



# Hematology



## HISTOLOGY

☒ Sheet

☐ Slide

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Number: **4**

Subject: **Platelets & lymphoid system**

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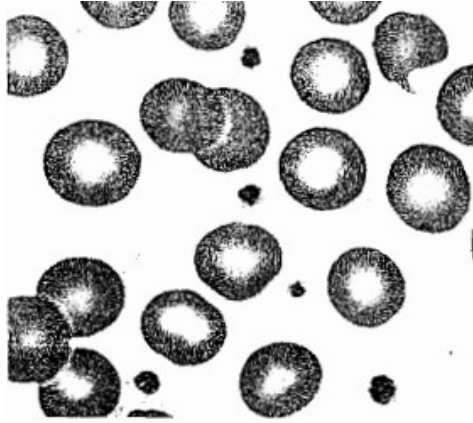
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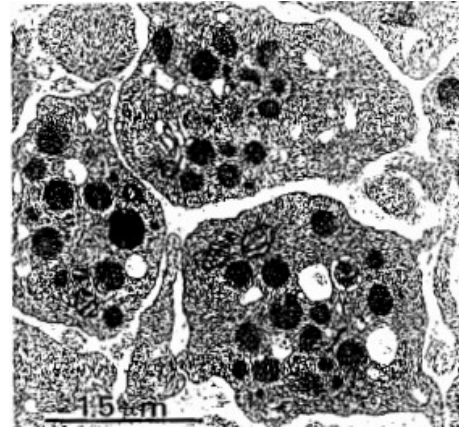
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## Platelets (Thrombocytes)

This is the appearance of the platelets in a blood film (notice the small granules).



This is the appearance of the platelets in electron microscope; the cytoplasm is filled with electron dense granules.



- ❖ *Platelets* are small non-nucleated cells, formed by fragmentation of the cytoplasm of **megakaryocytes**; these cells are huge cell present in the bone marrow which were originally **megakaryoblasts**.



- ❖ Diameter: 1.5-3.5  $\mu\text{m}$ .
- ❖ Number: 150,000 – 400,000/ $\text{mm}^3$ . If you count the platelets of a patient and you find the platelets count **less than 100,000**; this patient will definitely suffer from **bleeding tendency**, which emphasizes the importance of platelets in **haemostasis**.
- ❖ Cytoplasm: either free of granules  $\rightarrow$  hyalomere, or contains granule  $\rightarrow$  granulomere.
- ❖ When we stain the cytoplasm with Leishman stain or Wright's stain (polychromatic stain) it appears **purple**.
- ❖ Granular cytoplasm is filled with organelles, and the most important organelles are **granules** of all types, which are:
  - **Alpha** granules: the largest, contain fibrinogen and some clotting factors (factor **5** & factor **8**) and also contain platelet factor 4  $\rightarrow$  *regulate vascular permeability*.
  - **Delta** / Dense granules: contain serotonin (absorbed from plasma), Adenosine triphosphate (ATP) and adenosine diphosphate (ADP).
  - **Lambda** granules (lysosomes): contain hydrolytic enzymes  $\rightarrow$  aid in clot resorption.

Thus, the platelets aid in clot **formation** and once the clot is formed & the job is done; the enzymes that come out of some platelets will aid in its

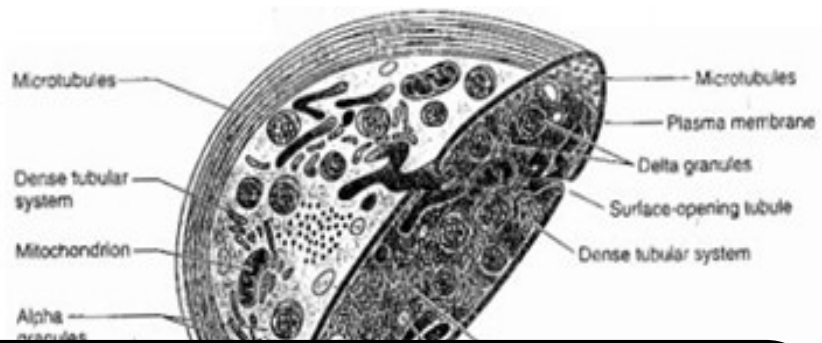
- ❖ Deep to the cell membrane there is a network of tubules; microtubules & microfilaments cytoskeleton, and inside there is a dense tubular system, both **preserve the shape of the platelets**.
- ❖ Function of the platelets: haemostasis.

