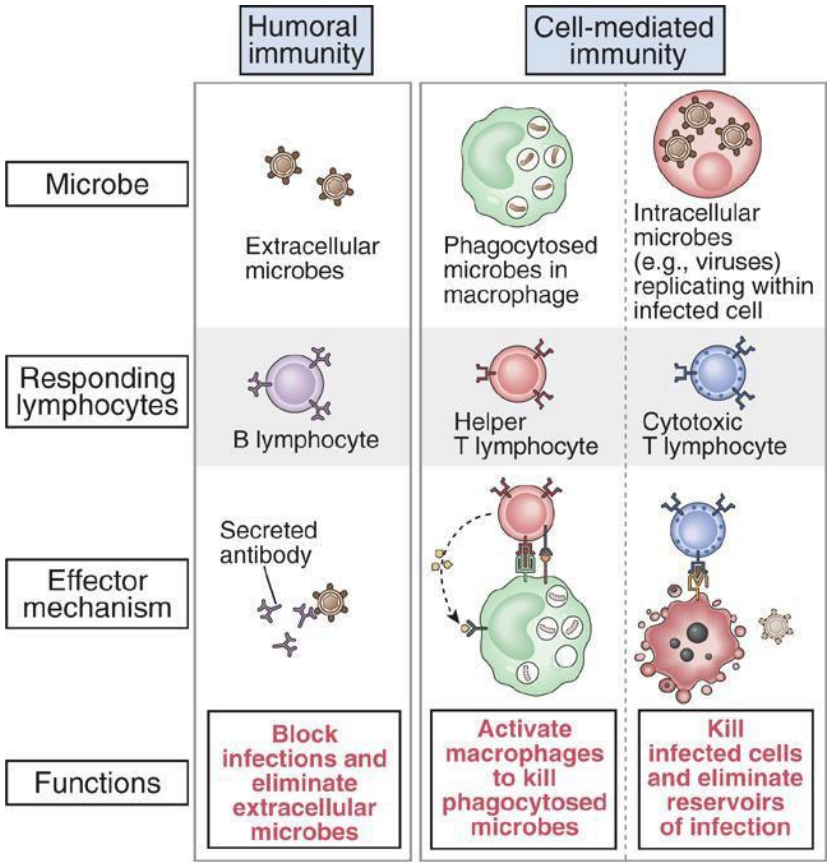


Figure 1 adaptive immune response.

Cellular adaptive response

A key to the adaptive immune response is the lymphocyte. There are several subtypes, however, these fall under two broad designations: **T lymphocytes** and **B lymphocytes** (commonly known as T cells and B cells).

Although both originate in the bone marrow, T cells mature in the thymus, whilst B cells mature in the bone marrow. During an organism's early development a large number of B- and T cells are produced, each of which has the ability to recognise a specific, and essentially unique, molecular target.

Adaptive immunity utilises many kinds of receptor to coordinate its activities. T cells carry **T-cell receptors (TCR)**, whilst B cells carry **B-cell receptors (BCR)**. In addition, another set of receptors, encoded by the major histocompatibility complex (MHC), play an important role in adaptive immunity. **MHC class I receptors** are displayed on a majority of body cells, whilst **MHC class II receptors** are restricted to antigen-presenting cells (APCs). Both of these receptor types interact with TCRs.

T cells do not recognize microorganisms in the extracellular fluids. Instead, T cell receptors bind to fragments of antigens (*epitopes*) that are presented on the surface of antigen presenting cells (APC).

There are three main types of APC:

- Macrophages
- Dendritic cells
- Naïve B cells

When T cells recognize an antigen presented by the APC, they can differentiate into several different types of T cells: (See Figure 2)

- ✓ **Cytotoxic T cells: (also called CD8+ T cells)**.

  Kill cells infected with intracellular pathogens such as viruses

  Helper T cells: (also called CD4+ T cells)

- Activate antigen and stimulate B cells to differentiate and produce antibodies .

