Chapter 4: Specific Immunity

Immunoglobulin Domains

The surface receptors of B and T cells are part of the immunoglobulin superfamily. These receptors are proteins composed of structural motifs known as immunoglobulin (Ig) domains. All molecules of the immunoglobulin superfamily extend from the surface of cells. They are flexible and include specialized domains; the antigen-binding site on B lymphocyte receptors is an example.

Members of this family include:
• Immunoglobulin (B cell receptor, BCR)
• T cell receptor (TCR)
• Major Histocompatibility Complex (MHC) molecules

Each domain is approximately 110 amino acids in length. The polypeptide chain of each domain folds into seven or eight antiparallel β-strands. These strands are arranged to form two opposing sheets, connected by a disulfide bond and stabilized by hydrophobic interactions. This compact structure is called the **immunoglobulin fold**.

Structure of BCR and TCR

The surface receptor of B cells is a membrane-bound immunoglobulin molecule (mIg). The BCR recognizes the conformational structure (shape) of antigenic epitopes. It is composed of two light chains and two heavy chains (Figure 1). The BCR associates with two Ig-α/Ig-β heterodimers (members of the immunoglobulin superfamily), which are responsible for transmitting the signal received by the mIg into the cell.

Immunoglobulins are also secreted by plasma cells. The extracellular portion of the BCR has a structure identical to that of secreted immunoglobulins (sIg). Membrane-bound immunoglobulins (mIg) differ from secreted immunoglobulins (sIg) in that they have transmembrane and cytoplasmic regions that anchor them to the membrane.

Different classes of immunoglobulins can be expressed on the same B cell and may indicate the developmental stage of the B cell. For example, a mature but naïve B cell expresses both mIgM and mIgD. The antigen specificity of all mIg molecules expressed on any given B cell is the same.

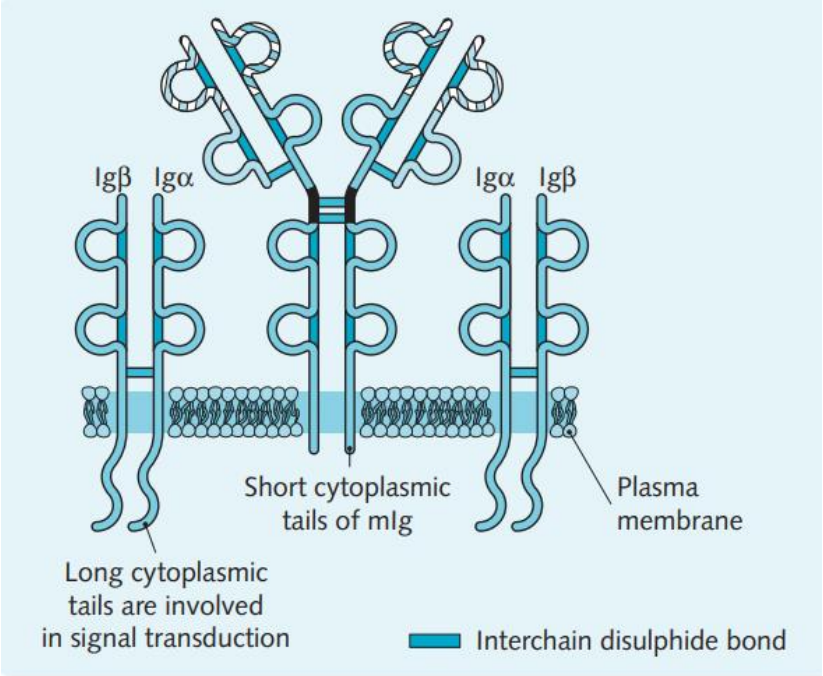

Figure 1: Molecular structure of B lymphocyte membrane receptor

The recognition of antigens by T cells differs from antigen recognition by B lymphocytes. T lymphocytes recognize peptide fragments of an antigen presented in association with Major Histocompatibility Complex (MHC) molecules. These antigen fragments are processed (prepared) by antigen-presenting cells (APCs) before being presented to the T cell.

The antigen receptor on the surface of T lymphocytes consists of the T cell receptor (TCR) associated with CD3. The TCR is a heterodimer composed of either α and β chains or γ and δ chains. Approximately 95% of T lymphocytes express αβ TCRs.

The TCR is structurally similar to the Fab region of immunoglobulins. Each TCR chain contains two immunoglobulin-like domains, one variable and one constant, connected by a disulfide bridge. As in the variable domains of immunoglobulins, three highly variable regions on each chain combine to form the antigen-binding site (Figure).

CD3 is composed of three polypeptide dimers, made up of four or five different peptide chains. The dimers are γε, δε, and ζζ (present in 90% of CD3 molecules) or ζη. The γ, δ, and ε chains are members of the immunoglobulin superfamily.

The TCR recognizes and binds the antigen, while CD3 is functionally similar to the Ig-α/Ig-β heterodimer in B cells and is involved in signal transduction (Figure2).

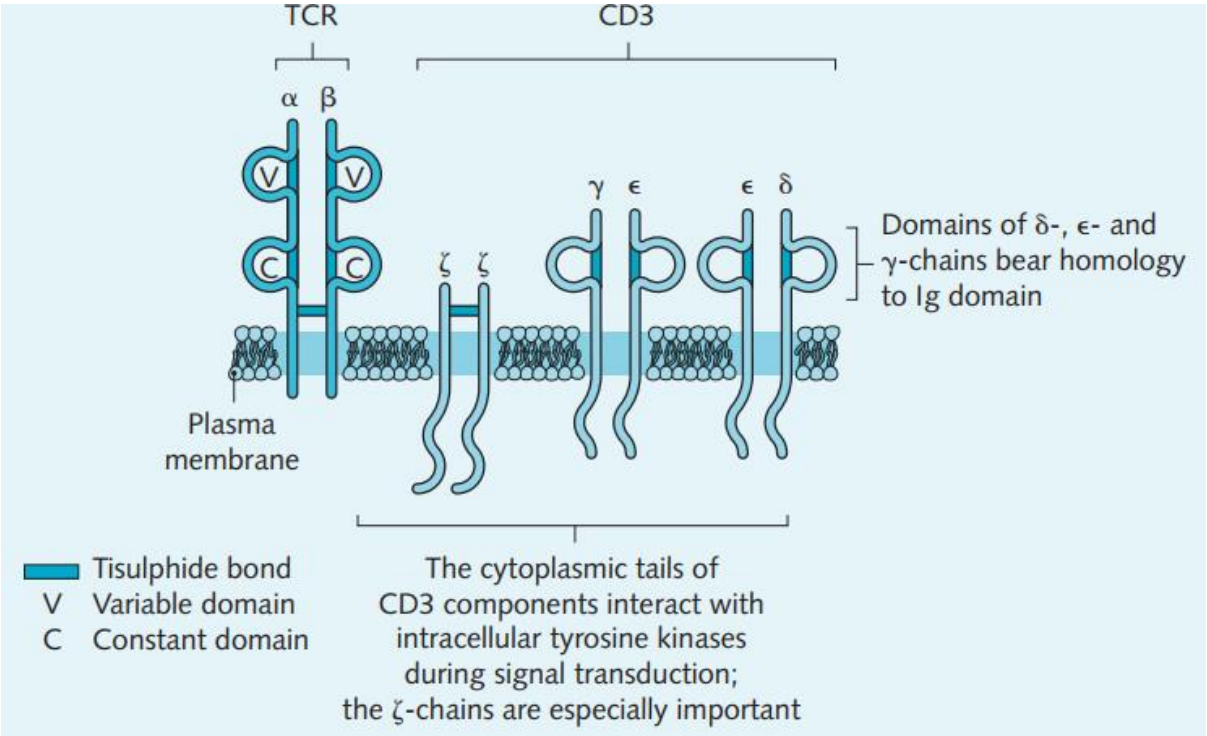

Figure 2: Molecular structure of T lymphocyte membrane receptor (TCR)

Structure and Function of MHC
Genetic Diversity

MHC genes exhibit a high degree of polymorphism, meaning they show considerable diversity (more than 100 alleles have been identified). As a result, most individuals are heterozygous at most MHC loci, and two randomly selected individuals are highly unlikely to share identical HLA alleles.

MHC diversity increases the likelihood that an individual will be able to mount an adaptive immune response against a pathogen. The genetic loci are closely linked, so a complete set of MHC genes is inherited from each parent in a "block transmission" manner.

The MHC genes are organized into three regions, each encoding one of the three classes of MHC molecules: Class I, Class II, and Class III. MHC alleles are codominantly expressed, meaning that both alleles inherited from the parents are expressed.

MHC Molecules

MHC molecules are membrane glycoproteins whose extracellular part consists of a heterodimer displaying two proximal domains (close to the cell membrane) folded according to the "immunoglobulin domain" structure, and two distal domains with a unique (variable) structure. Each distal domain features a platform of β-pleated sheets topped by an α-helix.

The pairing of the two distal domains creates a central groove where a peptide can be embedded: this is known as the peptide-binding groove. The binding operates according to the "lock-and-key" model, requiring sufficient complementarity between the physical and chemical properties of the groove and those of the peptide.

The groove is designed to hold a peptide, with two to four of its amino acids fitting into "anchor pockets" located at the base of the groove. This is only possible if these anchor amino acids have appropriate physico-chemical characteristics.

The peptide is held in place by non-covalent bonds distributed along the entire groove, which also serves to stabilize the HLA molecule.