How does it help in haemostasis? Does it help *stop bleeding from a large artery*? *No*, but when there is a *bleeding from a ruptured small artery* (arteriole) or capillary, platelets will aggregate forming a **plug** which will close the ruptured blood vessel and hence stop bleeding.

What simulates **adhesion** of the platelets in the wall of the blood vessel?

When damage happens to the endothelium of the blood vessel, **collagen will show up**; so the platelets will adhere to the collagen. This plug of platelets will stop the bleeding and later on, will be replaced by a blood clot.

Therefore, the platelets don't only form a **plug**. They also make the fibrin aggregate on its surface as well (forming **clot & thrombus**).



What is the difference between a platelets plug and a blood clot?

**Platelets plug** is only platelets.

Fibrin above the platelets + RBCs = **blood clot**.

Layers of WBCs above the blood clot + fibrin + RBCs → **thrombus**.

So the **clot** is part of the thrombus and is formed on the surface of the platelets.

## *Lymphoid (Immune) System*

- ❖ Lymphatic system is composed of organs; either *encapsulated* lymphatic organs or *non-encapsulated*.

### *1. Encapsulated:* thymus, lymph node & spleen.

These three organs have a *basic structure*; they are surrounded by a *capsule* (dense connective tissue) and this capsule sends *septa* to the inside, divides the organ into compartments. These septa are called *trabeculae*. Then, there's a *network of reticular cells and fibers*. So three components form the structure of spleen, lymph node & thymus:

*Capsule* → *trabeculae* → *network of reticular cells and fibers*.

Inside this network of the lymphatic organs there are; B lymphocytes, T lymphocytes, their "maid"; antigen presenting cells and macrophages.

These are the major components of any lymphatic organ.

### *2. Non -encapsulated:* we call them diffuse lymphatic tissue or mucosa associated lymphatic tissue (MALT), found in the wall of the stomach, the wall of the bronchi and the urinary tract.

They take the form of a lymphatic nodule/follicle, and are not surrounded by a capsule. Remember: in the gut, the collection of lymphatic nodules is called Peyer's patches. Peyer's patches are an example of diffuse *non-encapsulated* lymphatic tissue.

- ❖ There are two types of immune systems responsible for two types of immune reactions; *innate* (non specific) & *adaptive* (specific).

- *Innate (non specific):* the components of this system are:

- *Macrophages*: most of them derived from monocyte.

- *Neutrophil* (microphage).

Both can phagocytose bacteria in a non specific way; they inhibit the bacteria which have 5-6 types of antigens on its surface (they don't recognize the antigens). Therefore, they inhibit the bacteria with all its antigens and the way they do that is not specific.

- *Natural killer cell*: type of lymphocyte that can swallow tumor cell, virally infected cell, and parasite in the blood in a non specific way.

- *Complement system* of blood-born macromolecules. One of its types helps in opsonization (cover the bacteria with complements or antibodies to ease its phagocytosis by neutrophils). If opsonization didn't occur, it means that these bacteria are severely pathogenic and they resist the phagocytosis by neutrophils.

- *Adaptive (specific): deal with specific invaders*, the components of this system are: **B lymphocytes**, **T lymphocytes** and their maid; **antigen presenting cells**.
  - Its ability improves with subsequent exposure.
  - The most important characteristic of this system is specificity, other characteristics are memory and self & non self recognition.

### Memory

If pathogenic bacteria entered your body one year ago and your body responded by an immune reaction that killed the bacteria, the same bacteria might enter later and it will find more potent reaction that will destroy it before the development of the symptoms. *How does that happen?*

When the bacteria enter for the first time, they have antigens on the surface, these antigens interact with B lymphocytes. B lymphocytes will be activated after this interaction and divide to give plasma cells that produce antibodies and B memory cells.

B memory cells will function as B lymphocytes. If the bacterial antigen enters again after one month, two months or a year; B memory cells will interact with it rapidly, and destroy it before the development of the symptoms. This is the importance of B memory cells.