- Activate macrophages to become more efficient at killing the pathogen

- Control intracellular bacterial infections (e.g. tuberculosis) that grow in intracellular membrane-bound vesicles of macrophages. The macrophages can't kill the bacteria but instead display the bacterial antigen on the surface so that it can be recognized by T cells

✓ **Regulatory T cells:**

Suppress lymphocytes and control the immune response

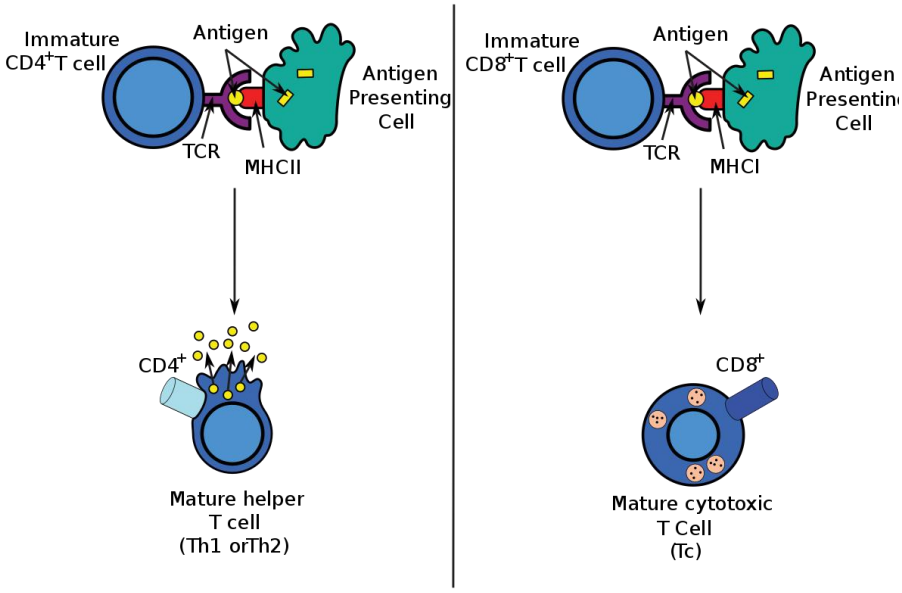


Figure 2 Antigen presentation stimulates T cells to become either "cytotoxic" CD8+ cells or "helper" CD4+ cells.

Humoral adaptive response

Humoral immunity is mediated by B cells. B cells react against foreign substances in the extracellular spaces of the body by producing and secreting

antibodies (Abs). These Abs are present in the biological fluids of the body (the humours); hence the term humoral immunity.

B cells display immunoglobulin molecules (antibodies) on their surface membranes, which act as receptors for the antigens. B cell antibody receptors can either bind to helper T cells that have interacted with an APC or bind to extracellular microorganisms such as bacteria.

Once an antigen binds to an antibody with the best "fit", the B cell differentiates into plasma cells or B memory cells.

- Plasma cells:

These cells operate as factories to manufacture the chosen antibody and then secrete those antibodies.

- B memory cells:

These cells mediate immunological memory. They respond rapidly on re-exposure to the antigen that originally induced them.

