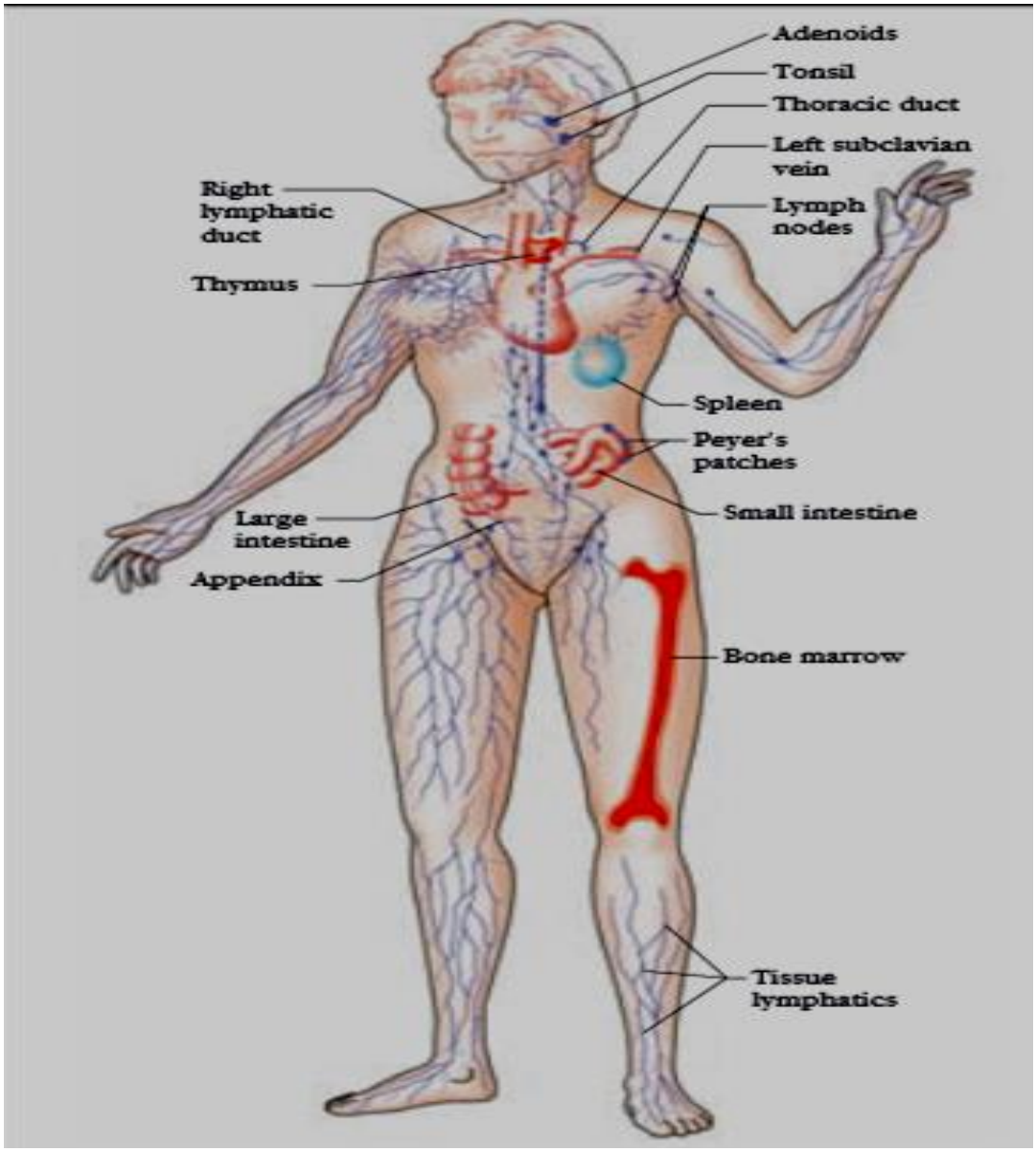
# Unit – 2 Immune System and Immune Responses

## Immune System: Organs & Cells involved in immune response

A number of morphologically and functionally diverse organs and tissues have various functions in the development of immune responses. These can be distinguished by function as the primary and secondary lymphoid organs.

