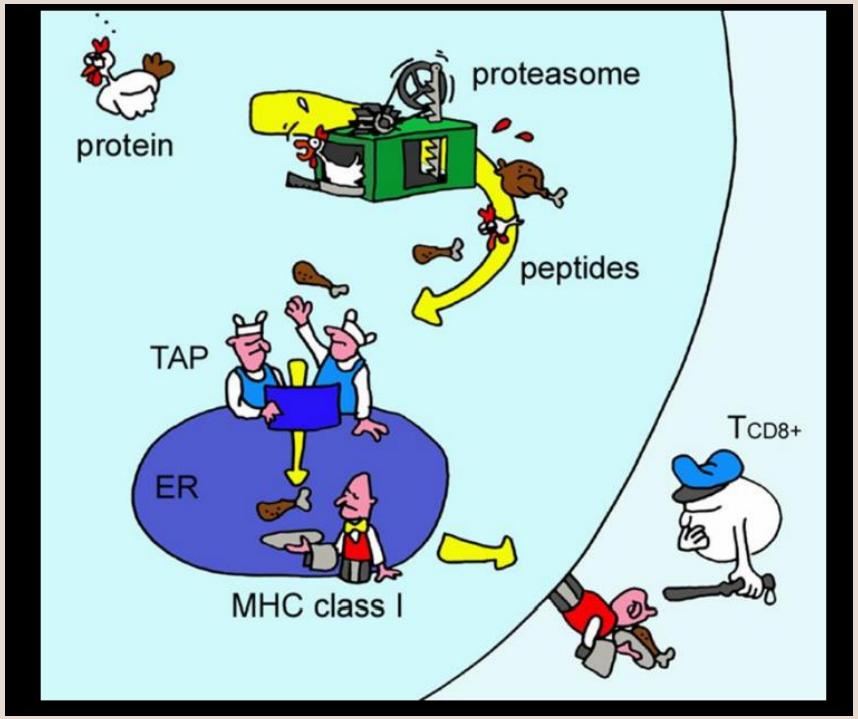
Lect.8 Antibody production

Contents

Lymphocytes Perform Adaptive Immune Functions

- The cells of the adaptive immune system, in contrast to those of the innate immune system, interact with the environmental agent in a highly discriminative way, i.e., they **display specificity, heterogeneity, and memory.**
- These functions are primarily carried out by two types of cells that are involved in the recognition of antigen:
  - (1) the thymus-dependent or T lymphocytes, which participate in cellular responses against intracellular pathogens, organ transplants, and malignant cells
  - (2) the bone marrow or bursal-dependent B lymphocytes, which provide humoral immunity, i.e., antibody-mediated immunity against extracellular pathogens, their toxins, and other environmental substances.
- third group of cells involved in the presentation of antigen to T cells, i.e., APCs, include dendritic cells, macrophages, and B cells
- APCs take up predominantly protein antigens, cut them into peptides, bind the peptides to major histocompatibility complex (MHC) molecules, and display these presented antigens on their cell surface, where they can be recognized and bound by antigen receptors on T lymphocytes.
- T lymphocytes are identified by a surface cluster of differentiation (CD) molecule named CD3 and are comprised of two major groups: the CD4 and CD8 populations



- T cells do not recognize intact antigen (the whole chicken)
- Interact only with antigen fragments — peptide (the drumstick)
- Peptides are only recognized when they are associated with self-MHC molecules (presented by a waiter)

Histocompatible: transplanted tissue is successfully accepted as self

• Histocompatibility antigens(مستضدات التوافق النسيجي) rejection of foreign tissue is the result of an immune response to cell-surface molecules

• Histocompatibility complex: a region of multiple loci that play major roles in determining whether transplant tissue is with histocompatibility or histo incompatibility

- Major vs minor – Major Histocompatibility Complex, MHC : rapid graft rejection –
- Minor Histocompatibility complex, mHC : slow graft rejection
- HLA: human leukocyte antigen, MHC antigens in human
- H-2: MHC antigen in mice T
- HLA or MHC gene/complex — DNA
- HLA or MHC antigen/molecule — protein

The MHC molecules are glycoproteins encoded in **a large cluster of genes located on chromosome 6.**
They were first identified by their potent effect on the immune response to transplanted tissue.
For that reason, the gene complex was termed the "**major histocompatibility complex."**

MHC genes (called the H-2 complex in mice) were first recognized in 1937 as a barrier to transplantation in mice.
In humans, these genes are often called human leukocyte antigens (HLA), as they were first discovered through antigenic differences between white blood cells from different individuals.
The HLA complex is located within the 6p21.3 region of the short arm of chromosome 6 and chromosome 17 in mice. In humans, it contains >240 genes of diverse functions.

MHC gene family is divided into three subgroups or classes, named I, II, and III. The classical loci, routinely studied in human medicine, are **HLA-A, HLA-B, and HLA-C for class I**, and **HLA-DRB1, HLA-DQB1, and HLA-DPB1 for class II**. HLA genes are closely linked to one another and are inherited en bloc as a genetic unit. The series of HLA alleles on a single chromosome 6 is called *haplotype*. Combination of maternal and paternal haplotypes inherited creates the individual's HLA genotype.