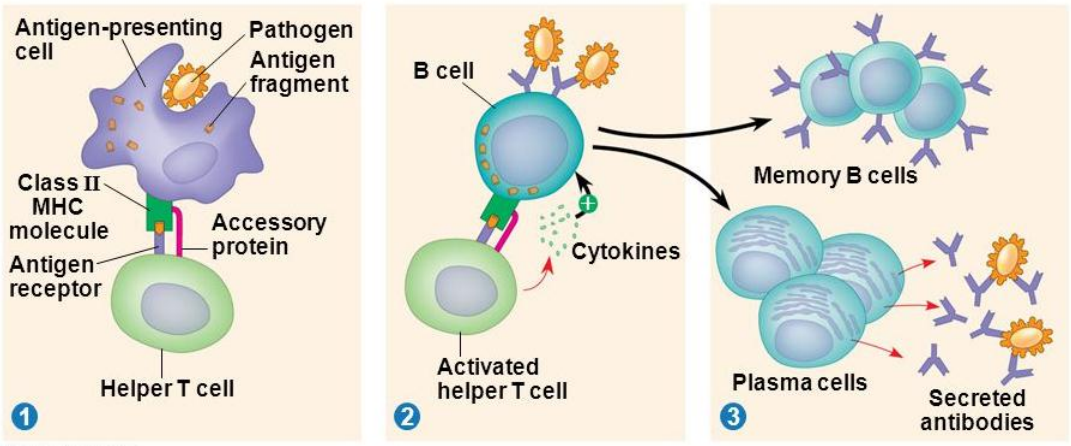


Figure 3 antigen presenting cells

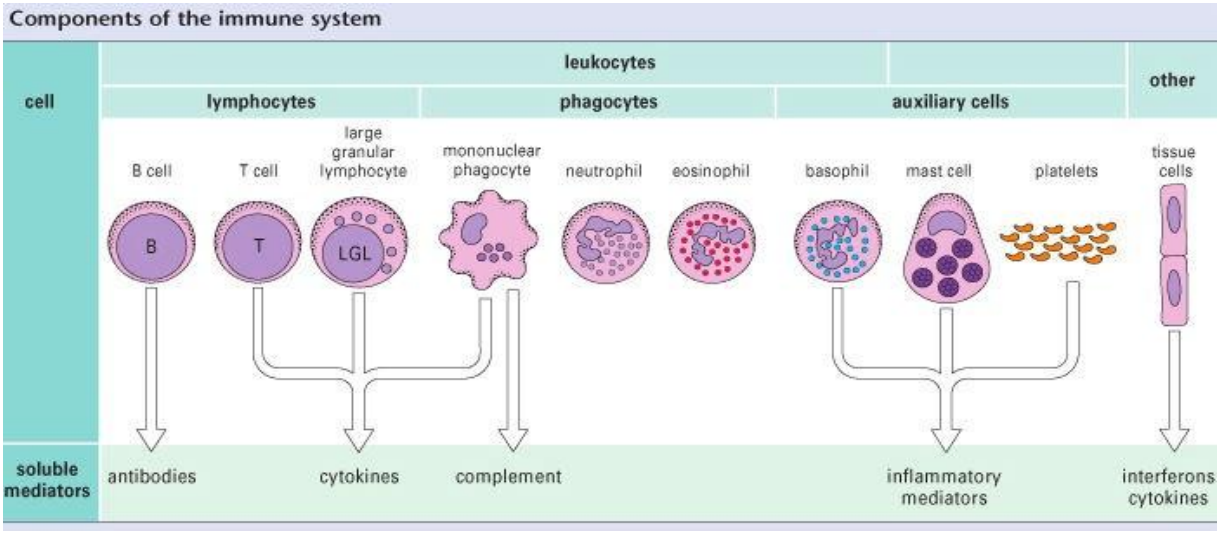
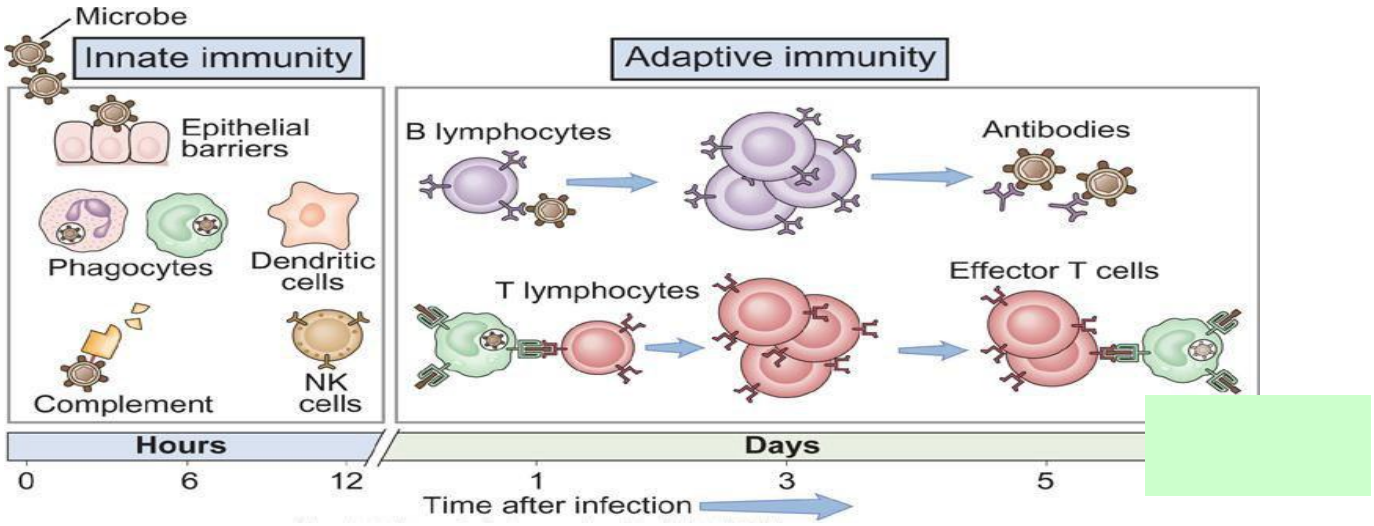