Again, B memory cell function to kill the antigen if it enters after a year, for the second, third, or fourth time. The reaction here is faster, more potent, and longer in duration.

That was -briefly- the concept of memory cell.

In any immune reaction, antigen interacts with B lymphocyte → B memory cell.

Antigen interacts with T lymphocyte → T memory cell.

Both **B & T**  
memory cells  
are present.

Both memory cells live in the body for years and are responsible for the immunity & the potent interaction that kills the microbe during the second or the third exposure before the development of the symptoms.

### Self and Non-Self Recognition

T and B lymphocytes almost always interact with foreign antigens and don't interact with self antigens (our cell proteins). If T and B lymphocytes interact with self antigens → autoimmune disease (the body attacks its own cells).

Again, The components of the adaptive system are **B lymphocytes**, **T lymphocytes** and their maid; **antigen presenting cells**. All of these cells are formed in the bone marrow.

- ❖ B lymphocytes are **formed** in bone marrow, and become mature/**immunocompetent** (can recognize its antigen and interact with it) also in bone marrow.
- ❖ T lymphocytes are **formed** in the bone marrow, they travel to the thymus and become **immunocompetent** there. Then they exit the thymus and go to secondary lymphatic organs (spleen, lymph node, and diffuse lymphatic tissue) and meet their antigens.
- ❖ Programming of lymphocytes means to become immunocompetent (recognize its antigen).
  - T lymphocyte is programmed in the *thymus*.
  - B lymphocyte is programmed in the *bone marrow*.

Both **thymus** and **bone marrow** are called primary lymphatic organs, while **spleen**, **lymph node**, and **diffuse lymphatic tissue** are called peripheral/secondary lymphatic organs.

Once an immunocompetent B lymphocyte exit the bone marrow & an immunocompetent T lymphocyte exit the thymus; they travel to the diffuse lymphatic tissue, the spleen, and lymph node to look for and meet their antigen.

Epitope: the sensitive part of the antigen (exogenous or endogenous antigen) that interact with antibody , usually composed of 8-11 amino acid (polypeptide) and found on the outer surface of the receptor.

On the surface of T and B lymphocyte there are receptors, those on the surface of B lymphocytes are immunoglobulins; we call them SIGs (specific immunoglobulins). B lymphocyte which is formed in bone marrow, produce thousands of antibodies (immunoglobulins) and insert them in its cell membrane to work as receptors.

While T lymphocyte produces TCRs (T cell receptors).

Both types (TCRs & SIGs) act as receptors even though their structures differ.

\*\*\*\*\*

After B lymphocyte is formed in the bone marrow and becomes immunocompetent, it will attack the place where it will meet its antigen.

It's not right to say that T or B lymphocytes will meet its antigen in the blood, *the meeting occurs in the peripheral lymphatic organs (spleen, lymph node, and diffuse lymphatic tissue).*

Before the lymphocyte meets its antigen, it's called a virgin or naïve cell.

When it meets its antigen and recognizes it, it starts to proliferate to form two types of cells: **Activated cell** (effector cell) & **Memory cell**.



- ❖ When B lymphocyte meet its antigen and start to divide it give an effector cell which is plasma cell that produces antibodies. Effector cell of T lymphocyte is a cytotoxic T cell or T helper cell each one have a certain function.
- ❖ Both T & B lymphocytes give memory cells. The most important characteristic of memory cell: it's responsible of killing the bacteria that carry the antigen if it enters the body for the second time and before the development of symptoms.

Why?

Because it interacts with the previously recognized antigen.

- ❖ It will interact with the antigen more rapidly with longer duration, and more potent interaction. Memory cell live for long durations in the body.

Memory cells (T & B) are **NOT** formed from the first immune reaction, it doesn't interact with the antigen (their mothers interact with it). So they are not directly involved in immune reaction from the first time. But when there is a second exposure to the antigen; memory cells play a major role.

## ***B-LYMPHOCYTES***

- ❖ Formed and become immunocompetent in the bone marrow.
- ❖ After it becomes immunocompetent it migrates from the bone marrow to the peripheral lymphatic organs (spleen, lymph node, and diffuse lymphatic tissue). It migrates to look for its antigen (bacteria, etc...)
- ❖ B lymphocyte is responsible for antibody mediated immunoreactions, *how does it happen?*

B lymphocyte recognizes the antigen, then when it proliferates and becomes activated it will bring into being a plasma cell that produces antibodies, & memory B cell.