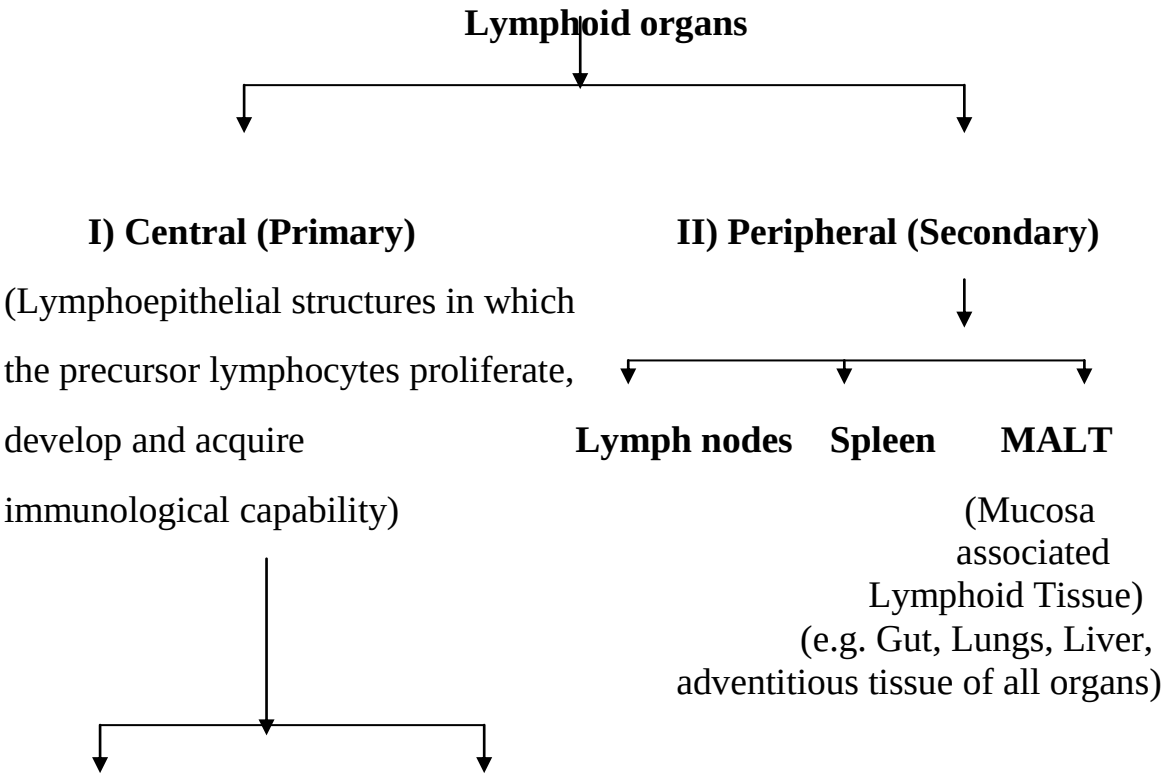
The thymus and bone marrow are the primary (or central) lymphoid organs, where maturation of lymphocytes takes place. The lymph nodes, spleen, and various mucosal associated lymphoid tissues (MALT) such as gut-associated lymphoid tissue (GALT) are the secondary (or peripheral) lymphoid organs, which trap antigen and provide sites for mature lymphocytes to interact with that antigen.



The **Lymphoid System** consists of –

**A) Lymphoid organs**

**B) Lymphoid cells (Lymphocytes and Plasma Cells)**




(The Thymus is responsible for CMI and the Bone Marrow is responsible for AMI. The lymphocytes after acquiring immunocompetence, migrate along blood and lymph streams, accumulate in the peripheral lymphoid organs and, following antigenic stimulus, effect the appropriate immune response.)

Immature lymphocytes generated in hematopoiesis mature and become committed to a particular antigenic specificity within the primary lymphoid organs. Lymphocytes matured within a primary lymphoid organs are called as immunocompetent cells (capable of mounting an immune response). T cells arise in the thymus, and B cells originate in bone marrow (for example in many mammals—humans and mice).

## I) **Central (Primary) Lymphoid organs**

There are two types of Central (Primary) Lymphoid organs.

1. Thymus
2. Bursa of Fabricius in Birds or Bone Marrow in Mammals

### 1. Thymus: -

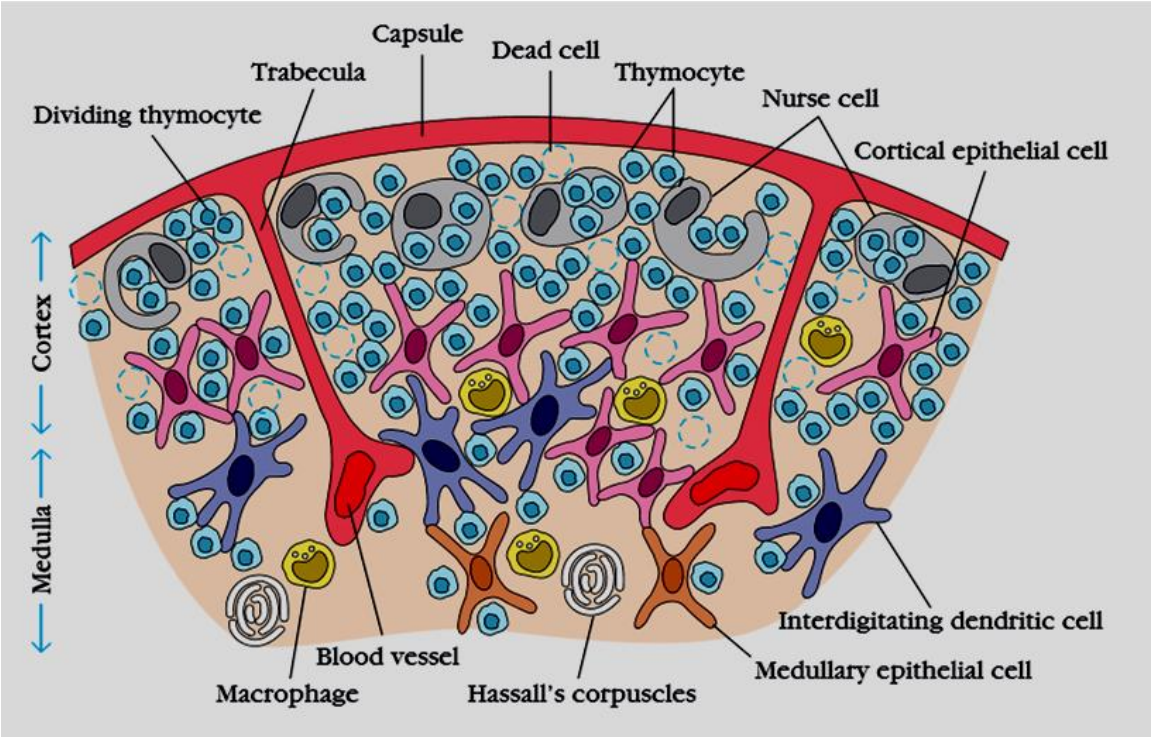
The thymus is located behind the upper part of the sternum. The thymus develops from the epithelium of the third and fourth pharyngeal pouches at about the sixth week of gestation. By the eighth week, mesenchymal stem cells (precursors of lymphocytes) from the yolk sac, liver and bone marrow reach the thymus and differentiate into the thymic lymphoid cells (thymocytes). It is thus the first organ in all animal species to become predominantly lymphoid. In human beings, the thymus reaches its maximal relative size just before birth. It continues to grow till about the $12^{th}$ year. After puberity, it undergoes spontaneous progressive involution, indicating that it functions best in early life.