Figure 4 components of immune system .



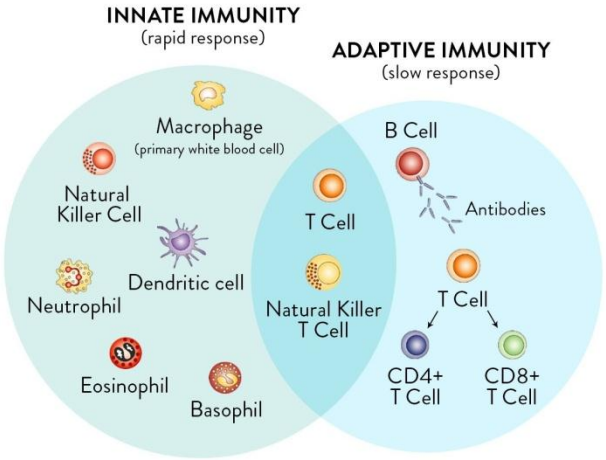


Figure 5 Innate and Adaptive immune system

The major histocomtpatibility complex (MHC)

The mammalian's immune system is capable of distinguishing self from non-self due to a group of protein markers (also called antigen here) known as the **major histocomtpatibility complex (MHC)**. In humans, they are called **human leukocyte antigen (HLA)**. The MHC in mice is called the Histocompatibility system 2 or just the H-2. Two organisms are said to be histocompatible if they can accept solid tissue transplants from each other and incompatible if they cannot.

"Histocompatibility" is a word that says pretty much what it means: "histo" means "tissue" and "compatibility" means "getting along." The *major histocompatibility complex* (MHC) genes were first identified in experiments investigating why the tissues and organs from one individual of a species were destroyed when introduced into another member of the same species.

The genes controlling the histocompatibility of tissue transplantation were localized to a large genetic region containing multiple loci; hence, the term "complex." The molecules encoded by these genes were found to have striking effects on histocompatibility, and to distinguish them from other molecules (encoded elsewhere in the genome) that had relatively minor effects on histocompatibility, these molecules were called the "major" histocompatibility molecules. Thus, the genes encoding these molecules were dubbed the "major histocompatibility complex." Because of the multiple loci present in the MHC, any one individual was found to express a Variety of different MHC molecules on his/her cells.