Table 1: Comparison between MHCI and MHCII molecules

	MHC Class I	MHC Class II
Expression	All nucleated cells	Professional antigen presenting cells (APCs)
Peptide Source	Intracellular peptide antigens	Extracellular engulphed peptide antigens
Peptide Length	8-10 amino acids	13-25 amino acids
Presentation Cell Type	Cytotoxic T cells	Helper T cells
Function	Activation of cytotoxic T cells to kill infected or cancerous cells	Activation of T helper cells to regulate the immune response

Class I MHC

Each Class I MHC molecule contains an α chain, anchored in the membrane, which has three extracellular domains. Its proximal domain, α3, associates non-covalently with β2-microglobulin, an invariant protein that forms an immunoglobulin-like domain and is encoded by a gene located outside the HLA gene complex.

The distal domains, α1 and α2, together form the peptide-binding groove. The ends of the α-helices in these domains are positioned close together, which closes the groove. As a result, the embedded peptide must be relatively small, typically about 9 amino acids in length, because both of its ends are trapped within the groove (Figure 3).

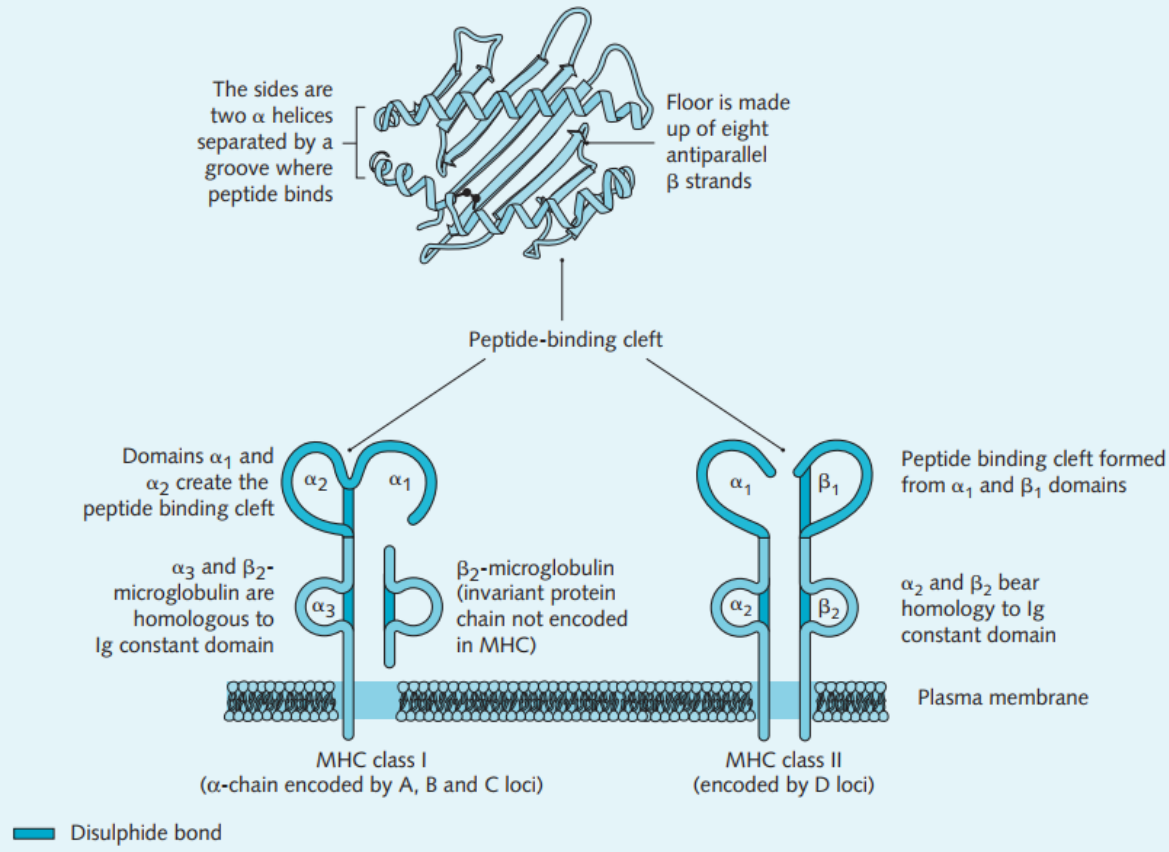

Figure 3: Molecular structure of Classe I and classe II MHC molecules

Class II MHC

Each Class II MHC molecule contains two chains, an α chain and a β chain, both anchored in the membrane, and each with two extracellular domains. The pairing of the distal domains, α1 and β1, forms the peptide-binding groove.

In contrast to Class I MHC, the ends of the α-helices in Class II MHC are less closely positioned, resulting in an open-ended groove. Therefore, the peptide can extend beyond the groove, allowing it to be longer — typically between 12 and 25 amino acids.

Nevertheless, the central portion of the peptide must still meet anchoring constraints, similar to those required for Class I MHC binding (Figure 3).

MHC Restriction

T lymphocytes are only capable of recognizing an antigen in the context of self-MHC molecules (MHC restriction). CD8+ T lymphocytes recognize antigens only when presented by MHC Class I molecules (Class I MHC restriction).
CD4+ T lymphocytes recognize antigens exclusively in association with MHC Class II molecules (Class II MHC restriction).

Antigen Processing by MHC Class I

The surface expression of both Class I and Class II MHC molecules depends on the embedding of a peptide; there are virtually no "empty" MHC molecules at the cell surface.

MHC Class I molecules present cytosolic (intracellular/endogenous) antigens. These antigens are proteins synthesized within the cytosol, including self-proteins that are at the end of their life cycle or defective (such as proteins that fail to fold properly or synthesis errors).

The cell processes both proteins encoded by its own genome and proteins present in the cytosol due to malignant transformation or viral infection in the same way.

Three key steps are involved in antigen processing for Class I MHC presentation (Figure 4):

- **Fragmentation**: Proteins targeted for elimination are first "tagged" by the attachment of ubiquitin and then degraded by the proteasome, which releases peptides of variable lengths.
- **Translocation**: The peptides generated by the proteasome are transported into the endoplasmic reticulum (ER) by the TAP transporter (Transporter Associated with Antigen Processing).
- **Loading**: One of the peptides entering the ER can be embedded into the peptide-binding groove of a newly forming Class I MHC molecule. This stabilizes the MHC molecule and allows its transport to the cell surface.

Pro-inflammatory cytokines, especially Interferon-γ (IFN-γ), enhance the efficiency of this processing pathway. IFN-γ induces the formation of the immunoproteasome, which generates peptides with better-fitting C-terminal ends for Class I MHC binding.

Thus, the collection of peptides displayed by Class I MHC molecules on the cell surface provides a nearly real-time sampling of endogenous protein synthesis, including both normal "self" proteins and abnormal "non-self" elements.

This specificity helps prevent collateral damage during adaptive immune responses: a cell with abnormal protein synthesis will be correctly targeted by CD8+ T lymphocytes specific for its peptides, while healthy neighboring cells (innocent bystanders) will remain unharmed.

Antigen Processing by MHC Class II

MHC Class II molecules present exogenous antigens that have been phagocytosed or endocytosed into vesicles (Figure 4).

During synthesis in the endoplasmic reticulum, the Class II MHC heterodimer associates with the invariant chain (Ii).
The invariant chain wraps around the MHC heterodimer, with two important consequences:

- It blocks the peptide-binding groove, preventing premature binding of peptides present in the ER;
- It directs the complex (Class II heterodimer + invariant chain) toward intracellular vesicles called endosomes.

The endosome mediates the uptake of exogenous proteins and then fuses with lysosomes, which deliver active proteases under acidic pH conditions.

The simultaneous transport of the (Class II + invariant chain) complex into an endosomal vesicle enables the lysosomal proteases to fragment the captured proteins inside the vesicle and progressively degrade the invariant chain.

A lysosome-derived peptide, generated from the degraded proteins, can then embed itself into the peptide-binding groove of the Class II MHC molecule. This allows the peptide-loaded Class II MHC molecule to be transported to the plasma membrane.

Thus, Class II MHC molecules present on the cell surface a "mixed" sample, originating from both recycled self transmembrane proteins and captured exogenous proteins. This sampling reflects the immediate microenvironment of the cell.