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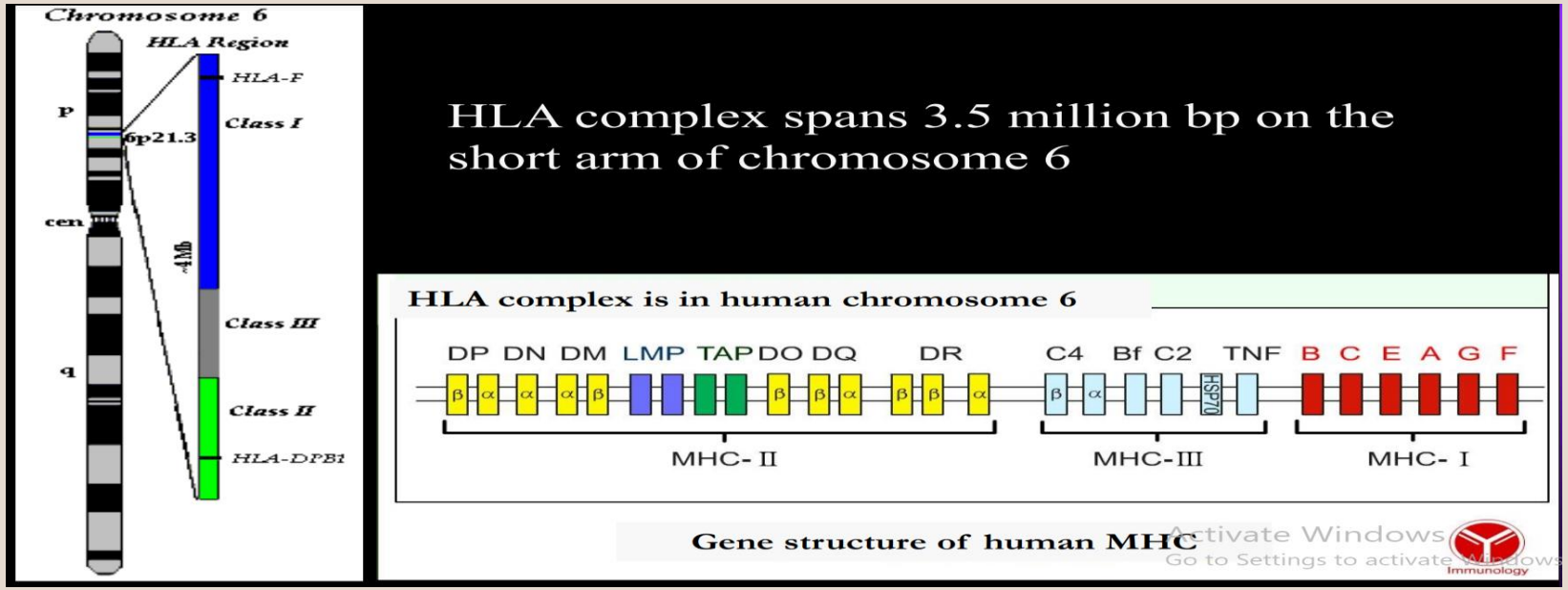


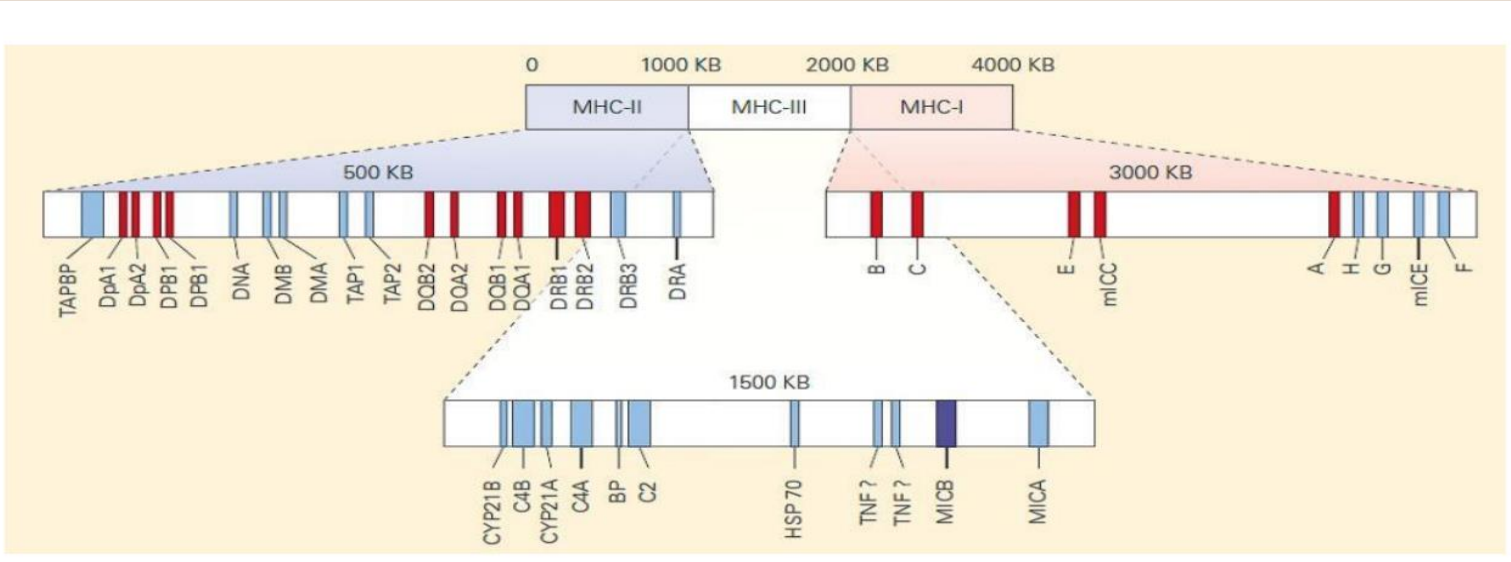


Figure 1. Genetic map of the MHC regions. This map has been simplified to demonstrate organizational themes within the MHC.