-The first descriptions of the MHC were made by British immunologist Peter Gorer in 1936. MHC genes were first identified in inbred mice

strains. Clarence Little transplanted tumors across differing strains and found rejection of transplanted tumors according to strains of host versus donor. George Snell selectively bred two mouse strains, attained a new strain nearly identical to one of the progenitor strains, but differing crucially in histocompatibility—that is, tissue compatibility upon transplantation—and thereupon identified an MHC locus. For this work, Snell was awarded the 1980 Nobel Prize in Physiology or Medicine, together with Baruj Benacerraf and Jean Dausset.

The Functions of MHC

MHC is the tissue-antigen that allows the immune system (more specifically T cells) to bind to, recognize, and tolerate itself (auto recognition). MHC is also the chaperone for intracellular peptides that are complexed with MHCs and presented to T cell receptors (TCRs) as potential foreign antigens. MHC interacts with the TCR and its co-receptors to optimize binding conditions for the TCR-antigen interaction, in terms of antigen binding affinity and specificity, and signal transduction effectiveness.

Essentially, the MHC-peptide complex is a complex of auto-antigen/allo-antigen. Upon binding, T cells should in principle tolerate the auto-antigen, but activate when exposed to the allo-antigen. Disease states occur when this principle is disrupted.

MHC role in antigen presentation

The events that occur inside a host cell after a protein antigen has entered it are summarized in (Figure 1) .In summary:

• The protein is broken down (catabolized or "processed") to peptides—linear fragments—of varying length.

• Some of these peptides bind to an MHC molecule inside the cell. This binding is selective; that is, not all the peptides formed bind to MHC molecules.

• The MHC molecule with bound peptide moves to the cell surface.

• The combination of peptide bound to an MHC molecule is recognized at the cell surface by a T cell that expresses the "appropriate" or "correct" TCR—one of the billions of different TCRs the host can generate.

Thus, MHC molecules have two key functions:

(1) to **selectively bind** to peptides produced when proteins are processed inside cells of the host

(2) to **present** peptides on the surface of a host cell to a T cell with the appropriate TCR.

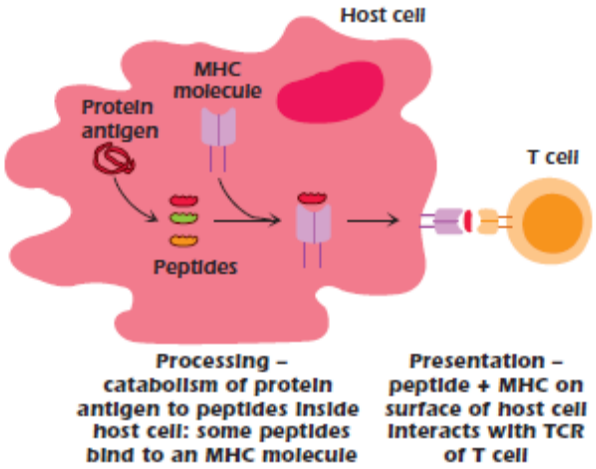


Figure 1 : The role of MHC in antigen presentation to T cells.

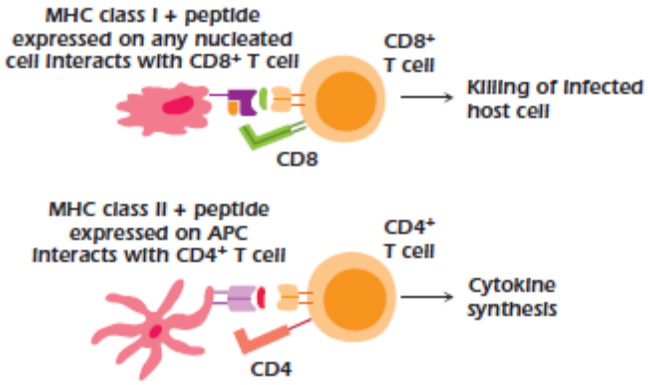


Figure 2 Cells expressing MHC class I interact with CD8+ T cells, which kill infected host cells; cells expressing MHC class II interact with CD4+ T cells, which synthesize cytokines.