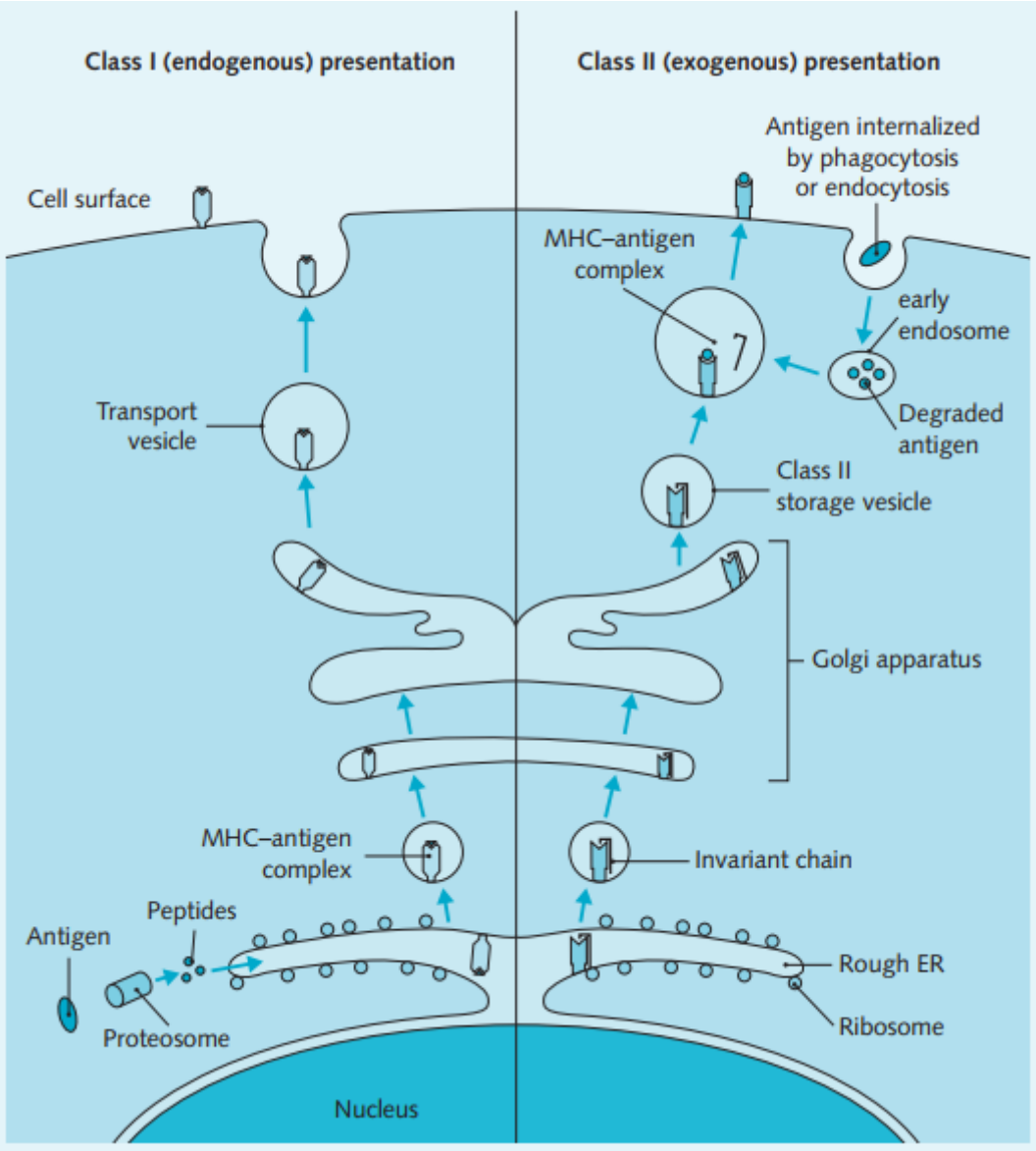

Figure 4: Antigen Processing Pathways for MHC Class I and Class II

Recognition of MHC Class I and II Molecules

During the antigen recognition stage, T lymphocytes examine the surface of the presenting cell. When an MHC molecule displays a peptide that matches the specificity of the T cell receptor (TCR), an activation signal is triggered in the T lymphocyte.
If the peptide does not match, the T lymphocyte disengages and remains inactive, continuing its search for a suitable target (Figure 5).

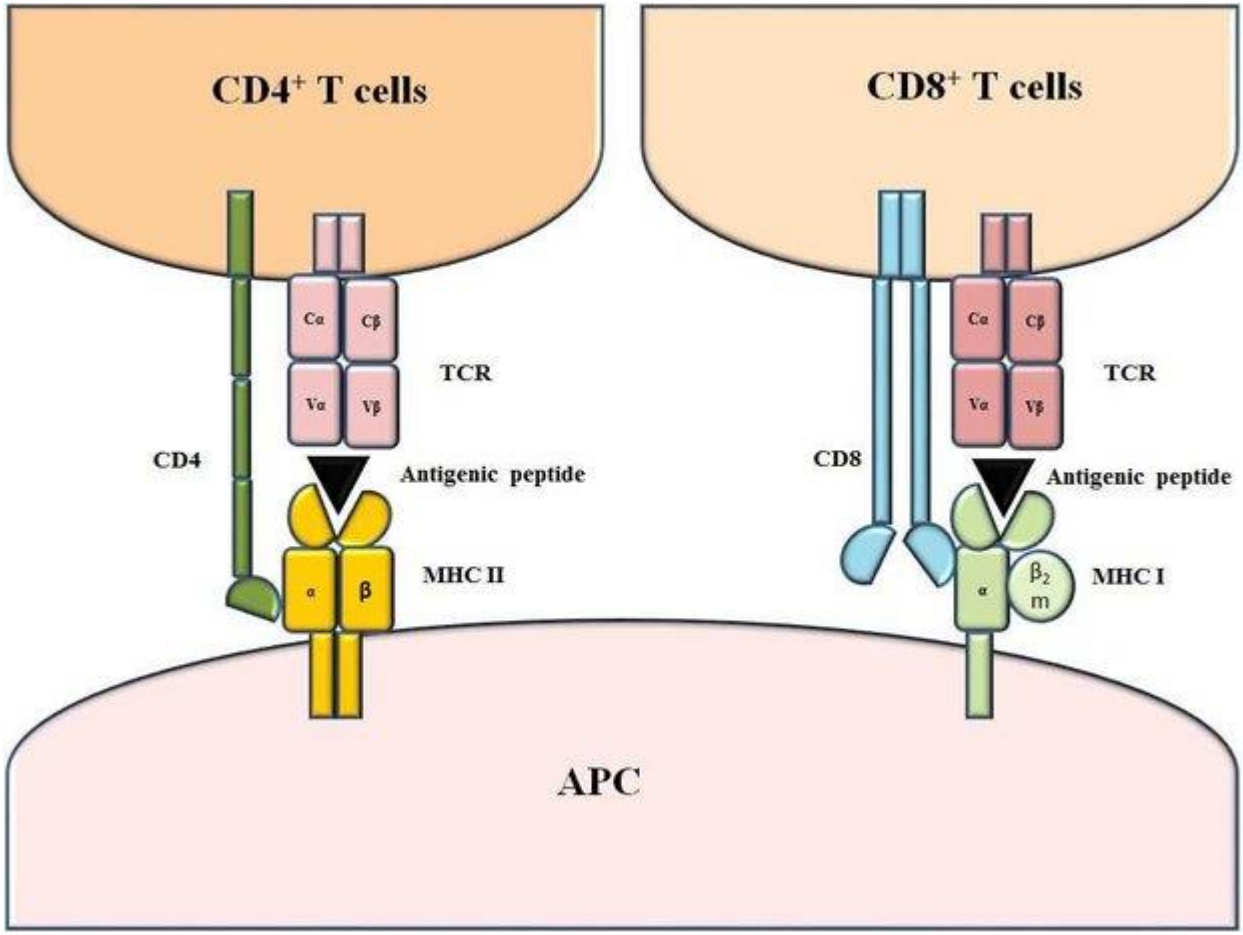

Figure 5: Recognition of MHC Class I and II Molecules

At the molecular level, a ternary complex is formed in which the antigenic peptide is "sandwiched" between the MHC molecule and the TCRαβ. The paratope of the TCRαβ is then in contact with:

- On one side, the amino acids in the central part of the peptide, accessible between the edges of the groove.
- On the other side, several amino acids from the α-helices that border the groove of the MHC molecule.

The process of T lymphocyte activation begins with a cognate signal, initiated by the interaction of the TCRαβ with its antigenic peptide embedded in the MHC molecule. This activation requires the involvement of a "co-receptor" (CD4 or CD8, depending on the T lymphocyte population considered), which interacts with the MHC molecule:

- The CD4 molecule binds to the non-polymorphic proximal domain of an MHC Class II molecule.
- The CD8 molecule binds to the non-polymorphic α3 domain of an MHC Class I molecule.

Thus, CD4+ T lymphocytes can respond to cells expressing Class II molecules, and CD8+ T lymphocytes can respond to any cell expressing Class I molecules.

Structure of Secretory Immunoglobulins (Antibodies)

Immunoglobulins are symmetrical molecules composed of four homologous polypeptide chains, grouped into pairs: two heavy chains (H) and two light chains (L). The heavy chains are linked to each other by one or more disulfide bonds. The light chains are attached to the heavy chains by a disulfide bond located near their constant and carboxy (C)-terminal ends (Figure 6).

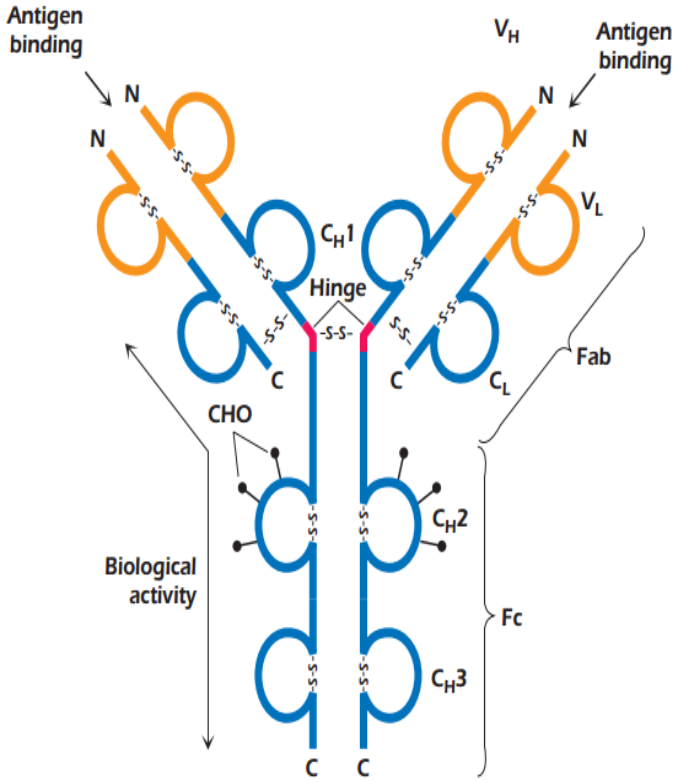

Figure 6: Antibody molecular structure

a) Heavy Chains

There are five types of heavy chains, designated by the Greek letters γ (gamma), α (alpha), μ (mu), δ (delta), and ε (epsilon), which define the five classes of immunoglobulins: IgG, IgA, IgM, IgD, and IgE. Some classes are further divided into subclasses, such as IgG (IgG1 to IgG4) and IgA (IgA1 and IgA2).

b) Light Chains

There are two types of light chains, κ (kappa) and λ (lambda), which can combine with any type of heavy chain. For a given immunoglobulin, the two light chains are always identical.