- The principal function of the MHC is to present antigen to T cells to discriminate between self (our cells and tissues) and nonself (the invaders or modified self).
- Two main characteristics of the MHC make it difficult for pathogens to evade immune responses:
- First, the MHC is polygenic. It contains several different MHC-I and MHC-II genes so that every individual possesses a set of MHC molecules with different ranges of peptide-binding specificities.
- Second, the MHC is extremely polymorphic. The MHC genes display the greatest degree of polymorphism in the human genome.
- Polymorphic sites are found predominantly in specific regions of the MHC-I and MHC-II molecules called domains. Although each HLA molecule shows slight differences in its amino acid sequence from one another, causing a slightly altered three-dimensional structure in the peptide-binding cleft, the basic structures of MHC-I and MHC-II molecules are very similar

MHC-I molecules

Class I molecules consist of two linked glycoprotein chains. An α chain (45 kDa) is associated with a much smaller chain called β2 - microglobulin (β2M) (12 kDa) . The α chain is inserted in the cell membrane. It consists of five domains: three extracellular domains called α1 , α2 , and α3 , each about 100 amino acids long; a transmembrane domain; and a cytoplasmic domain. The antigen binding site is formed by the α1 and α2 domains. The β2M chain consists of a single domain and stabilizes the structure. The folding of the α1 and α 2 domains creates a long cleft or groove that is the site at which peptide antigens bind to the MHC-I molecule and are presented to the CD8 lymphocyte.

MHC-II molecules

MHC class II molecules consist of two chains called α and β. Each chain has two extracellular domains (one constant and one variable), a connecting peptide, a transmembrane domain, and a cytoplasmic domain. A third invariant chain, called the Ii or γ chain, is associated with the assembly of class II molecules within cells . MHC-II molecules are presented to the CD4 lymphocyte

MHC Class III Molecules The remaining genes within the MHComplex are located within the class III region They code for proteins with many diverse functions. Some are important in the defense of the body such as the genes for the complement components C4, FB, and C2. They also include genes that encode tumor necrosis factor-α (TNF-α), several lymphotoxins, and some NK cell receptors.

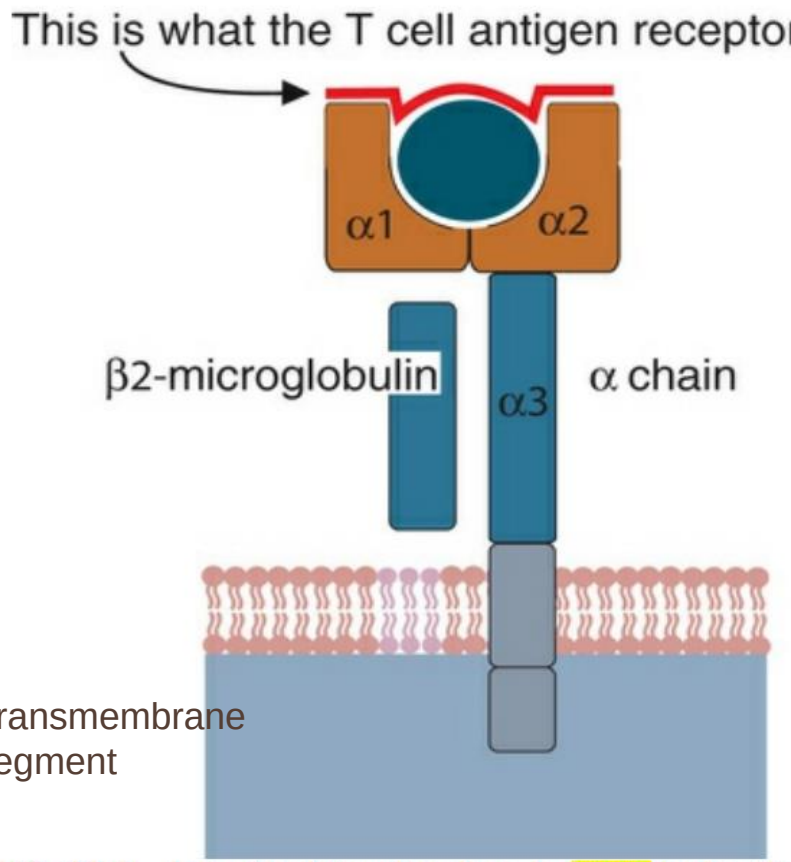


FIG. 11.3 The structure of a class Ia MHC molecule on a cell membrane. Its antigen-binding site is formed by the folding of both its α_1 and α_2 domains.