The thymus is the site of T-cell development and maturation. It is a flat, bilobed organ situated above the heart. Each lobe is surrounded by a capsule and is divided into lobules, which are separated from each other by strands of connective tissue called trabeculae. Each lobule is organized into two compartments: the outer compartment, or cortex, is densely (more number) packed with immature T cells, called thymocytes, whereas the inner compartment, or medulla, is sparsely (less number) populated with thymocytes.

Both the cortex and medulla of the thymus are crisscrossed by a three dimensional stromal-cell network composed of epithelial cells, dendritic cells, and macrophages, which make up the framework of the organ and contribute to the growth and maturation of thymocytes. Many of these stromal cells interact physically with the developing thymocytes. Some thymic epithelial cells in the outer cortex, called nurse cells, have long membrane extensions that surround as many as 50 thymocytes, forming large multicellular complexes. Other cortical epithelial cells have long interconnecting cytoplasmic extensions that form a network and have been shown to interact with numerous thymocytes as they traverse the cortex.

The function of the thymus is to generate and select a stock of T cells that will protect the body from infection. As thymocytes develop, an enormous diversity of T-cell receptors is generated by a random process that produces some T cells with receptors capable of recognizing antigen-MHC complexes.

However, most of the T-cell receptors produced by this random process are incapable of recognizing antigen-MHC complexes and a small portion react with combinations of self antigen-MHC complexes. Using mechanisms, the thymus induces the death of those T cells that cannot recognize antigen-MHC complexes and those that react with self antigen-MHC complexes and create a danger of causing autoimmune diseases. More than 95% of all thymocytes die by apoptosis in the thymus without ever reaching maturity.




Scientists Good (1954) and Miller (1961) studied the role of thymus organ in the development of cell-mediated immunity (CMI). The primary function of the thymus is the production of **thymic lymphocytes.** It is the major site for lymphocyte proliferation in the body. However, of the lymphocytes produced, only about **one percent** leaves the thymus. The rest are destroyed locally. The reason for this wasteful process is not known.

In the thymus, the lymphocytes acquire new surface antigens (**Thy antigens).** Lymphocytes produced in the thymus are called as **Thymus (T) dependent lymphocytes or 'T' cells.**

The thymus confers immunological competence on the lymphocytes during their stay in the organ. Prethymic lymphocytes are not immunocompetent. In the thymus they are **'educated'** so that they become capable of mounting cell mediated immune (CMI) response against appropriate antigens.

## 2. Bone Marrow in Mammals / Bursa of Fabricius in Birds

In human and mice, bone marrow is the site of B-cell origin and development. Arising from lymphoid progenitors, immature B cells proliferate and differentiate within the bone marrow, and stromal cells within the bone marrow interact directly with the B cells and secrete various cytokines that are required for development. Like thymic selection during T-cell maturation, a selection process within the bone marrow eliminates B cells with self-reactive antibody receptors.

Bone marrow is not the site of B-cell development in all species. In birds, a lymphoid organ called the bursa of Fabricius, a lymphoid tissue associated with the gut, is the primary site of B-cell maturation. This is a lymphoepithelial organ arising as a pouch from the dorsal part of the cloaca in birds. It becomes a lymphoid organ by about the $15^{th}$ day of embryonation, develops full functional ability near hatching and starts involuting by 7 – 13 weeks of age, corresponding to the age of puberty. The bursa is also a site of lymphocytic proliferation and differentiation.

Stem cells from the yolk sac, liver and bone marrow enter the bursa, proliferate and develop into immunocompetent **'bursal lymphocytes' or 'B' cells (B for Bursa or Bone marrow).**

In mammals such as primates and rodents, there is no bursa and no single counterpart to it as a primary lymphoid organ. In cattle and sheep, the primary lymphoid tissue hosting the maturation, proliferation, and diversification of B cells early in gestation is the fetal spleen. Later in gestation, this function is assumed by a patch of tissue embedded in the wall of the intestine called the ileal Peyer's patch, which contains a large number B cells. The rabbit, too, uses gut-associated tissues such as the appendix as primary lymphoid tissue for important steps in the proliferation and diversification of B cells.