Both the heavy and light chains of the immunoglobulin molecule consist of domains, each about 110 amino acids long, stabilized by intracatenary disulfide bonds. The light chains have two domains, while the heavy chains have four (in IgD, IgG, and IgA) or five (in IgM and IgE) domains. The amino (N)-terminal domains of the heavy and light chains vary considerably from one antibody to another, and are called VH (variable heavy) and VL (variable light). The C-terminal domains of both the light and heavy chains are constant and are referred to as CL (constant light) and CH1, CH2, CH3 (constant heavy 1, 2, and 3), and sometimes CH4.

The VH and VL domains are paired, as are the CH1 and CL domains. The two CH3 domains of the heavy chains interact with each other, while the sugar composition of the CH2 domains prevents such interaction.

The immunoglobulin molecule has two distinct regions:

- The association of VH-VL forms the antigen-binding site of the antibody.
- The Fab (Fragment antibody or antigen binding) is the association between the VH-VL-CH1-CL domains. Each immunoglobulin monomer thus has two Fab fragments.

- The constant part of the two heavy chains, consisting of the CH2-CH3 domains, and sometimes CH4, forms the Fc fragment.

Antibody Variability

1. **Isotypic Variation**: Each immunoglobulin chain defines an isotype with an amino acid structure unique to each species. For example, when a human immunoglobulin is injected into an animal, it induces an immune response against the injected immunoglobulin.
2. **Allotypic Variation**: Allotypic variation (allotypes) concerns a few amino acids and reflects genetic variations (polymorphism) within the same species, often involving the constant regions of the heavy chains.
3. **Idiotypic Variation**: Modifications in the amino acid sequence of the variable region, particularly in the hypervariable region directly responsible for the specificity of the antibody site, define idiotypes that arise during the maturation of B lymphocytes in the bone marrow.

Functions of Immunoglobulins (Antibodies)

1. **Opsonization**: Phagocytic cells have antibody (Fc) receptors, so the antibody can facilitate the phagocytosis of the antigen.
2. **Agglutination**: The antigen and antibody (IgG or IgM) agglutinate because the immunoglobulin can bind to multiple epitopes simultaneously. IgM is more effective due to its high valency (10 antigen-binding sites).
3. **Neutralization**: Binding to pathogens or their toxins prevents their attachment to cells.
4. **Antibody-Dependent Cellular Cytotoxicity (ADCC)**: The antibody-antigen complex can bind to cytotoxic cells (e.g., cytotoxic T cells, NK cells) via the Fc component of the antibody, targeting the antigen for destruction.
5. **Complement Activation**: IgG and IgM can activate the classical pathway; IgA can activate the alternative pathway.
6. **Mast Cell Degranulation**: The crosslinking of IgE bound to mast cells and basophils triggers degranulation and histamine release.
7. **Protection of the Newborn**: The transplacental passage of IgG and the secretion of sIgA in breast milk protect the newborn.

Types of Immunoglobulins

1. **IgG**
    - IgG are monomers and are evenly distributed in both intra- and extravascular compartments. They constitute the majority class during the secondary immune response and make up 75% of plasma immunoglobulins (Figure 6).
2. **IgA**
    - IgA are predominant in mucosal secretions (saliva, milk, bronchial secretions) and are more than 80% present as dimers (two units linked by a J chain) (Figure 6).
3. **IgM**
    - IgM are pentameric in structure and are confined to the intravascular compartment. They represent most of the so-called "natural" antibodies and are predominant in the primary immune response. On the surface of B lymphocytes, IgM exists as a monomer forming the BCR (B Cell Receptor) (Figure 6).

4. **IgD**
    - IgD constitutes less than 1% of plasma immunoglobulins. The function of secreted IgD is not well understood, but membrane-bound IgD, associated with membrane-bound IgM on naïve B lymphocytes, plays a role in antigen receptor activity, differentiation, memory, and tolerance processes (Figure 6).
5. **IgE**
    - IgE are monomers with four constant domains. They are present either in serum or bound to the surface of mast cells and basophils via a high-affinity receptor (FcεRI). IgE plays a key role in antiparasitic immunity and immediate hypersensitivity reactions (allergic reactions) (Figure 6).

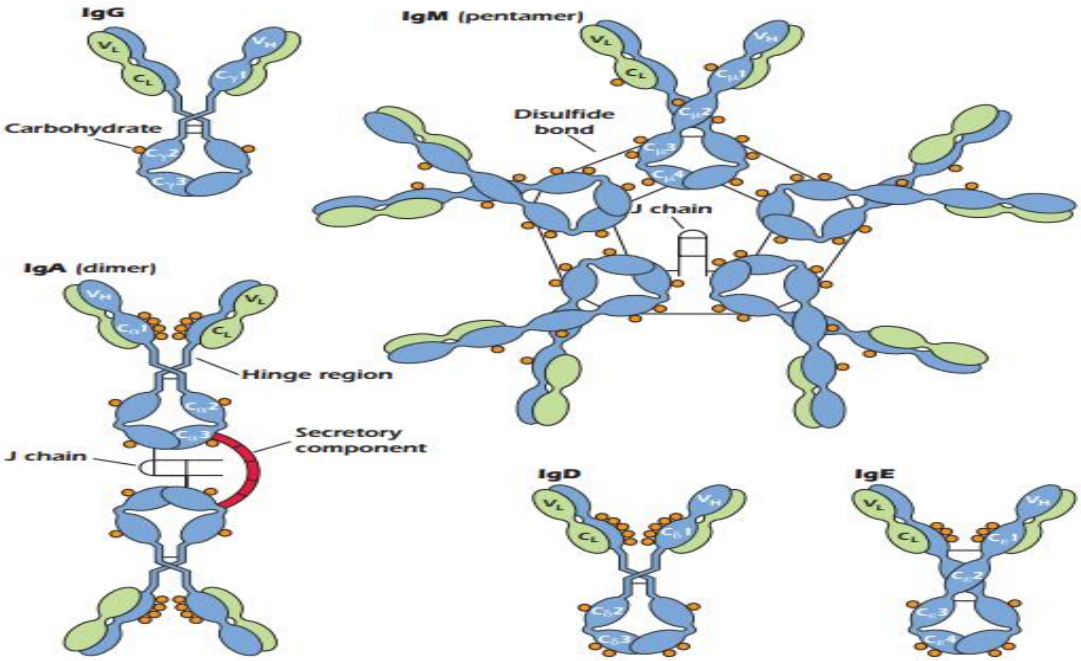

Figure 6: Different Immunoglobulins isotypes (Antibodies)

Humoral Immune Response

B lymphocytes represent about 5 to 15% of circulating lymphocytes and are defined by the presence of surface immunoglobulins (Ig). After activation by encountering an antigen for which they express specific receptors, B lymphocytes can either rapidly differentiate into short-lived IgM-secreting plasma cells or differentiate into memory B cells or long-lived plasma cells.

Memory B Cells

These cells constitute a minority group of long-lived cells capable of persisting without proliferating (from several months to several decades in humans). Their generation occurs after interaction between a naïve B cell, its corresponding antigen, and a follicular helper T cell (TFH) within secondary follicles. Follicular dendritic cells also contribute to this process through the signals they deliver.

Memory B cells are capable of generating a rapid and robust response to pathogens for which they are specific. Indeed, they can quickly and efficiently present antigens to T lymphocytes during a secondary response, proliferate, and subsequently differentiate into plasma cells. They all share a high capacity to respond upon re-exposure to the same antigen, resulting in the production of high levels of antibodies that facilitate rapid pathogen elimination.

Regulatory B Lymphocytes

These more recently discovered cells produce, among other cytokines, **IL-10**, and play important roles in regulating the immune response.

Plasma Cells

These cells, which express the CD38 and CD138 receptors, are the effector cells of the humoral immune response. They are specialized in the production and secretion of antibodies distributed throughout the body. The lifespan of these secreting cells can be either short or long, depending on the type of signals received during antigenic stimulation.

These cells play an essential role as the first line of defense against certain microorganisms such as **encapsulated bacteria**. This function is mainly ensured by **marginal zone (MZ) B cells**. These peripheral B cells predominantly produce natural, **IgM-type** antibodies, which are polyreactive and of low affinity. Their functions are diverse: elimination of cell debris, transport of cytokines, and formation of antigen-antibody complexes that are presented to follicular B cells by follicular dendritic cells.

A. Encounter with the Antigen

The probability that a B lymphocyte becomes activated by encountering a native antigen in the body is very low. However, this encounter is facilitated in a specific anatomical environment found in **secondary lymphoid organs** (lymph nodes, spleen, and Peyer's patches), which are the primary sites for thymus-dependent B cell activation.

These structures have a **highly organized microarchitecture**, rich in both B and T lymphocytes, and are extensively vascularized. This setup allows for the **continuous recirculation** of naïve lymphocytes between the blood and the B and T cell zones of these organs.

In the lymph node, **naïve B cells** (those that have not yet encountered an antigen) enter from the bloodstream through the walls of **post-capillary venules**. B lymphocytes then migrate to the **cortical area**, known as the **B cell zone**, where they remain for about one day unless they encounter their specific antigen and become activated. Otherwise, they return to circulation, making space for other B cells of different specificities, thereby **increasing the likelihood** of antigen-specific encounters despite the small size of lymph nodes.