MHC Class I

MHC class I molecules interact with CD8, whose expression defines the subset of T cells called **CD8+ T cells**. Thus, to expand on the definition of MHC restriction of T-cell responses we introduced earlier, we say that *the responses of CD8+ T cells are restricted by MHC class I molecules*. MHC class I molecules are expressed on all nucleated cells (thus, not on red blood cells), any of which may be infected by a pathogen such as a virus, bacterium, or parasite. The main function of CD8+ T cells is to kill athogeninfected host cells, as well as tumors and transplanted tissue. Thus, MHC class I molecules and CD8+ T cells play critical roles in the responses to pathogens that infect host cells. In addition to their interaction with CD8 expressed on CD8+ T cells, MHC class I molecules also interact with molecules expressed on natural killer (NK) cells. This interaction prevents NK cells from killing normal host cells.

Structure of MHC class I: (Figure 3)

- Class-I MHC gene encodes a glycoprotein molecule which expressed on the surface of all nucleated cells and platelets.
- MHC-I molecule contains a 45KDa α-chain associated non-covalentely with a 12KDa β2 microglobulin molecule.
- Association of α-chain and β2 microglobulin is required for expression of class-I MHC molecule on cell membrane.

α-chain of MHC-I:

- The α-chain is a transmembrane glycoprotein encoded by polymorphic gene within A, B and C region of Human HLA complex
- The α-chain is anchored in the plasma membrane by its hydrophobic trans-membrane segment and hydrophilic cytoplasmic tail.
- α-chain is made up of 3 domains (α1,α2 and α3). Each domain containing approximately 90 aminoacids, a transmsmbrane domain of about 25 hydrophobic aminoacids followed by short stretch of charged (hydrophilic) aminoacids of cytoplasmic tails of 3o aminoacids.
- α1 and α2 domains interacts to form a deep groove on the top which is a **peptide binding clift**. It can binds antigen of 8-10 animoacids long.
- α3 and β2 are organized into β-pleated sheets, each formed by antiparallel β-strand of aminoacids, this structure is known as immunoglobulin fold. Because of this structure α-chain and β2 microglobulin are classified as member of immunoglobulin super-family receptor.

β2 microglobulin of MHC-I:

- β2 microglobulin is a protein encoded by a highly conserved gene located on different chromosome
- β2 microglobulin is similar in size and organization to α3 domain.
- B2 microglobulin does not contain transmembrane region and is non-covalently linked with α-chain.

Functions of MHC class I:

- The Major function of MHC-I is to bind peptide antigens and present to CD8+ T cells (T helper cells)
- CD8 T cells are specific for MHC-I antigen
- MHC-I bind endogenous antigen and present to T helper cells.
- MHC-I molecules are found on the surface of all nucleated cells.

MHC Class II

MHC class II molecules interact with CD4, whose expression defines the subset of T cells called **CD4+ T cells**. *The responses of CD4+ T cells are restricted by MHC class II molecules*.

MHC class II molecules have a more limited distribution than MHC class I molecules: They are expressed ***constitutively*** (that is, under baseline conditions) only on ***antigen-presenting cells*** (APCs) but can be induced on other cell types. APCs are cells that take up antigen and present it to T cells. In humans, the principal APCs that express MHC class II are dendritic cells, macrophages, and B lymphocytes; thymic epithelial cells also express MHC class II molecules. In the absence of inducing factors, most cells (for

example, liver and kidney tissue cells) express MHC class I but not MHC class II molecules; by contrast, APCs constitutively expresses *both* MHC class I and class II molecules.