## II) Peripheral (Secondary) lymphoid organs

Lymph nodes and the spleen are the most highly organized of the secondary lymphoid organs; they consist of not only lymphoid follicles, but additional distinct regions of T-cell and B-cell activity, and they are surrounded by a fibrous capsule. Less-organized lymphoid tissue, collectively called mucosal-associated lymphoid tissue (MALT), is found in various body sites. MALT includes Peyer's patches (in the small intestine), the tonsils, and the appendix, as well as numerous lymphoid follicles within the lamina propria of the

intestines and in the mucous membranes lining the upper airways, bronchi, and genital tract.

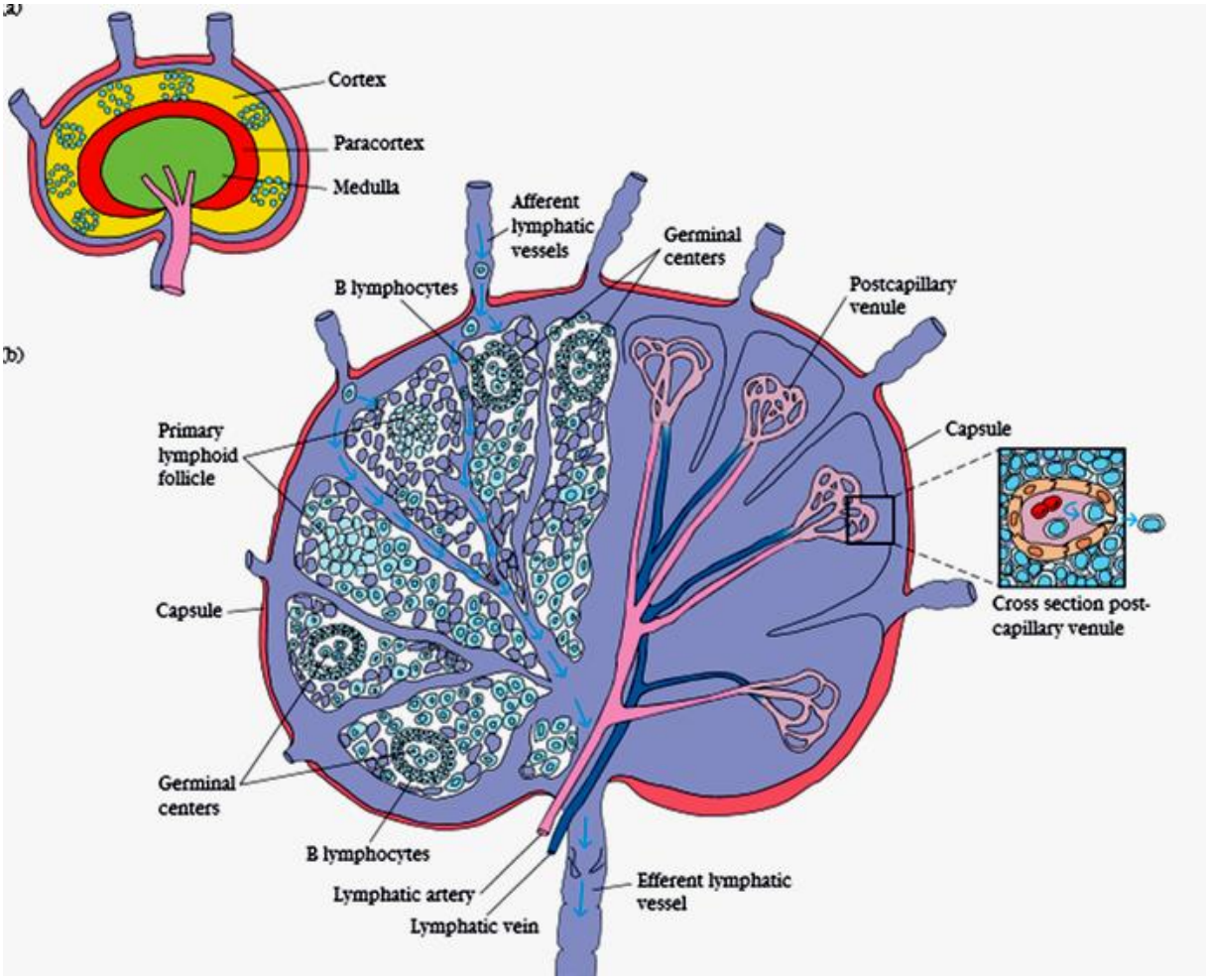
There are mainly three types of peripheral lymphoid organs ---

1. **Lymph nodes**
2. **Spleen**
3. **Mucosa Associated Lymphoid Tissue (MALT)**

### 1. Lymph nodes: -

Lymph nodes are the sites where immune responses are mounted to antigens in lymph. They are encapsulated bean-shaped structures containing a reticular network packed with lymphocytes, macrophages, and dendritic cells. Clustered at junctions of the lymphatic vessels, lymph nodes are the first organized lymphoid structure to come in contact with antigens that enter the tissue spaces. As lymph percolates through a node, any particulate antigen that is brought in with the lymph is trapped by the cellular network of phagocytic cells and dendritic cells (follicular and interdigitating). The overall architecture of a lymph node supports an ideal microenvironment for lymphocytes to effectively interact and respond to trapped antigens.