The main organizers of lymphoid tissue in these organs are **chemokines**, which act via specific receptors to guide the selective migration of lymphocytes. For instance:

- B lymphocytes express **CXCR5**, which attracts them to the **B cell zone** of the lymph node, where **stromal cells** produce **CXCL13**, the ligand for CXCR5.
- T lymphocytes express **CCR7**, which attracts them to the **paracortical (T cell) zone**, adjacent to the B cell zone. This region's stromal cells secrete **CCL19** and **CCL21**, ligands for CCR7.

When an antigen enters the body through the skin, it reaches the lymph node via the lymphatic circulation. There, it is captured by B lymphocytes that recognize the **native antigen** in various forms:

- Soluble form
- As part of **free immune complexes**
- **Bound to the membrane** of **antigen-presenting cells (APCs)**, such as **follicular dendritic cells** or **marginal sinus macrophages** of the lymph node (see Figure 7).

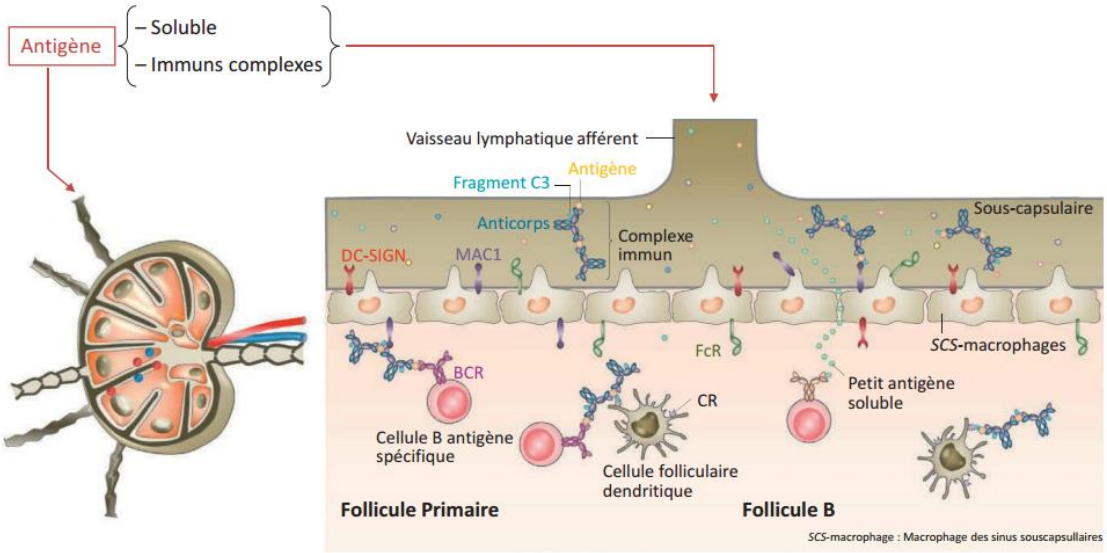

Figure 14.1
Interaction entre l'antigène et le lymphocyte B.

Figure 7: B cells recognition of antigens (Ag)

When a **B lymphocyte** recognizes the antigen for which it is specific, the binding occurs via the **B cell receptor (BCR)**. The subsequent activation of the B cell induces the expression of **CCR7**, which promotes its **migration to the interface** between the B and T cell zones.

At the same time, **antigen presentation** by **interdigitating dendritic cells** in the **T cell/paracortical zone** of the lymph node enables the **activation of helper T lymphocytes** that are specific for the same antigen. The presence of **TGF-β**, **IL-12**, **IL-23**, and **ICOS** promotes the **differentiation of helper T cells into follicular T helper cells (Th)**, which express **BCL6** and produce **IL-21**. These Th cells **lose expression of CCR7** and instead **express CXCR5**, allowing them to **migrate toward the B cell zone** to encounter the activated B lymphocyte.

This meeting occurs at the **junction between the B and T cell zones** of the lymph node (see Figure 8).

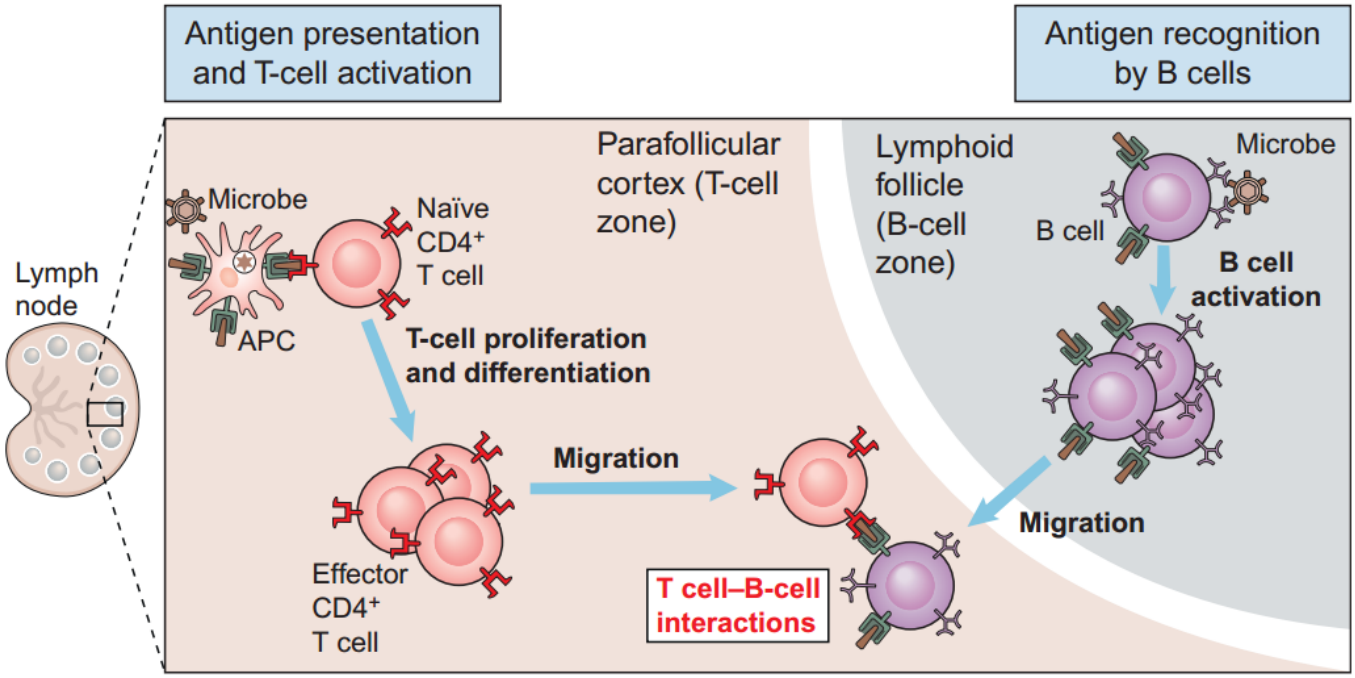

Figure 8: Antigen recognition by T and B cells in the lymph node.

At this stage, an **immunological synapse** forms, resulting in **reciprocal activation of B and T lymphocytes**, both specific to the same antigen. This is known as "**cross-presentation**" and involves the presentation of the antigen by the **B lymphocyte to the follicular T lymphocyte**. This process is essential for the activation of B lymphocytes specific for a **T-dependent (TD) antigen (Figure 9)**.

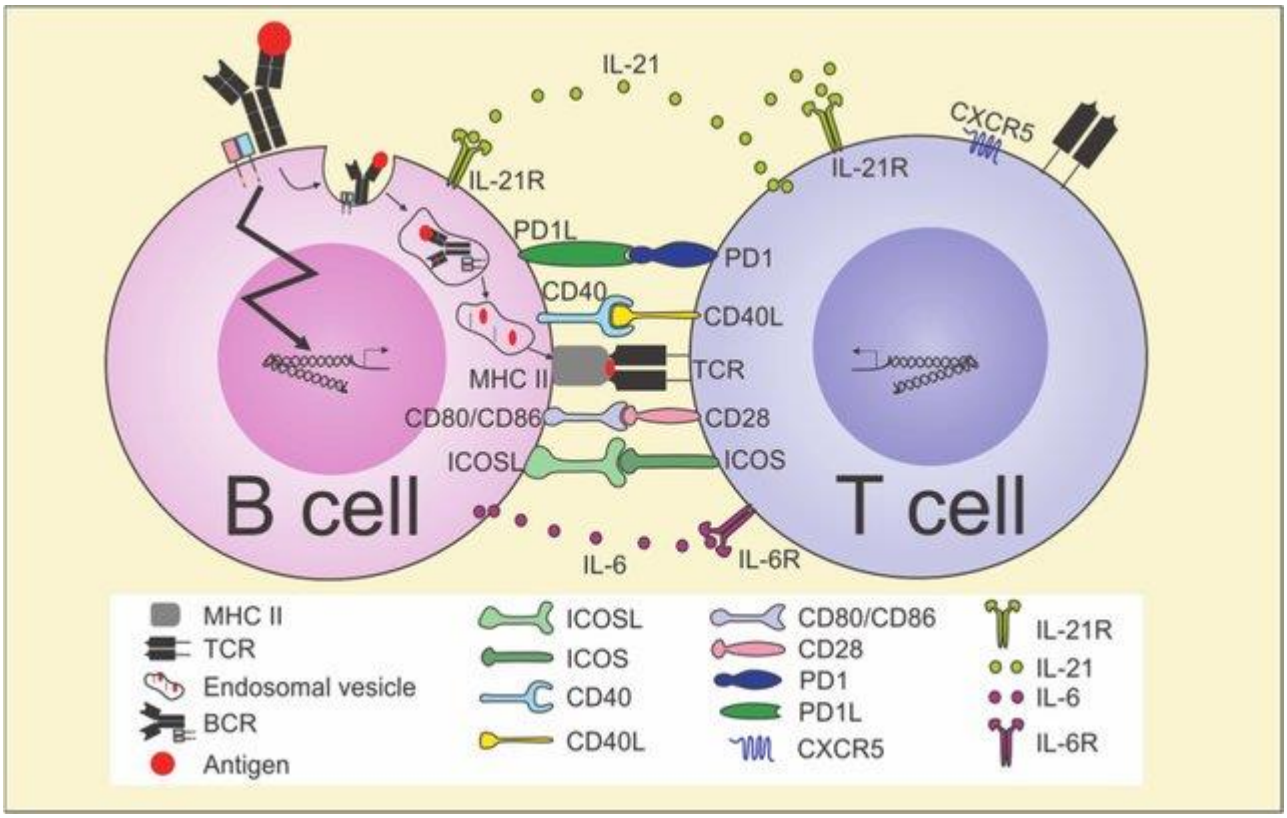

Figure 9: Immunological synapse and cross-presentation between B and T lymphocytes