Plasma cells are formed in the spleen & lymph nodes and a small amount will stay there. Most plasma cells that are formed in the antibody mediated immunoreactions travel to the bone marrow which is the major source of antibodies.

### ***How are the receptors (SIGs) formed?***

Receptor of B lymphocytes are specific immunoglobulins (SIGs). During its formation and programming in the bone marrow; each B lymphocyte produces 50,000-100,000 IgM & IgE and insert them on the cell surface.

- Immunoglobulins/antibodies are Y shaped structures.

\* The upper part (X) interacts with the epitope of the antigen.

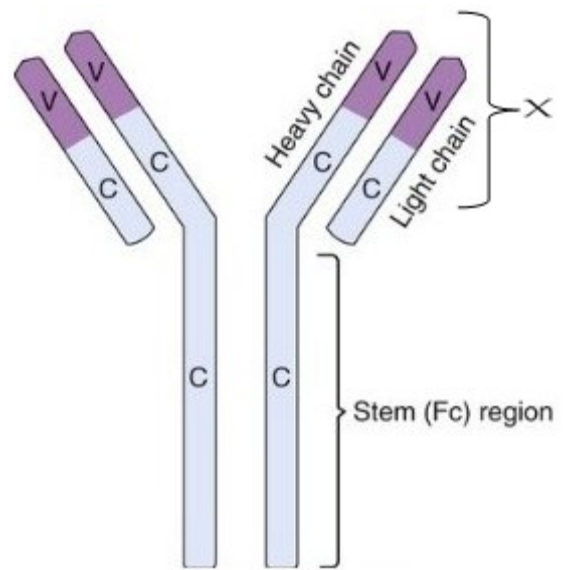
\* The stem (Fc segment) is embedded in the cell membrane and composed of two types of immunoglobulins: Ig beta & Ig alpha.

Function of the stem: when the epitope interacts with the antibody the signal is transformed and enters through the stem.

So Ig beta & Ig alpha transform the signal to the inside of the cell.

Once the signal reaches the inside of a lymphocyte, series of reactions occur & lead to the activation of B lymphocyte.

When it's activated it enlarges and proliferates to give plasma cell and B memory cell.



## *Class Switching*

*Does a B lymphocyte (after it exits the bone marrow) continue to produce IgM & IgE?*

No, it can produce other types of antibodies / immunoglobulins.

*What determine this process?*

T helper cell (found next to the B cell), which produces substances called cytokines (protein hormones). It either stimulates or inhibits certain cell functions (increases cell activity or decreases it).

Types of cytokines are: interleukin family & interferons.

Cytokines are produced from T cells to reach B cells, which in turn produce other types of immunoglobulins. For example: when a parasite enters the body (plasmodium malaria), T helper cell recognizes it immediately. When it recognizes the antigen of the malaria it starts producing different types of cytokines: IL-4 & IL-5. These interleukins exit the T helper and go to the B cell and signals it to produce **IgE** instead of IgG & IgM. IgE exits the B cell and travels to the surface of the **mast cell** to stimulate the release its granules. The mast cells also contain toxic material that kills the parasite.



T helper cell can affect the B cell and make it produce types of immunoglobulins according to the type of the microbe/organism/antigen that enters the body.