Morphologically, a lymph node can be divided into three roughly concentric regions: the cortex, the paracortex, and the medulla, each of which supports a distinct microenvironment. The outermost layer, the cortex, contains lymphocytes (mostly B cells), macro-phages, and follicular dendritic cells arranged in primary follicles. After antigenic challenge, the primary follicles enlarge into secondary follicles, each containing a germinal center. In children with B-cell deficiencies, the cortex lacks primary follicles and germinal centers. Below the cortex is the paracortex, which is populated largely by T lymphocytes and also contains interdigitating dendritic cells thought to have migrated from tissues to the node. These interdigitating dendritic cells express high levels of class II MHC molecules, which are necessary for presenting antigen to $T_H$ cells. The paracortex is therefore sometimes referred to as a thymus-dependent area in contrast to the cortex, which is a thymus-independent area.




The innermost layer of a lymph node, the medulla, is less populated with lymphoid-lineage cells; of those present, many are plasma cells actively secreting antibody molecules. As antigen is carried into a regional node by the lymph, it is trapped, processed, and presented together with class II MHC molecules by interdigitating dendritic cells in the paracortex, resulting in the activation of $T_H$ cells. The initial activation of B cells is also thought to take place within the T-cell-rich paracortex. Once activated $T_H$ and B cells, form small foci consisting largely of proliferating B cells at the edges of the paracortex. Some B cells within the foci differentiate into plasma cells secreting IgM and IgG. These foci reach maximum size within 4–6 days of antigen challenge. Within 4–7 days of antigen challenge, a few B cells and $T_H$ cells migrate to the primary follicles of the cortex. It is not known what causes this migration. Within a primary follicle, cellular interactions between follicular dendritic cells, B cells, and $T_H$ cells take place, leading to development of a secondary follicle with a central germinal center. Some of the plasma cells

generated in the germinal center move to the medullary areas of the lymph node, and many migrate to bone marrow.

Afferent lymphatic vessels penetrate the capsule of a lymph node at numerous sites and empty lymph into the subcapsular sinus. Lymph coming from the tissues percolates slowly inward through the cortex, paracortex, and medulla, allowing phagocytic cells and dendritic cells to trap any bacteria or particulate material (e.g., antigen-antibody complexes) carried by the lymph. After infection or the introduction of other antigens into the body, the lymph leaving a node through its single efferent lymphatic vessel is enriched with antibodies newly secreted by medullary plasma cells and also has a fiftyfold higher concentration of lymphocytes than the afferent lymph.

The increase in lymphocytes in lymph leaving a node is due in part to lymphocyte proliferation within the node in response to antigen. Most of the increase, however, represents blood-borne lymphocytes that migrate into the node by passing between specialized endothelial cells that line the postcapillary venules of the node. Estimates are that 25% of the lymphocytes leaving a lymph node have migrated across this endothelial layer and entered the node from the blood.

Because antigenic stimulation within a node can increase this migration tenfold, the concentration of lymphocytes in a node that is actively responding can increase greatly, and the node swells visibly. Factors released in lymph nodes during antigen stimulation help this increased migration.

**2. Spleen: -**

The spleen plays a major role in increasing immune responses to antigens in the blood stream. It is a large, ovoid secondary lymphoid organ situated high in the left abdominal cavity. While lymph nodes are specialized for trapping antigen from local tissues, the spleen specializes in filtering blood and trapping blood-borne antigens; thus, it can respond to systemic infections. Unlike the lymph nodes, the spleen is not supplied by lymphatic vessels. Instead, blood-borne antigens and lymphocytes are carried into the spleen through the splenic artery. Experiments with radioactively labeled lymphocytes show that more recirculating lymphocytes pass daily through the spleen than through all the lymph nodes combined.

The spleen is surrounded by a capsule that extends a number of projections (trabeculae) into the interior to form a compartmentalized structure.