B. Interactions between T and B lymphocytes during T-dependent responses

The interaction between the **antigen and the BCR** alone is not sufficient to activate the B cell and trigger antibody production. B lymphocytes require a **second signal** provided by **follicular helper T cells (Th)** in the context of **T–B cooperation**, where the **B cell acts as an antigen-presenting cell (APC)** to the **T cell**, which has already been activated by the same antigen.

1. The reaction begins with the **specific binding of the BCR to the antigen**, in its native form or as presented by a **follicular dendritic cell** or a **subcapsular macrophage**. This antigen-binding step is followed by **internalization of the BCR–antigen complex** into endocytic vesicles where the antigen is degraded, generating **peptides** that can bind to **MHC class II molecules** expressed by the B cell.
2. These **peptides are then displayed on the B cell membrane** and presented to a **CD4+ follicular helper T cell** that has already been activated. The **B and T cells then activate each other** and begin to **proliferate**. Effective B cell activation requires the combination of **two signals**:
    - The **first signal**, received through the BCR, is **antigen-specific**.
    - The **second signal** results from interactions between **membrane-bound receptors/ligands** or **soluble cytokines**, which are **not antigen-specific**.

2. Accessory Molecules in B Cell Activation

During their activation, **B lymphocytes** also begin to express new molecules, such as **CD80/CD86**. These bind to **CD28** on the **T lymphocyte**, providing a **co-stimulatory signal** that activates the T cell and induces the expression of **CD40 ligand (CD40L)**. CD40L then binds to **CD40** on the B cell, delivering a **reciprocal co-stimulatory signal**.

The **CD40 (on B cell)/CD40L (on T cell)** interaction is **essential** for:

- B cell proliferation,
- **Germinal center formation**, and
- **Isotype switching** (class switch recombination).

Other membrane interactions are also involved in **B–T cooperation**, especially:

- The **ICOS/ICOS ligand** interaction, which plays a **major role** in the **differentiation** and **migration** of **follicular helper T cells (Tfh)**, and their **cytokine production**.
- Proteins from the **SLAM (Signaling Lymphocyte Activation Molecule)** family, expressed on both **B and follicular T cells**, interact mutually and **support Tfh development** and **germinal center formation**.
- **Adhesion molecules**, such as the **ICAM-1/LFA-1** pair, tightly link **B and T lymphocytes**, prolonging contact time, which is **critical for effective activation**.

During this close interaction, the B lymphocyte also receives **soluble signals** from the T cell that are vital for:

- **Proliferation and survival** (e.g., **IL-4**, **BAFF**),
- **Maturation** (e.g., **IL-21**, which plays roles in **class switching**, **affinity maturation**, and **plasma cell differentiation**).

Activation of B lymphocytes by **follicular T cells** leads to their differentiation into **short-lived plasmablasts**, which produce **low-affinity antigen-specific IgM** antibodies and are responsible for the **primary antibody response**.

A small fraction of activated B cells, known as **founder cells**, migrate into a **primary follicle** to form a **germinal center**, which characterizes the **secondary lymphoid follicle**, a site of **active cell division** (Figure 10).

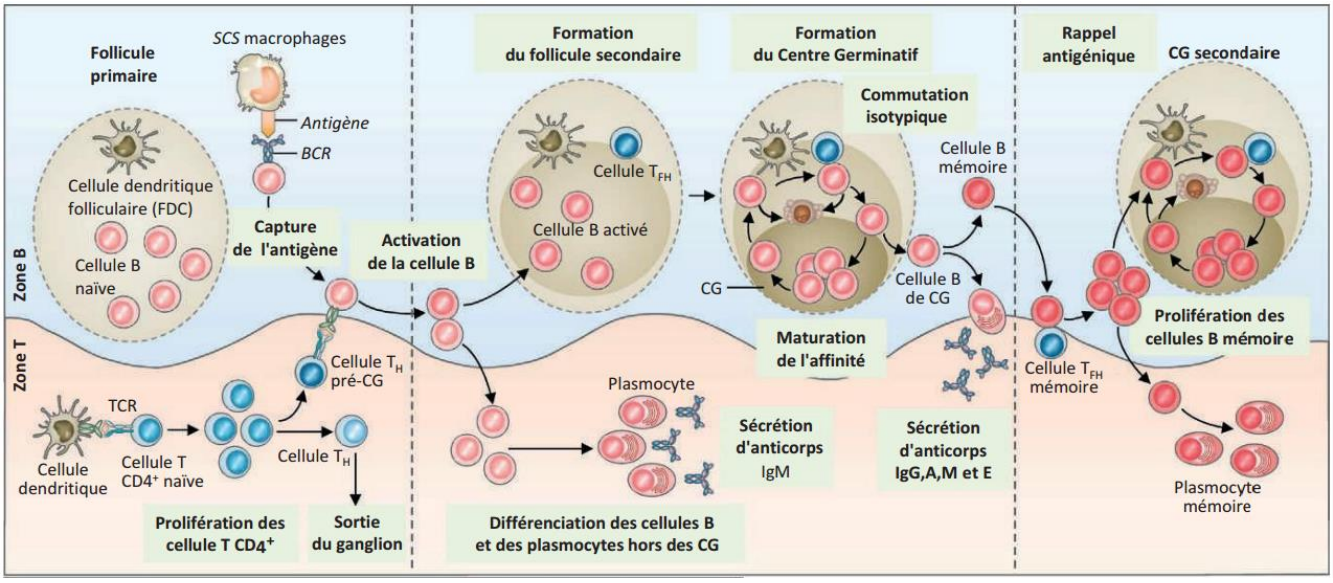

Figure 10: Development of humoral immune response inside lymph nods

T Cell-Independent B Cell Response

The vast majority of **protein antigens** (T cell-dependent antigens) require the help of **helper T cells** to trigger an effective **humoral immune response**. However, many **bacterial antigens** are capable of inducing an antibody response **in the absence of T lymphocytes**. These antigens are called **T cell-independent antigens**.

Two categories of T-independent (TI) antigens are distinguished based on either their **structural characteristics** or the **cellular mechanisms** involved in B cell activation:

1. Type 1 T-Independent Antigens (TI-1)

TI-1 antigens are capable of **directly activating B lymphocyte proliferation**.

- At **high concentrations**, they induce **polyclonal proliferation**, meaning **non-specific activation** of many B cell clones.
- At **low concentrations**, they activate **only B cells whose receptors are specific** for the antigen.

This activation **does not occur via the BCR** (B cell receptor), but rather through **danger signal receptors** such as **TLRs (Toll-like receptors)**. Therefore, it is **independent of the Btk signaling molecule (Figure 11)**.

Certain molecules, like **some plant lectins**, can by themselves induce **proliferation and antibody production** from mature B cells.

2. Type 2 T-Independent Antigens (TI-2)

Some macromolecules, including **polymerized proteins**, **polysaccharides**, or **lipids**, contain **repetitive molecular motifs** that can **interact with multiple immunoglobulin receptors** on the B cell surface and **cross-link them**.

These **repetitive structures** allow for the **cross-linking of BCRs**, resulting in a **sustained signal** that requires the **Btk kinase (Figure 11)**.
This mechanism enables a **fast and effective response** against many **extracellular pathogens** with **polysaccharide-rich walls**, which are often **resistant to phagocytosis**.

TI-2 antigens mainly activate **B1 cells**, which:

- Express **high levels of membrane IgM**,
- Are predominantly located in the **marginal zone of the spleen**.

TI-2 antigen responses also require support from **non-T helper cells** of the **myeloid lineage**. These cells contribute through:

- **CD40/CD40L interactions**,
- Secretion of **soluble mediators** like **BAFF**, **APRIL**, and **IL-21**.