## ***T-LYMPHOCYTES***

- ❖ Originate in the bone marrow and become immunocompetent in the thymus, then it exits the thymus and migrates to the peripheral lymphatic organs (spleen, lymph node ...)
- ❖ It resides in a certain region in the lymph node, where? Between the cortex and the medulla → *deep cortex or paracortex*
- ❖ Where is it found in the spleen? In a region called *peri-arteriolar lymphatic sheaths*.
- ❖ If we remove the thymus from a young experimental animal, we will notice atrophy in parts of lymph nodes and parts of spleen ; atrophy in the deep cortex of the lymph node (where the T cells are found) and atrophy in parts of the spleen where the T cells are found. This assures the importance of the thymus in programming the T lymphocytes.
- ❖ Histologically, we can't differentiate between T and B lymphocytes. But there are four major functional differences:
  1. Different **receptors**; receptors of B cells are SIGs (IgM & IgE), and receptors of T cells are TCRs. These receptors differ in the structure.
  2. T cells can only recognize the epitopes (antigens) **presented** to them by other cells (antigen presenting cells) which might be a macrophage that swallowed the antigen and processed it in a certain way then presented it to the T cell, while B cells can interact with antigens that swim in the lymph or the blood without being presented to it by an APC.
  3. T lymphocyte interacts only with **protein antigen** while B lymphocyte interacts with protein, sugar residues, etc...
  4. T lymphocytes perform their function only at a short **distant**; it gets closer to its antigen on the surface of tumor cell or virally infected cell. They have to be close to it and produce toxic material to destroy the foreign cell. While B lymphocytes just send its antibodies.
- ❖ Whether we have **SIGs** (specific immunoglobulins) on the surface of B lymphocytes or **TCRs**, they both act as antigen receptors and interact with it.
- ❖ TCRs (the receptors on the surface of T lymphocytes) can recognize the epitope (the sensitive part of the antigen) **only if the epitope is a polypeptide** (the antigen is a protein) and **if the epitope is bound to MHC** (major histocompatibility complex).

So the receptors of T lymphocytes will NOT recognize the epitope of an antigen unless it was a polypeptide and bound to other protein on the cell surface, this protein is a macroprotein called **MHC** and it's of two types: MHC I & MHC II.

For example, cytotoxic T cells usually approach a tumor cell and recognize it as a foreign cell then destroys it. This cancer cell has antigen on its surface (tumor protein) and this antigen will bind to other protein which is MHC. ***Cytotoxic T cell will recognize MHC and the antigen on the surface of the cancer cell.***

Cytotoxic cell will not recognize the antigen, phagocytose it and destroy it unless it binds MHC I.

Again, for lymphocyte to recognize the antigen on the cell surface (bacteria, tumor cell, or virally infected cell), it has to be bound to another protein; MHC I or MHC II.

**MHC I** is a glycoprotein; the antigen binds to it so the lymphocyte can recognize the antigen. MHC I is found in all the nucleated cells, except the many nucleated.

MHC II and MHC-I are found on the cell membrane of the antigen presenting cell. *All* our cells have **MHC I**, while **antigen presenting cells** have **MHC I** as well as **MHC II**.

To be activated; T cell have to recognize and interact, not only with the epitope of the antigen; but also with MHC. If there is epitope bounded to MHC on the cell surface → T cell receptor recognizes and interacts with the epitope and the MHC, ***if it didn't recognize the MHC; the reaction is defective.*** Therefore, it has to recognize the antigen and the protein bounded to it; MHC.

***T cell capacity against the epitope is MHC restricted.***

Cell ability to recognize the antigen is determined by the presence or the absence of MHC. No MHC → no normal interaction with the epitope.

The epitope have to be bound to the MHC.

- Types of T lymphocytes:

1. ***T- Helper cell.*** It works by producing cytokines (affect other cells in the immune reaction). It has two types: T- Helper 1 & T- Helper 2.

***T- Helper 1*** produces cytokines if the antigen was viral or bacterial; its function is restricted to ***bacterial or viral attack***. If a bacteria or a virus enters the body, B lymphocyte will produce antibody against it. What signals it to produce the proper antibody? Cytokines of T- Helper 1.

Again, in the case of bacterial or viral infection, T- Helper cell type 1 has a role. How? The bacteria have antigen on their surface; we need antibody to interact with them and kill them. Plasma cell from B cell will produce antibody against the bacteria or the virus, ***what activates the B cell? T helper cell.***

T- Helper will produce cytokines (IL4, IL5) that leave the T cell and go to the B and tell it to produce antibody against that virus.

If other types of invaders enter the body (*parasite or bacteria that attack the mucosa*), this requires the incorporation of *T Helper 2*. It's necessary to activate the immune reaction that will produce *IgE* in case of *parasitic infections* and *IgA* in case of *mucosal infection*.