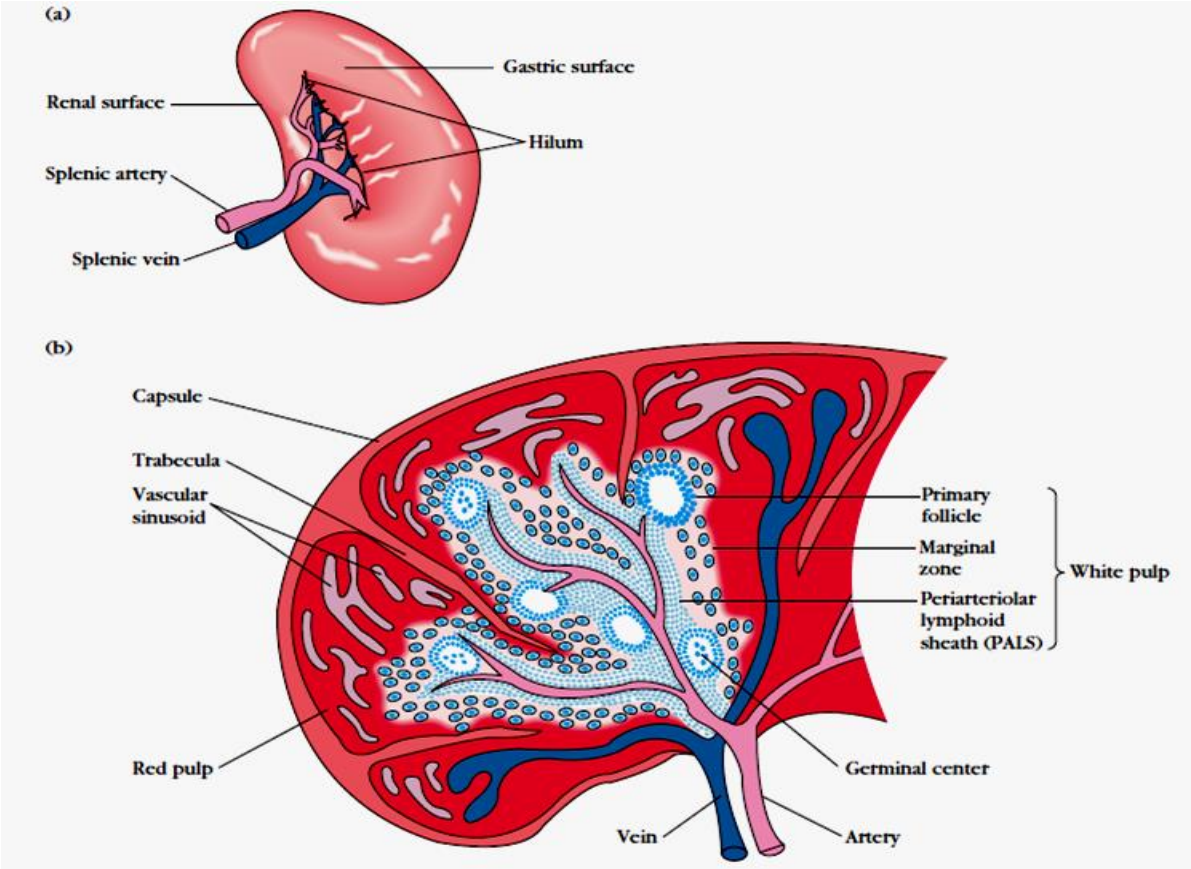
The compartments are of two types, the red pulp and white pulp, which are separated by a diffuse marginal zone.

The splenic **red pulp** consists of a network of sinusoids populated by macrophages and numerous red blood cells (erythrocytes) and few lymphocytes; it is the site where old and defective red blood cells are destroyed and removed. Many of the macrophages within the red pulp contain engulfed red blood cells or iron pigments from degraded hemoglobin.

The splenic **white pulp** surrounds the branches of the splenic artery, forming a **periarteriolar lymphoid sheath (PALS)** populated mainly by T lymphocytes. Primary lymphoid follicles are attached to the PALS. These follicles are rich in B cells and some of them contain germinal centers. The **marginal zone,** located peripheral to the PALS, is populated by lymphocytes and macrophages.

Blood-borne antigens and lymphocytes enter the spleen through the splenic artery, which empties into the marginal zone. In the marginal zone, antigen is trapped by inter-digitating dendritic cells, which carry it to the PALS. Lymphocytes in the blood also enter sinuses in the marginal zone and migrate to the PALS.

The initial activation of B and T cells takes place in the T cell-rich PALS. Here interdigitating dendritic cells capture antigen and present it combined with class II MHC molecules to $T_H$ cells. Once activated, these $T_H$ cells can then activate B cells. The activated B cells, together with some $T_H$ cells, then migrate to primary follicles in the marginal zone. Upon antigenic challenge, these primary follicles develop into characteristic secondary follicles containing germinal centers (like those in the lymph nodes), where rapidly dividing B cells (centroblasts) and plasma cells are surrounded by dense clusters of concentrically arranged lymphocytes.