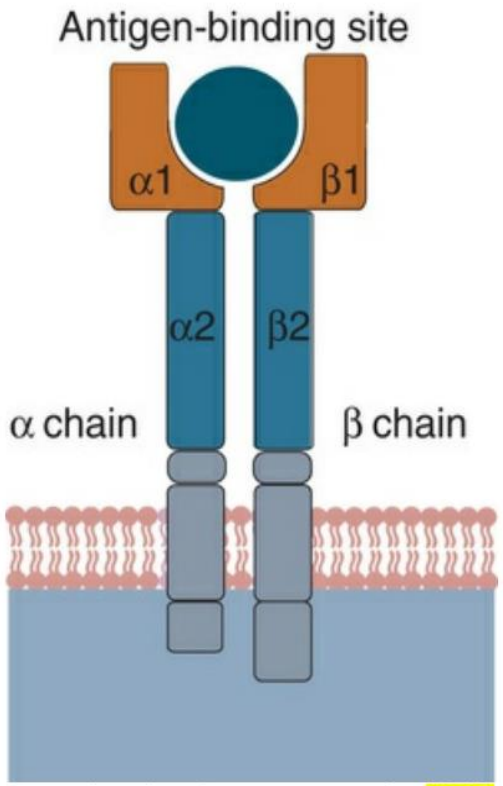


FIG. 11.6 Diagram showing the structure of a MHC class II antigen located on a cell surface. Note that the antigen-binding site is formed by the variable domains from both peptide chains.

Features of MHC-I and MHC-II molecules

- The level of MHC molecule expression plays an important role in T cell activation and therefore differences in levels of expression are significant.
- Expression of MHC Molecules MHC-I MHC- I proteins are expressed on all nucleated cells, in contrast to MHC-II molecules, which are restricted to antigen-presenting cells (APCs) Lymphocytes, macrophages, dendritic cells, Langherans cells, and some endothelial cells are the predominant cells that express MHC-II.
- Nonnucleated cells such as mammalian red blood cells express little or no MHC-I and thus, pathogens within red blood cells can go undetected by cytotoxic T cells, e.g., malaria

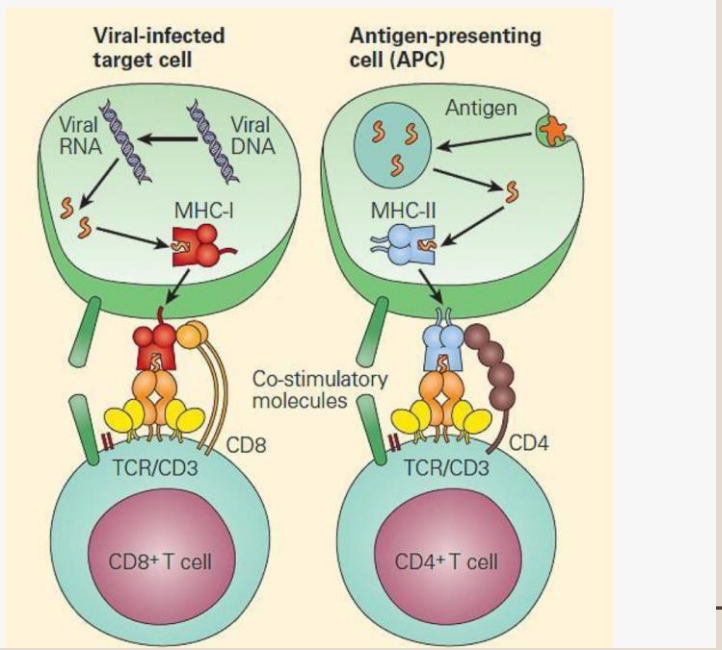
Feature	MHC-I	MHC-II
Polypeptide chains	A single a chain (44–47 kD) noncova-lently linked to the b2-microglobulin chain (12 kD)	A single a chain (32–34 kD) non-covalently linked to a single b chain (29–32 kD)
Distribution	All nucleated cells	Antigen-presenting cells
Composition of antigen-binding clefts	α1 and α12 domains	α1 and β1 domains
Binding site for T cell co-receptor	CD8 binds to the α3 region	CD4 binds to the β2 region
Size of peptide-binding cleft	Accommodates peptides of 8–11 residues	Accommodates peptides of 13–25 residues or more
Nomenclature in the human	HLA-A, HLA-B, HLA-C	HLA-DR, HLA-DQ, HLA-DP

•T cells recognize foreign antigens in the form of short peptides that have been processed and dis-played on the cell surface bound to MHC-I or MHC-II molecules

•Antigens are often categorized according to whether they are derived from (1) viruses, intracellular bacteria, or protozoan parasites (endogenous pathogens); or (2) exogenous pathogens that replicate outside of the cell.

•Intracellular antigens are presented to T cells by any nucleated cell because MHC-I expression is ubiquitous.