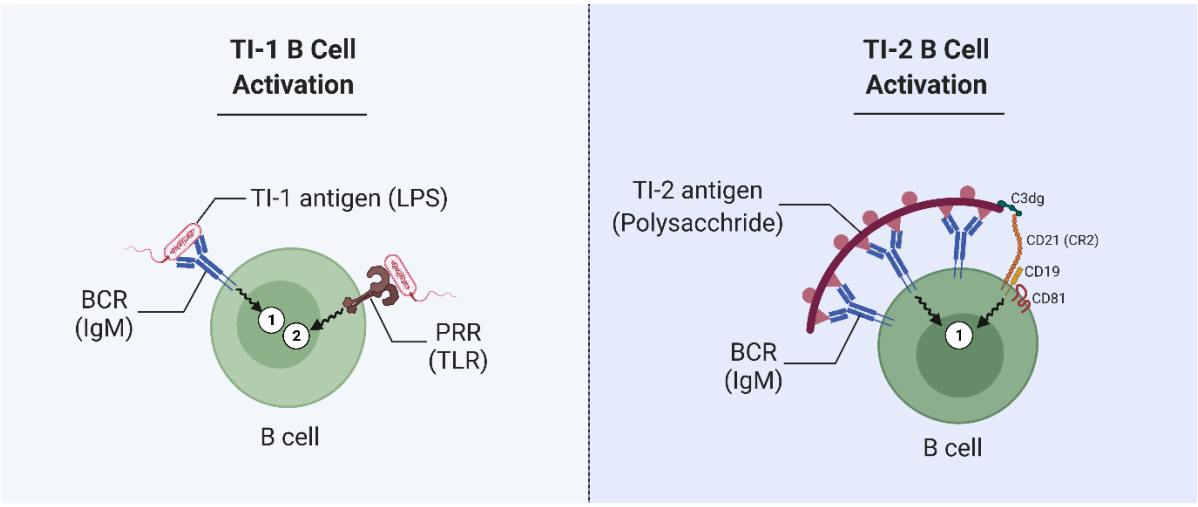

Figure 11: B Cell thymo independent response to type 1 and 2 antigens

In general, the **T cell-independent response** does **not involve affinity maturation** or **isotype switching**. It also **does not lead to the generation of memory B cells**.

Cell-Mediated Specific Immunity

Cell-mediated specific immunity is driven by **T lymphocytes** and is involved in eliminating:

- **Intracellular pathogens** and infected cells (mainly viruses, mycobacteria, and fungi),
- **Tumor cells**,
- **Foreign grafts**.

The **thymus** plays a key role in this type of immunity as it is the site where T lymphocytes mature.

Development of T Lymphocytes

T cell precursors are produced in the **bone marrow** and migrate to the **thymus** for development and maturation. The goal is to select T cells with receptors that can recognize foreign antigens **presented in the context of MHC molecules**. Cells with non-functional receptors or that are strongly self-reactive are eliminated.

Positive Selection

Positive selection occurs in the **thymic cortex**. T cells that can bind **self-MHC molecules** are allowed to survive (positively selected), whereas those that do not recognize self-MHC are eliminated.

- T cells interacting with **MHC class I** lose their CD4 expression → become **CD8+ cytotoxic T cells**.

- T cells interacting with **MHC class II** lose their CD8 expression → become **CD4+ helper T cells**. This is called **MHC restriction**.

Negative Selection

T cells that were positively selected but bind **too strongly to self-antigens** undergo **negative selection** to prevent autoimmunity. Thymic epithelial cells express a wide variety of tissue-specific antigens, allowing the deletion of many self-reactive T cells.

Activation of T Lymphocytes

Antigen-presenting cells (APCs) are the only cells capable of activating **naïve T lymphocytes**. This interaction occurs in **secondary lymphoid organs**.

- **Dendritic cells** process and present peptides from captured antigens via **MHC-peptide complexes**.
- Naïve T cells scan these APCs via weak adhesive interactions (e.g., ICAM-3, CD2).
- If no high-affinity TCR-MHC interaction occurs, the T cell leaves the lymph node.
- If a specific peptide-MHC complex is recognized with sufficient affinity, **T cell activation** begins through **clonal selection**.

A. First Signal: TCR Engagement

This is the binding of the **T Cell Receptor (TCR)** to a specific **peptide-MHC complex**. It must be **strong and sustained** for successful activation.

- **CD4/CD8 co-receptors** bind MHC class II/I, respectively, enhancing the signal.
- Adhesion molecules (e.g., **CD2**, **LFA-1**) promote stable contact and **formation of the immunological synapse**, which includes:
    - Central: TCR, CD4/CD8, CD2, CD28
    - Peripheral: Adhesion molecules

The **TCR-CD3 complex** transmits the signal via **ITAM motifs** in its cytoplasmic domain.

B. Second Signal: Costimulation

A second signal is essential to **prevent anergy or premature apoptosis**.

- **CD80/CD86** on dendritic cells bind **CD28** on T cells.
- This enhances IL-2 production, which is vital for T cell proliferation.
- Without CD28 signaling, T cells enter **anergy** (non-responsive state).
- **CD40L** on T cells binds **CD40** on APCs, boosting CD80/86 expression → amplifies CD28 signaling (positive feedback loop).
- To prevent overactivation, **CTLA-4** (expressed later) binds CD80/86 with **higher affinity** than CD28 but delivers an **inhibitory signal**.

This balance ensures a robust yet controlled T cell response.

C. Third Signal: Cytokine Environment

The **third signal** involves **cytokines** in the lymph node microenvironment, mainly from dendritic cells and other immune cells. These cytokines guide **functional differentiation** of activated T cells into subsets (e.g., Th1, Th2, Th17, Treg).

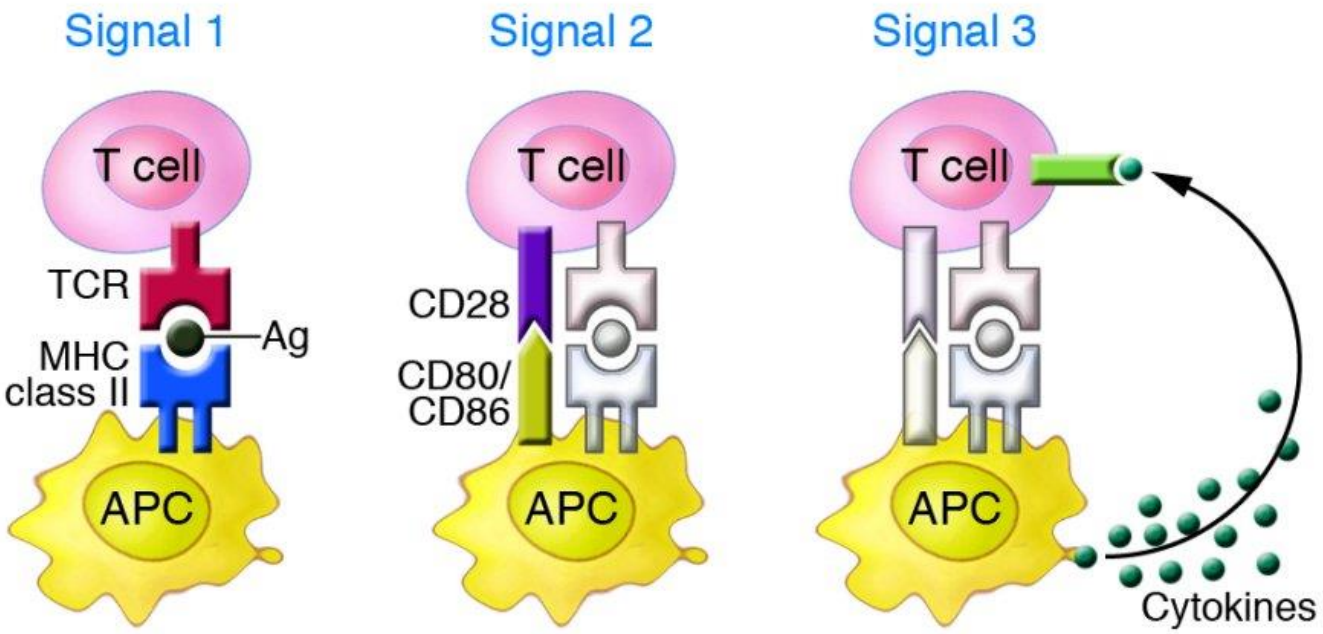

Figure 12: Activation of T-cells and their interaction with dendritic cells

Functional Differentiation of CD4+ T Lymphocytes

After **antigen recognition** and **activation**, CD4+ T lymphocytes proliferate. Some of the activated clones differentiate into **effector/helper T lymphocytes** (Th cells), or under certain conditions, into **regulatory T cells** (induced Tregs, iTreg) (Figure 13).