**Schematic diagram of splenic architecture**

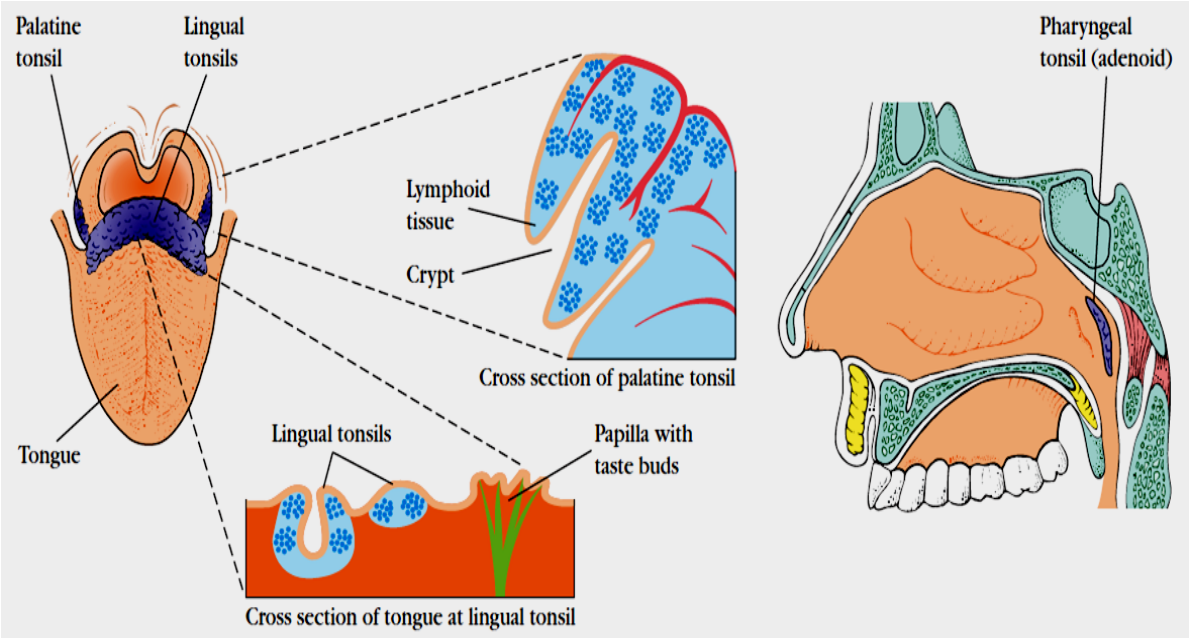
## 3. Mucosa Associated Lymphoid Tissue (MALT)

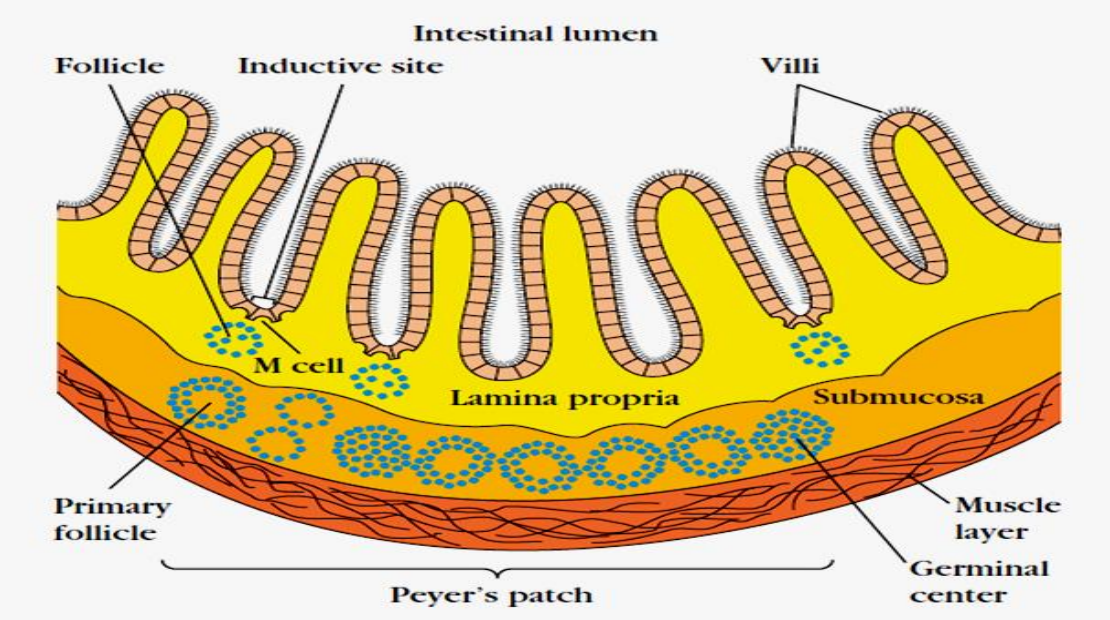
The mucosa lining the alimentary, respiratory, genitourinary and other lumina and surfaces are constantly exposed to numerous antigens. These areas contain more collection of lymphoid cells, either specialized aggregates like the Peyer's patches or scattered isolated lymphoid follicles --- collectively called the **Mucosa Associated Lymphoid Tissue (MALT).** Such lymphoid tissues in the gut, from the adenoids and tonsils to the follicles in the colon, are called the **Gut Associated Lymphoid Tissue (GALT)** and those in the respiratory tract the **Bronchus Associated Lymphoid Tissue (BALT).**