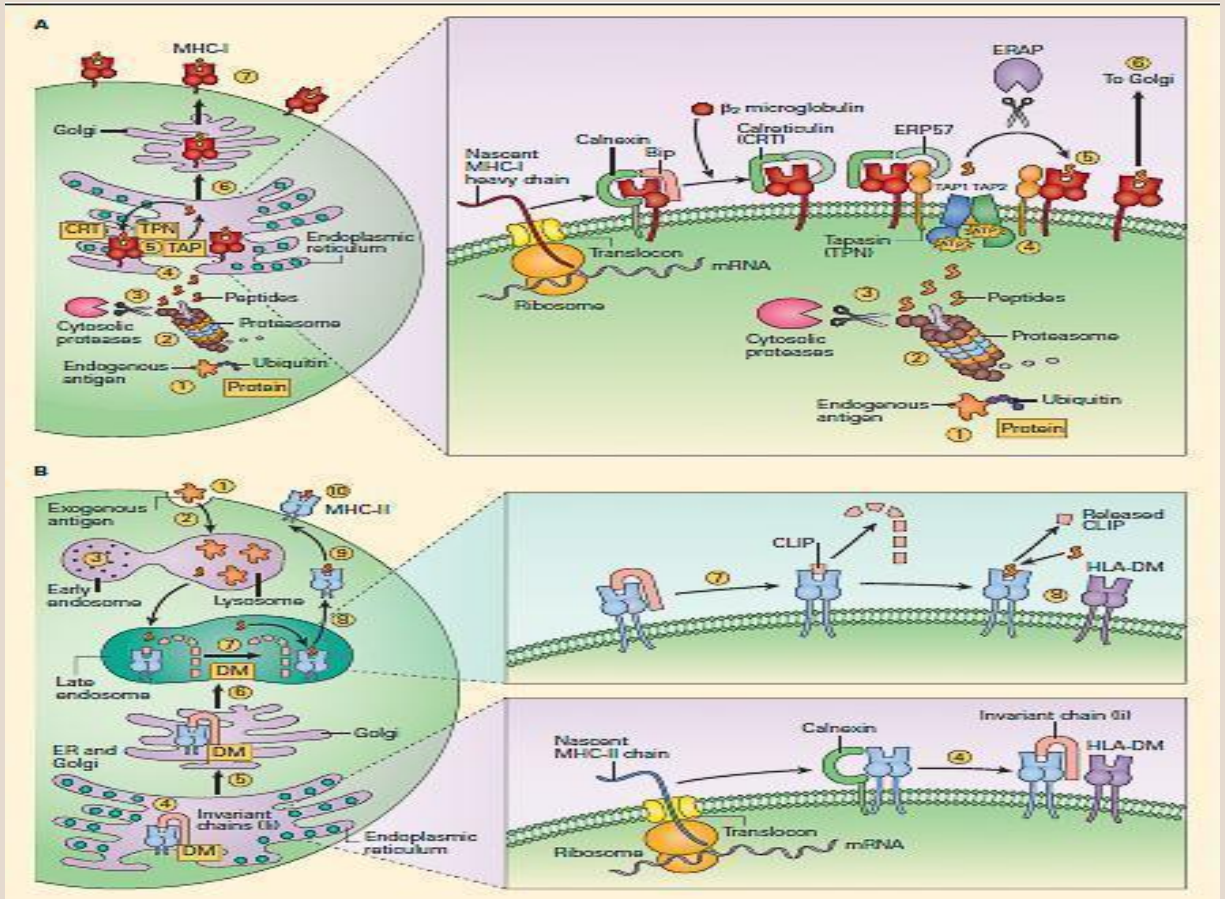
•In contrast, exogenous antigens are taken up by professional APCs, which process the antigens and present them in the context of MHC-II. An important function of a professional APC, e.g., dendritic cell (DC), is to deliver a second signal (costimulation) to the T cell to alert it to the presence of infection.

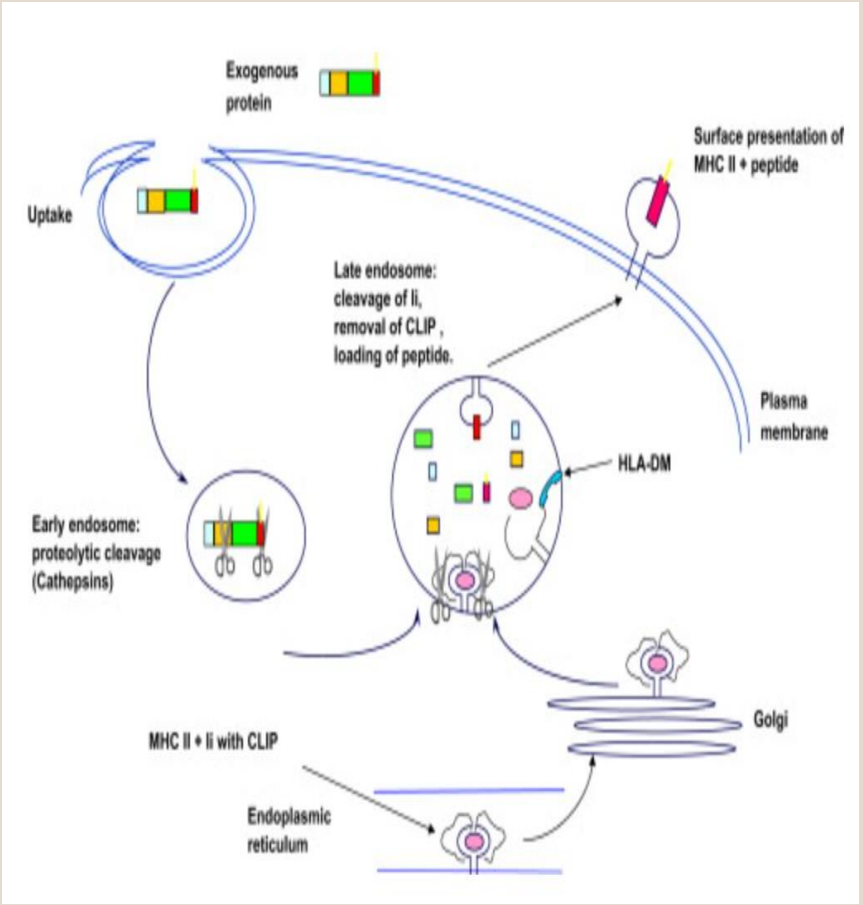


•**Endogenous antigens,** including misfolded proteins and pathogen-derived peptides, are processed by the proteasome synthesis and peptide loading of MHC-I through the endogenous pathway as bellow is presented:

1. Endogenous proteins (e.g., a self protein or a viral protein) synthesized in the cytoplasm are modified initially by ubiquitin helps to regulate the processes of other proteins. following which they are processed by the proteasomes
2. In the proteasomes, complex of proteases typically generates peptides of four to twenty amino acids with a hydrophobic carboxy terminus. After trimming of the peptide by cytosolic proteases, the antigenic peptides are translocated to the endoplasmic reticulum by the transporters associated with antigen processing (TAP1 and TAP2 molecule). **The TAP1 protein assembles with another protein called TAP2 to form a protein complex called transporter associated with antigen processing (TAP) complex**
3. **The MHC-I alpha chain,** which is initially formed as a linear peptide in the ER, is then folded with the help of several chaperones (calnexin, calreticulin [CRT]). (**promote the folding and oligomeric assembly of the majority of newly synthesized glycoproteins in the endoplasmatic reticulum of eukaryotic cells).**
4. Binding immunoglobulin protein (BiP) and endoplasmic reticulum protein 57 (ERP57), during which the b2 microglobulin is added to the alpha chain, complete the synthesis of the complete MHC-I molecule.
5. The complex is held together by tapasin (TPN), which facilitates transfer of the peptide to the antigen-binding cleft.
6. The peptide-loaded MHC-I complex is then transferred to the Golgi and then transported to the surface of the cell .

1. Exogenous proteins are taken up and processed in the early endosomal compartment and cleaved into peptides by cathepsins and other acid proteases
2. MHC-II molecules are formed in the endoplasmic reticulum with the help of the chaperone calnexin (4) **and are held ready by the invariant chain (Ii);** the complex is later fused with the HLA-DM (DM)
3. After passage of the Ii-loaded MHC-II-DM complex through the Golgi into the late endosomes
4. the invariant chain is cleaved by acid proteases, leaving a residual peptide referred to as the class II-associated invariant chain peptide (CLIP) in the MHC-II cleft .
5. The MHC molecule loaded with peptide is transported and expressed on the cell surface (10


A
MHC-I
Golgi
CRT
TPN
TAP
Endoplasmic reticulum
Peptides
Proteasome
Cytosolic proteases
Endogenous antigen
Ubiquitin
Protein
ERAP
To Golgi
β2 microglobulin
Calnexin
Calreticulin (CRT)
ERP57
Nascent MHC-I heavy chain
BiP
TAP1 TAP2
Tapasin (TPN)
Translocon
mRNA
Ribosome
B
Exogenous antigen
MHC-II
Early endosome
Lysosome
Late endosome
DM
Golgi
ER and Golgi
Invariant chains (Ii)
Endoplasmic reticulum
CLIP
Released CLIP
HLA-DM
Nascent MHC-II chain
Invariant chain (Ii)


Exogenous protein
Surface presentation of MHC II + peptide
Uptake
Late endosome: cleavage of Ii, removal of CLIP , loading of peptide.
Plasma membrane
HLA-DM
Early endosome: proteolytic cleavage (Cathepsins)
Golgi
MHC II + Ii with CLIP
Endoplasmic reticulum

Cellular events in antibody production

Antibodies are produced by plasma cells which are derived from B-lymphocytes (B cells). B-cells carrying a matching antigen receptor on their surface are selected by antigen and undergo clonal expansion and differentiation into plasma cells which secrete antibody of the same specificity. Under the influence of helper T-cells of matching antigenic specificity, clonal expansion also results in the production of memory B cells.

 Initial exposure to antigen results in a primary response. Subsequent exposure results in a secondary response which is controlled by T-cell factors. Secondary antibody responses differ from primary responses in their time course, height of response, and class and affinity of antibody produced.

 Most antigens are T-dependent and require T-cell help to the B-cell for clonal expansion and antibody production. Antigen presenting cells are also required to allow the T-cell to recognize antigen. Some T-independent antigens do occur but produce only primary responses

Immature B cells are produced in the bone marrow of most mammals.

Their development occurs through several stages, each representing a change in the genome content at the antibody loci.

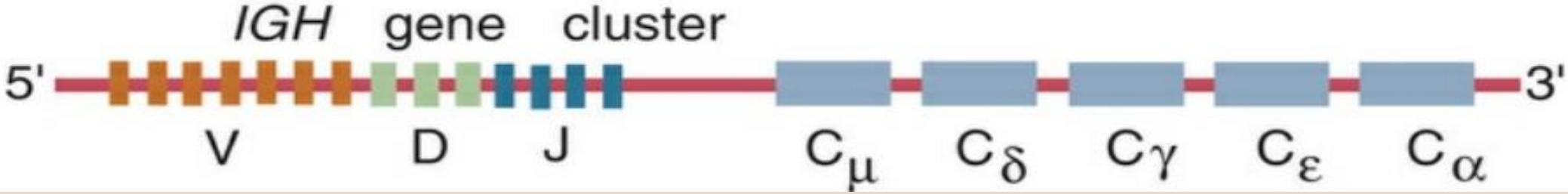
An antibody is composed of two identical light (L) and two identical heavy (H) chains, and the genes specifying them are found in the V (variable) region and C (constant) region.