*T helper cell works via cytokines, which affect other cells (including B lymphocyte).*

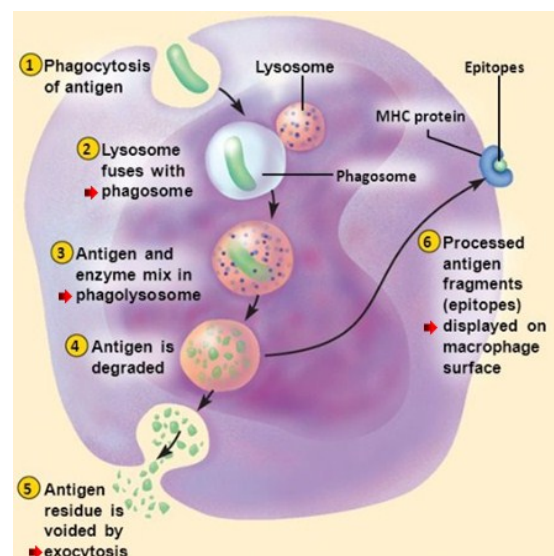
2. *Cytotoxic T cells* always kill the *abnormal cells* such as cells containing a virus. These cells have an abnormal nucleic acid (viral nucleic acid). For the T lymphocyte to start the immune reaction; the first step is to recognize the cell as *foreign*. What helps T lymphocyte to recognize cell as foreign? *The antigen on the cell surface is bound to MHC I*, if the antigen isn't bound to MHC I, it will not recognize it as foreign.
3. *Suppressor T cells decrease the activity of T and B lymphocytes.*
4. *T memory cells carry immunological memory.* Carries a memory of an antigen that enters the body one year ago/one month ago. T memory cells interact with the antigen when it enters the body for the second/third time, by fast & potent interaction that lasts for days before the development of symptoms. This is the most important characteristic of the memory cells.

## Antigen Processing

Antigen presenting cells might be macrophages. They have MHC on their surface (MHC I & MHC II). When a bacterial antigen gets closer (the antigen has several epitopes), the first thing antigen presenting cells do, is *phagocytosis* for the antigen and the MHC I, inside one vesicle → *phagosome*. The phagosome joins the lysosome (contains hydrolytic enzymes to digest the antigen) → *phagolysosome*.

Inside the phagolysosome; parts of the antigen join the MHC. Finally, *exocytosis* (insertion into the cell membrane) occurs to get the MHC with parts of the antigen on the cell surface.

If we have MHC II on the cell surface, T helper cell or B lymphocyte can easily recognize it. Antigen presenting cell



phagocytose and process (digest) the antigen then present it *bounded to the MHC*.

- ❖ If the antigen source is from bacteria (outside the body) → *exogenous* antigen, it binds to *MHC type II*.
- ❖ If the antigen is produced from body cells:
  - ✓ Cell was affected by a *virus* and started to produce abnormal proteins → *endogenous* antigen. In a virally infected cell in the liver, the antigen on its surface (abnormal protein) is bounded to *MHC I*.
  - ✓ A cell was affected by *cancer* & starts to produce abnormal protein → *endogenous* antigen, attach to the surface of the tumor cell by *MHC I*.If the antigen didn't bind to MHC I; cytotoxic T cell will not recognize it and kill it.
- ❖ In the antigen presenting cells (macrophage) the antigen is present on the surface bound to *MHC II*, whereas in cancer cells and virally infected cells, the antigen (abnormal protein) is bounded to *MHC I*.
- ❖ We identified two types of antigen; each type has a “glue” to adhere it on the cell surface:
  - \**Endogenous* antigens’ (from cancer cell or virally infected cell) “glue” is *MHC I*.
  - \**Exogenous* antigens (from exogenous bacteria) adhere to the surface of APC by *MHC II*.If the antigen didn't adhere to the cell surface via MHC I or MHC II → NO immune reaction, and thus NO recognition.  
For an immune reaction to occur; T or B lymphocyte is supposed to recognize the antigen. Unless the antigen is bounded to the MHC; the lymphocyte will not recognize it or interact with it.  
So the *function of MHC*: **is to present the antigen to the proper cell.**
- ❖ T cell can produce TCRs (receptors on the surface of T lymphocyte), in addition to it, T cell can produce other protein → *CD* which has two types *CD 4* and *CD 8*.
  - \**CD 4* is a protein adherent to the surface of T *Helper* cell.
  - \**CD 8* is adherent to the surface of *cytotoxic* T cell.Both have a role in immune reaction.
- ❖ If the epitope is present alone on the cell surface → no immune reaction.  
The **epitope** on the cell surface has to be **bound to MHC**.
- \* **Exogenous antigen → presented on APC → by MHC II.**
- \* **Endogenous antigen → presented on the abnormal cell → by MHC I.**