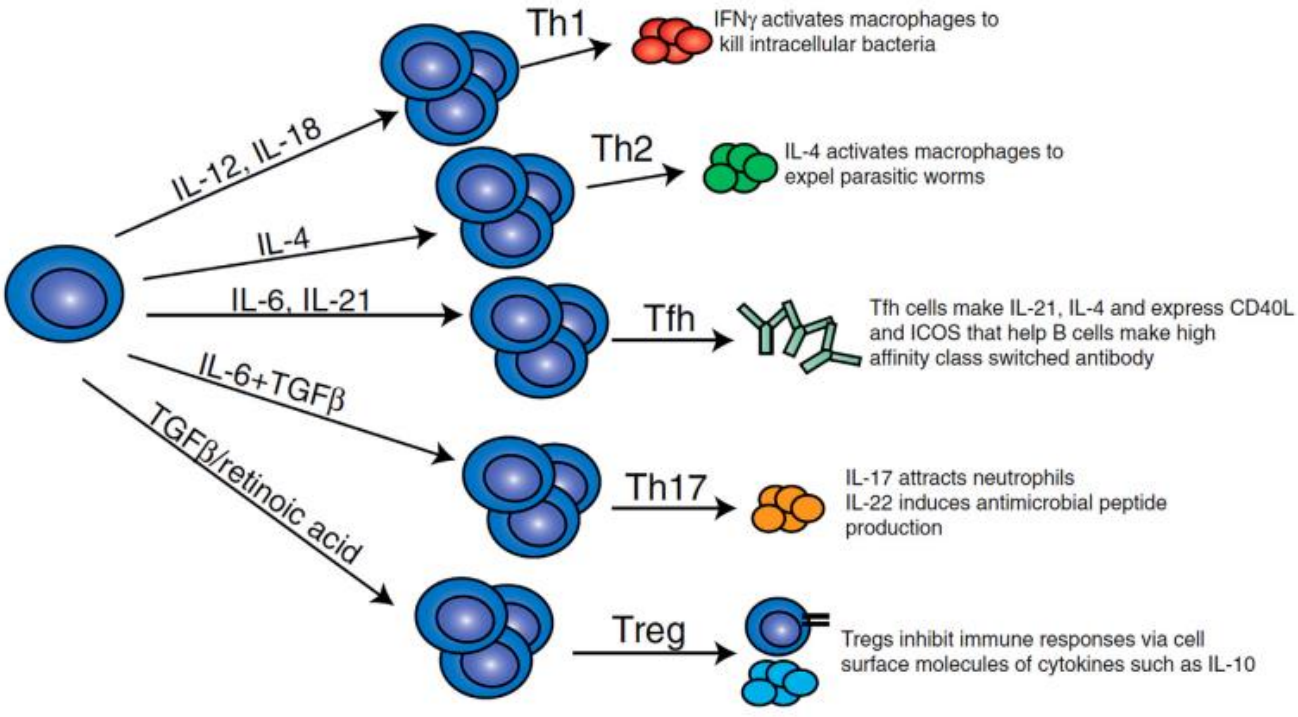

Figure 13 : T CD4+ cell differentiation

Helper T cells (Th) play a crucial role in developing immune responses:

- They determine which **epitopes** are targeted by the immune system through their interactions with antigens presented by **MHC class II** molecules on APCs.
- They shape the **type of immune response** (e.g., cytotoxic T cell or antibody response).
- They are essential for the **normal function of B cells**.

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1. Th1 Lymphocytes

These CD4+ T cells mainly secrete **interferon-gamma (IFN-γ)**, **tumor necrosis factor-alpha (TNF-α)**, and **interleukin-2 (IL-2)**.
They promote strong **cell-mediated immune responses** against **viruses** and **bacteria**.

2. Th2 Lymphocytes

Th2 cells primarily secrete **IL-4, IL-5**, and **IL-13**.
They induce **IgE production** and stimulate **eosinophil activity**, helping eliminate **extracellular parasites** like helminths.

3. Th17 Lymphocytes

Recently characterized CD4+ T cells that secrete **IL-17, IL-22** (Th17 "signature" cytokines), and **IL-21**.
They are important for controlling **extracellular bacterial** and **fungal infections**, especially by recruiting and activating **phagocytes**, particularly **neutrophils**.

Note: Most immune responses involve multiple Th subtypes. For example, *Staphylococcus* (an extracellular bacterium) triggers both Th2 and Th17 responses.
In contrast, some responses are highly polarized, e.g., immunity against *Mycobacterium tuberculosis* (an intracellular bacterium) relies almost exclusively on **Th1 cells**.

CD8+ Cytotoxic T Cell Response

Naïve **CD8+ T lymphocytes** must be **activated** to differentiate into **cytotoxic T lymphocytes (CTLs)** capable of inducing **apoptosis** in target cells.

Their activation depends on signals from two key cellular partners:

- **Dendritic Cells (DCs)**
- **CD4+ Th1 cells**

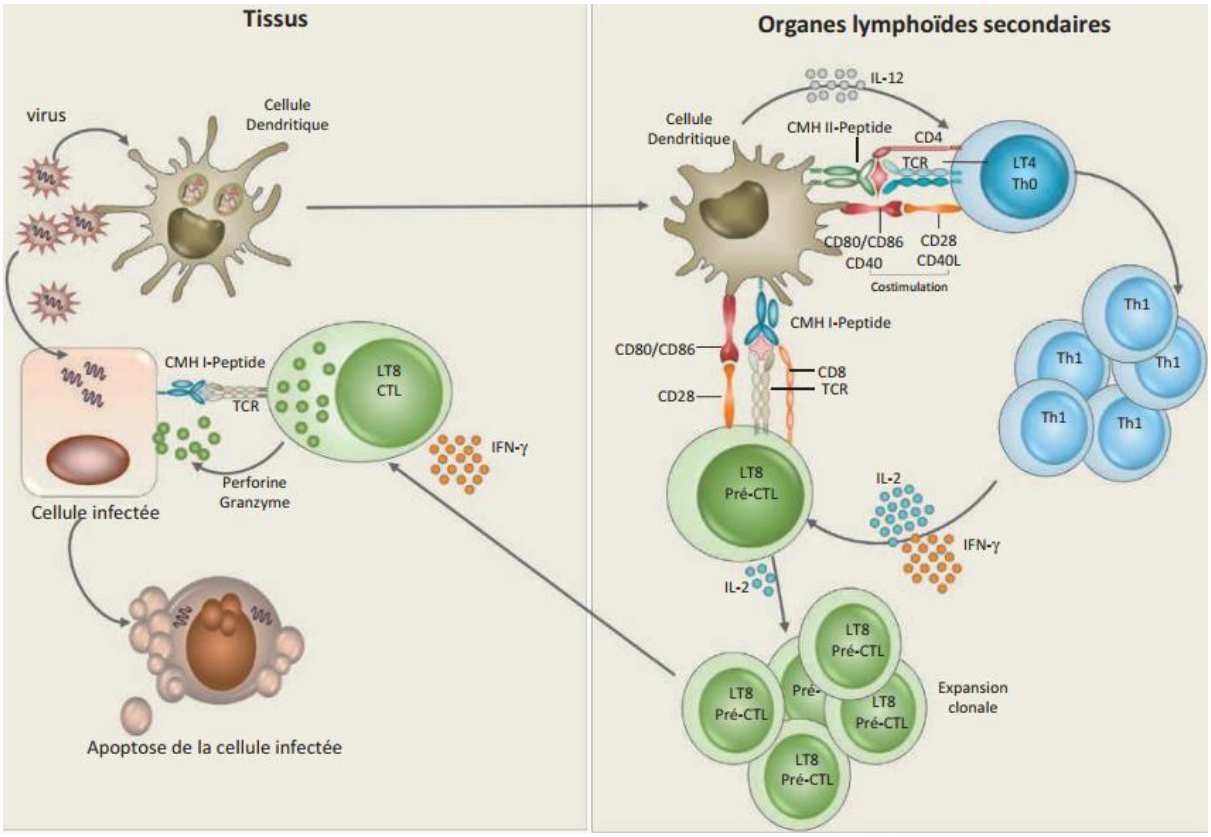
CD4+ Th1 cells are generated after their own activation via recognition of **MHC II-peptide complexes** (viral or tumor antigens) presented by DCs.

CD8+ T Cell Activation Steps:

1. First Signal – TCR Engagement:
Naïve CD8+ T cells recognize specific antigenic peptides presented by **MHC class I** molecules on DCs in secondary lymphoid organs.

2. Second Signal – Costimulation:
Provided by interaction between **B7 molecules (CD80/CD86)** on DCs and **CD28** on T cells.
Without this signal, CD8+ T cells become anergic or undergo apoptosis.

3. Third Signal – Cytokines:
Provided mainly by **Th1 CD4+ T cells**, including **IL-2** and **IFN-γ**.
Th1 cells recognize their own antigens on DCs and express **CD40L**, which binds **CD40** on DCs.
This interaction stimulates cytokine production that supports **CD8+ T cell proliferation and differentiation into CTLs**.



CD4+/CD8+ T Cell Cooperation and Clonal Expansion

This cooperation between **CD4+ and CD8+ T cells** leads, primarily through the action of **IL-2**, to the **proliferation of CD8+ T cells** (**clonal expansion**), generating a large number of **antigen-specific CD8+ T lymphocytes**.

It's worth noting that activated CD8+ T cells can also produce IL-2 themselves, albeit in smaller quantities, which still contributes to their own expansion.

The clonal expansion phase of a naïve CD8+ T cell gives rise, over **4–5 days**, to a large population of effector CD8+ T cells, estimated at **10^3 to 10^5 daughter cells**.

This stage is crucial for mounting a **CD8+ T cell immune response** that outpaces the **spread of the virus or tumor**.
Indeed, since the **naïve CD8+ T cell repertoire** is vast, the frequency of a CD8+ T cell specific for a given **MHC/peptide complex** is **very low** (1 in 10^4 to 1 in 10^5).

IFN-γ plays a key role in the **optimal differentiation** of CD8+ T cells into **cytotoxic T lymphocytes (CTLs)**. It promotes the **intracellular production of proteins** that, when released by the CTL, **induce apoptosis** of target cells in peripheral tissues.

In **viral infections**, the cytokine help provided by CD4+ T cells can be replaced by **pro-inflammatory cytokines** such as **IL-12** and **IFN-α/β**, secreted by innate immune cells like **DCs, macrophages**, and **granulocytes**.

B. Effector Functions of CTLs in Tissues

In peripheral tissues, **CTLs** specifically recognize and **destroy infected or tumor cells** presenting **peptide antigens bound to MHC class I** molecules.

At this point, CTLs **no longer require costimulation** or **help from CD4+ T cells**.

A CTL operates through **multiple successive cycles** involving the following steps:

1. **Conjugation** with the target cell
2. **Recognition and activation** (via TCR and co-activation molecules)
3. **Degranulation** to release **cytotoxic molecules** that kill the target
4. **Dissociation** from the dead cell
5. **Recirculation** in search of a new target
   (See figure X – assumed to be a diagram)

A single CTL is programmed to **destroy multiple targets** before eventually undergoing **apoptosis itself** – a mechanism similar to **natural killer (NK) cells**.