The heavy-chain V region has three segments, V, D and J. These segments recombine randomly in a process called VDJ recombination to produce a unique variable domain in the immunoglobulin of each individual B cell.

V(D)J recombination

V(D)J recombination is a site-specific recombination process that occurs early in the development of B and T lymphocytes.

it is required for assembling complete antigen receptor genes from separately encoded germ-line variable (V), diversity (D), and joining (J) segments. Completion of this process is of fundamental importance in generating a diverse repertoire of immunoglobulins and T-cell receptors



B Cell Activation and Plasma Cell Differentiation

B Cell Activation

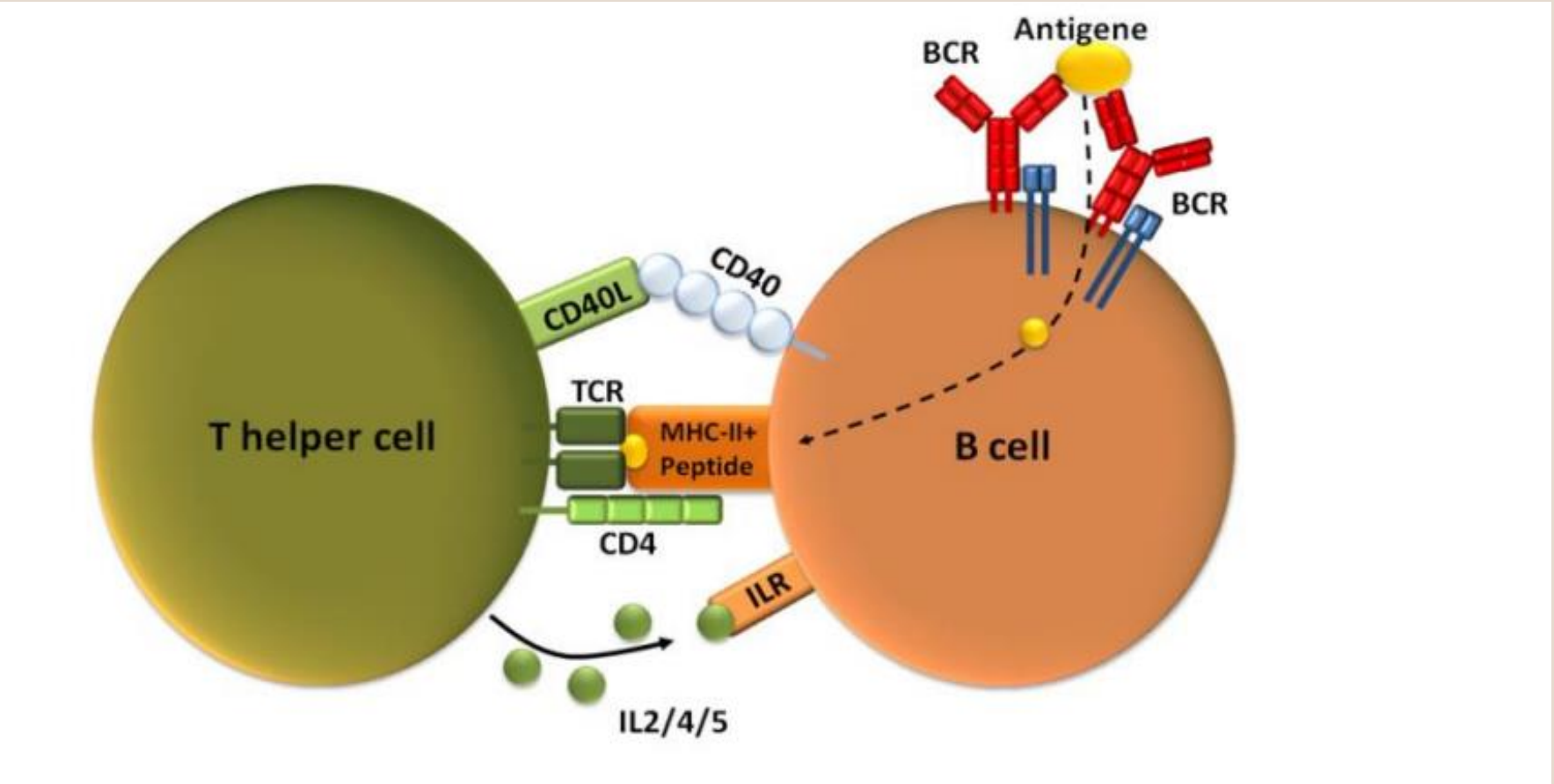
•Resting B cells become activated by antigen via the BCR and/or by microbiological side products (pathogen associated molecular patterns; PAMP) via Pattern recognition receptors such as toll like receptors (TLRs) and start to proliferate.

•Protein antigens become internalized, digested and presented to T cells as peptides via MHCII.

•Associated B cell / T cell interaction provides co-stimulation to B cells via CD40, which becomes activated on B cells via CD40 ligand (CD40L) expressed on T cells.

•T cells also provide cytokines to B cells that support their survival (IL-4), differentiation into plasma cells (IL-21) or class switch recombination.