**Exogenous** antigen: presented on the surface of antigen presenting cell.

**Endogenous** antigen (from a cancer cell): bound to MHC I on the surface of the cancer cell.

- ❖ Not all antigen presenting cells are produced from monocytes; some are of monocyte origin, other of non-monocyte origin.

Antigen presenting cells ingest/process the antigen, *the most important function of the antigen presenting cell → it display the antigen bounded to the MHC on its surface*, for T helper (T lymphocytes) to recognize it.

- ❖ Antigen presenting cells of *monocyte origin*: **macrophage** and **dendritic cell**. Dendritic cells have many types: langerhans cell in the skin, follicular dendritic cell; found in the lymphatic follicle/nodules of the spleen & lymph node, and interdigitating dendritic cell.

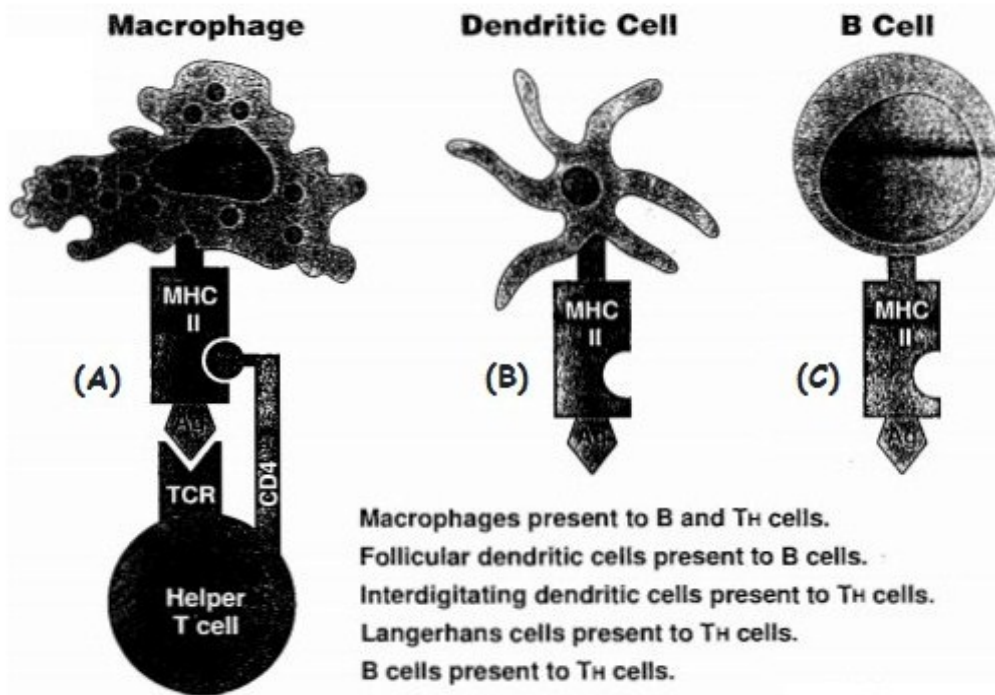
- ❖ *Non-monocyte origin*: **B cell**, **epithelial reticular cell in thymus**.

Exception: B lymphocytes are supposed to interact with antigen presenting cells and sometimes it works as an antigen presenting cell.

Epithelial reticular cells in thymus also work as antigen presenting cells and are not derived from monocytes.

- ❖ T helper cells produce **cytokines** (*some types of antigen presenting cells can produce it too*). Cytokines work as hormones; either increase the activity of the cell or decrease it (stimulate or inhibit the activity of certain cells).
- ❖ Cytokine family is a big family. Both interleukin and interferon are considered part of that cytokine family. Interferon was discovered many years ago and has been used in the treatment of sarcoma and viral hepatitis.