In response to activation, CD4+ T cells synthesize a vast array of cytokines, and hence cooperate with multiple types of cells, including helping B cells synthesize antibody. Thus, MHC class II molecules and CD4+ T cells play critical roles in the responses to agents—pathogens and antigens—that are taken into APCs.

Structure of MHC class II: (Figure 3)

- Class-II MHC is the glycoprotein molecule expressed primarily on antigen presenting cells such as macrophages, dendritic cells and B-cells.
- MHC-II molecules contains two different polypeptide chains, 1 33 KDa α-chain and 28KDa β-chain which are associated by non-covalent interactions.

α-chain and β-chain of MHC-II:

- α-chain and β-chain of MHC-II is a membrane bound glycoprotein that contains external domains, a transmembrane segment and acytoplasmic tail.
- α-chain and β-chain are made up of two domains (α1 and α2) and (β1 and β2) respectively.

- The peptide biding cleft is a open ended groove formed between α-chain and β-chain at proximal end. The cleft can bind antigenic peptide of 13-18 aminoacids long.

Functions of MHC class II:

- The Major function of MHC-II is to bind peptide antigen and present to CD4 T cells.
- MHC-II are found on the surface of Antigen presenting cells (APCs).
- CD4+T-cells are specific for MHC-II
- Activates B cells for antibody production
- MHC-II plays a significant role in graft versus host response and in mixed lymphocyte reaction (MLR) because the immune response gene is identical to MHC-II in human.

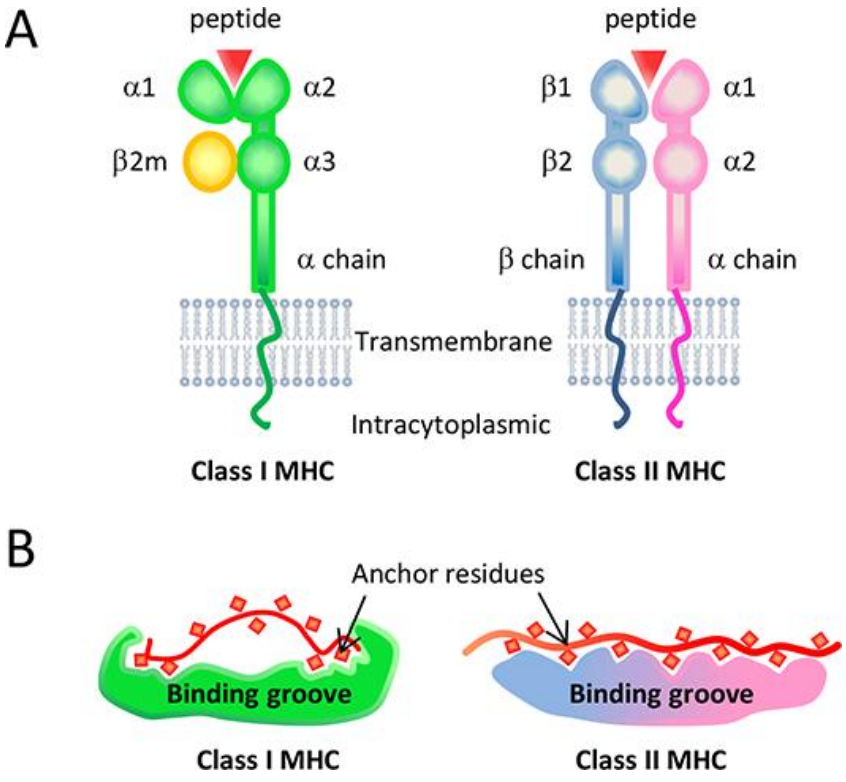


Figure 3 : Structure of MHC molecules and its binding sites.

MHC class III

- MHC III are a diverse group of molecules that serves a wide variety of functions in the immune system.
- MHC III are not a mark on the cell surface.

Functions of MHC class-III:

- Involved in complement activation
- Involved in inflammation caused by cytokines, tumor necrosis factors etc