When helper T cells “help” B cells they promote several different B cell activities. These signals result in increased expression of IgM BCR and MHC class II, as well as receptors for IL-4, IL-5, IL-6, tumor necrosis factor-α (TNF-α), and transforming growth factor-β (TGF-β), and start the process that leads to B cell division and differentiation into antibody-secreting cells. They stimulate B cell proliferation and survival through CD40L, IL-21, and IL-4. The “help” also triggers somatic mutation within germinal centers and thus changes antibody binding affinity

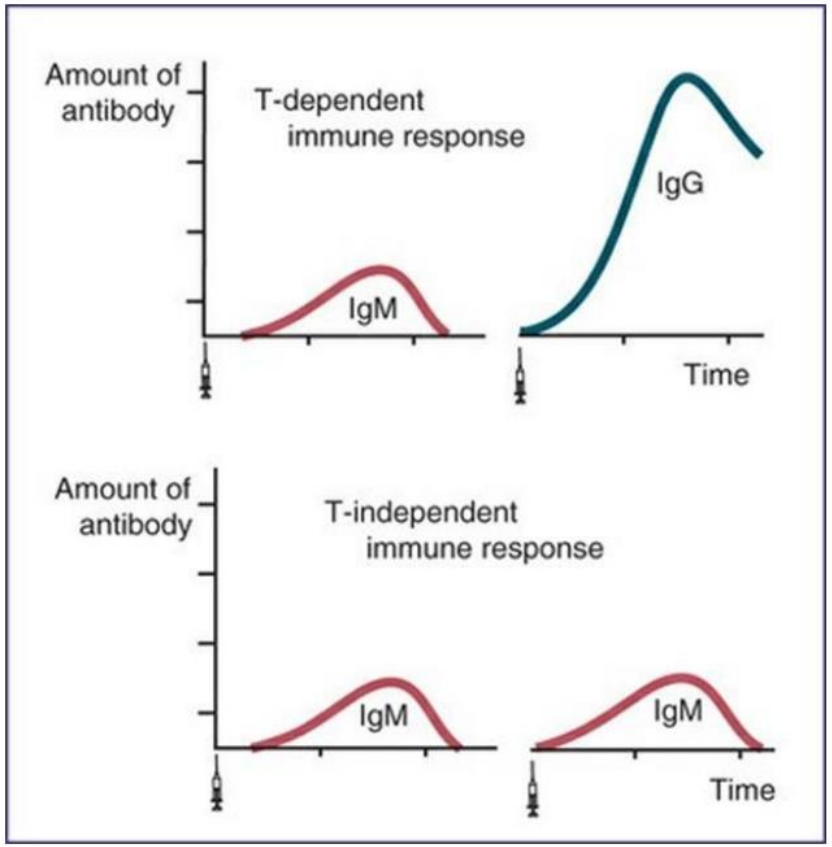

Antigene
BCR
BCR
CD40L
CD40
TCR
T helper cell
MHC-II+
Peptide
B cell
CD4
ILR
IL2/4/5

Activation of B cells via the BCR and/or PAMPs elicits proliferation of B cells.

- **T-independent**

Certain antigens can induce antibody formation in the absence of helper T cells.
These so-called T-independent antigens are usually simple repeating polymers such as Escherichia coli lipopolysaccharide, polymerized salmonella flagellin, and pneumococcal polysaccharide.
T-independent antigens bind directly to B cell TLRs and cross-link several BCRs, providing a sufficient signal for B cell proliferation.
Characteristically, T-independent antigens only trigger IgM responses and fail to generate memory cells.
Antibodies raised against the intact molecule will bind only to the intact molecule and may be unable to bind proteolytic fragments such as those produced by macrophage processing. T-independent antigens give rise only to primary type responses because they lack T-cell help. T independent antigens cannot induce an immunoglobulin switch or immunological memory, as demonstrated by a secondary antibody response.
T-dependent

Both specific B and Th lymphocytes are required before antibody can be produced. In addition, since T-cells can only recognise antigen on cell surfaces, initial stimulation of the T-cell may require the presence of an additional antigen presenting cell (APC), usually a macrophage or other cell of the mononuclear phagocyte system. Initial antigenic stimulation of the T-cell will also IgG IgM. In primary response T-cell clonal expansion with production of both effector and memory T-cells. In the secondary response there are increased numbers of both lymphocyte populations.


Amount of antibody
T-dependent immune response
IgG
IgM
Time
Amount of antibody
T-independent immune response
IgM
IgM
Time

The plasma cell and antibody secretion

- Each B cell produces a single species of antibody, each with a unique antigen-binding site.
- When a naïve or memory B cell is activated by antigen (with the aid of a helper T cell), it proliferates and differentiates into an antibody-secreting effector cell.
- effector cells make and secrete large amounts of soluble (rather than membrane-bound) antibody, which has the same unique antigen-binding site as the cell-surface antibody that served earlier as the antigen receptor.
- Effector B cells can begin secreting antibody while they are still small lymphocytes, but the end stage of their maturation pathway is a large *plasma cell* , which continuously secretes antibodies at the astonishing rate of about 2000 molecules per second.
- Plasma cells seem to have committed so much of their protein-synthesizing machinery to making antibody that they are incapable of further growth and division.
- Although many die after several days, some survive in the bone marrow for months or years and continue to secrete antibodies into the blood.