**(A):** Antigen presenting cell (*macrophage*).

*How does it play a role in immune reactions?*

It phagocytoses the antigen, digests it, then adheres some parts of it with MHC and displays both on its surface. Notice the antigen which is part of the bacteria, the MHC on the surface of the macrophage. The antigen is presented to the T Helper cell.

Remember: T helper cells have receptors on their surface that interact with the antigen hence the antigen is bound to the MHC. No MHC → no interaction.

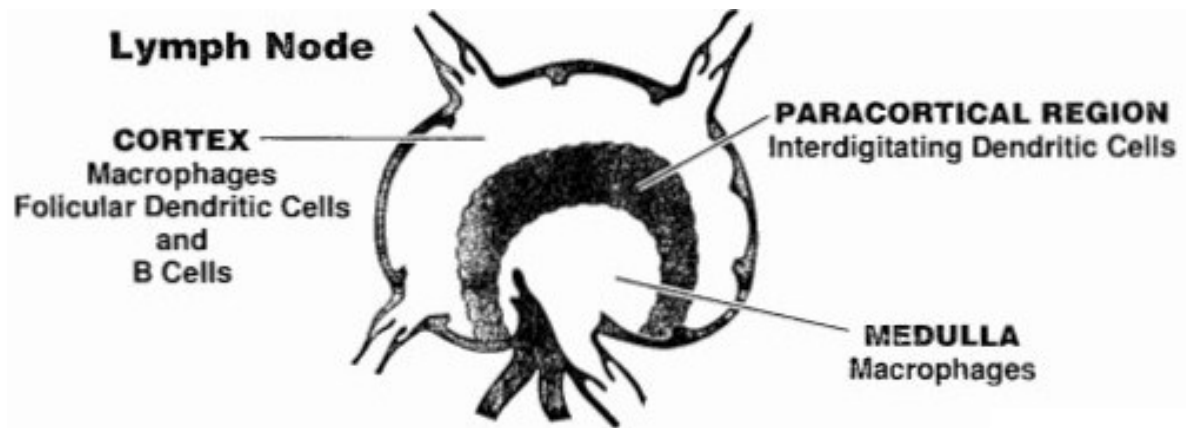
Notice the other protein on the surface of T Helper cell; CD4. It's necessary for the immunoreactions. During recognition process, it has a receptor on the surface of MHC. Therefore, the immunoreaction does not only involve *TCR* recognizing the antigen bounded to MHC II, but also CD4 has to recognize MHC II.

**(B):** *Dendritic cells are another type of antigen presenting cells* (langerhans cell, follicular dendritic cell in lymph node & spleen, and interdigitating dendritic cell). They work in the same way following the same principle.

They take the antigen, adhere it to MHC II then present it to the T helper cell.

**(C):** B lymphocyte can work as an antigen presenting cell; swallow the antigen, adhere it to MHC II then present it to T Helper cell.





❖ This figure presents a *section of lymph node*.

\* *How do lymph nodes differ from spleen and thymus?*

Lymph nodes have afferent and efferent lymphatic vessels, whereas spleen and thymus have only *efferent lymphatic vessels*.

A lymph node is composed of two parts: *outer cortex* + *inner medulla*. Each part contains certain lymphatic tissue.

Between the cortex and the medulla there is a region called *deep cortex* or *paracortex*. This region is where *T lymphocyte* reside (when T lymphocyte become immune competent it migrate from the thymus and lives here).

*Cortex* contains lymphatic nodules/follicles and each lymphatic nodule has *B lymphocytes* as the main cell. B lymphocytes can't work without T helper cells.

Lymphatic nodule/follicle contains B lymphocytes, T helper cells and the *maid of B lymphocyte*; a type of antigen presenting cell called follicular dendritic cell.

Deep cortex contains *T cells*, and its maid; type of dendritic cell called interdigitating dendritic cells.

The medulla contains the products of the immune reaction. When immune reaction happen in the cortex, B lymphocyte recognizes its antigen and then divides to give plasma and memory cells, both go to the medulla (they don't stay in the medulla) then most of them go to the blood. *Memory cell* circulate in the *blood*, enter other *lymph node* and recognize an antigen. *Plasma cells* go to the *bone marrow* to produce antibodies. (**Bone marrow is the major source of antibodies in our body**).

*Best Regards*