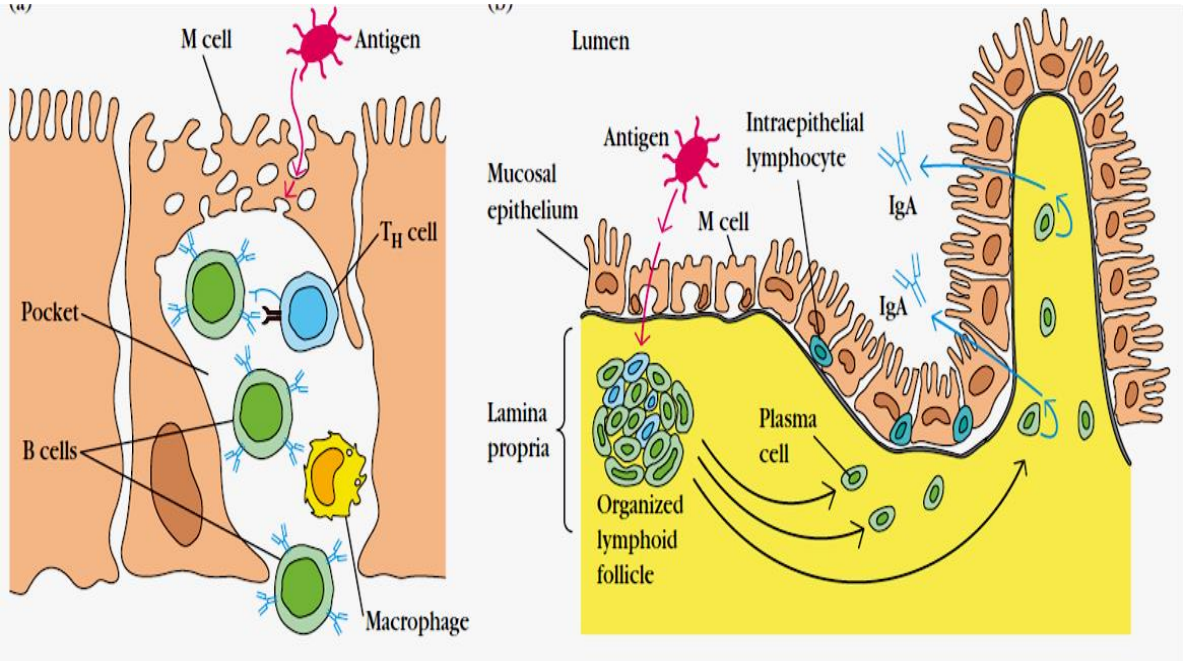
The mucous membranes lining the digestive, respiratory, and urogenital systems have a combined surface area of about 400 $m^2$ (nearly the size of a basketball court) and are the major sites of entry for most pathogens. These susceptible membrane surfaces are protected by a group of organized lymphoid tissues mentioned earlier and known collectively as **mucosal-associated lymphoid tissue (MALT).**

Structurally, these tissues range from loose, hardly organized clusters of lymphoid cells in the lamina propria of intestinal villi to well-organized structures such as the familiar tonsils and appendix, as well as Peyer's patches, which are found within the submucosal layer of the intestinal lining. The functional importance of MALT in the body's defense is indicated by its large population of antibody-producing plasma cells, whose number far exceeds that of plasma cells in the spleen, lymph nodes, and bone marrow combined.

The **tonsils** are found in three locations: lingual at the base of the tongue; palatine at the sides of the back of the mouth; and pharyngeal (adenoids) in the roof of the nasopharynx. All three tonsil groups are nodular structures consisting of a meshwork of reticular cells and fibers mixed together with lymphocytes, macrophages, granulocytes, and mast cells. The B cells are organized into follicles and germinal centers; the latter are surrounded by regions showing T-cell activity. The tonsils defend against antigens entering through the nasal and oral epithelial routes. The best studied of the mucous membranes is the one that lines the gastrointestinal tract. This tissue, like that of the respiratory and urogenital tracts, has the capacity to endocytose antigen from the lumen. Immune reactions are initiated against pathogens and antibody can be generated and exported to the lumen to combat the invading organisms.






As shown in above Figures, lymphoid cells are found in various regions within this tissue. The outer mucosal epithelial layer contains so-called **intraepithelial lymphocytes (IELs).** Many of these lymphocytes are T cells that express unusual receptors (T-cell receptors, or TCRs), which exhibit limited diversity for antigen. Although this population of T cells is well situated to encounter antigens that enter through the intestinal mucous epithelium. Their actual function remains largely unknown. The lamina propria, which lies under the epithelial layer, contains large numbers of B cells, plasma cells, activated $T_H$ cells, and macrophages in loose clusters.

Histologic sections have revealed more than 15,000 lymphoid follicles within the intestinal lamina propria of a healthy child. The submucosal layer beneath the lamina propria contains Peyer's patches, nodules of 30–40 lymphoid follicles. Like lymphoid follicles in other sites, those that compose Peyer's patches can develop into secondary follicles with germinal centers.

The epithelial cells of mucous membranes play an important role in promoting the immune response by delivering small samples of foreign antigen from the lumina of the respiratory, digestive, and urogenital tracts to the underlying mucosal-associated lymphoid tissue.

This antigen transport is carried out by specialized **M cells.** The structure of the M cell is striking: these are flattened epithelial cells lacking the microvilli that characterize the rest of the mucous epithelium. In addition, M cells have a deep invagination, or pocket, in the basolateral plasma membrane; this pocket is filled with a cluster of B cells, T cells, and macrophages. Luminal antigens are endocytosed into vesicles that are transported from the luminal membrane to the underlying pocket membrane. The vesicles then fuse with the pocket membrane, delivering the potentially response-activating antigens to the clusters of lymphocytes contained within the pocket. M cells are located in so-called **inductive sites**—small regions of a mucous membrane that lie over organized lymphoid follicle.

Antigens transported across the mucous membrane by M cells can activate B cells within these lymphoid follicles. The activated B cells differentiate into plasma cells, which leave the follicles and secrete the IgA class of antibodies. These antibodies then are transported across the epithelial cells and released as **secretory IgA** into the lumen, where they can interact with antigens.

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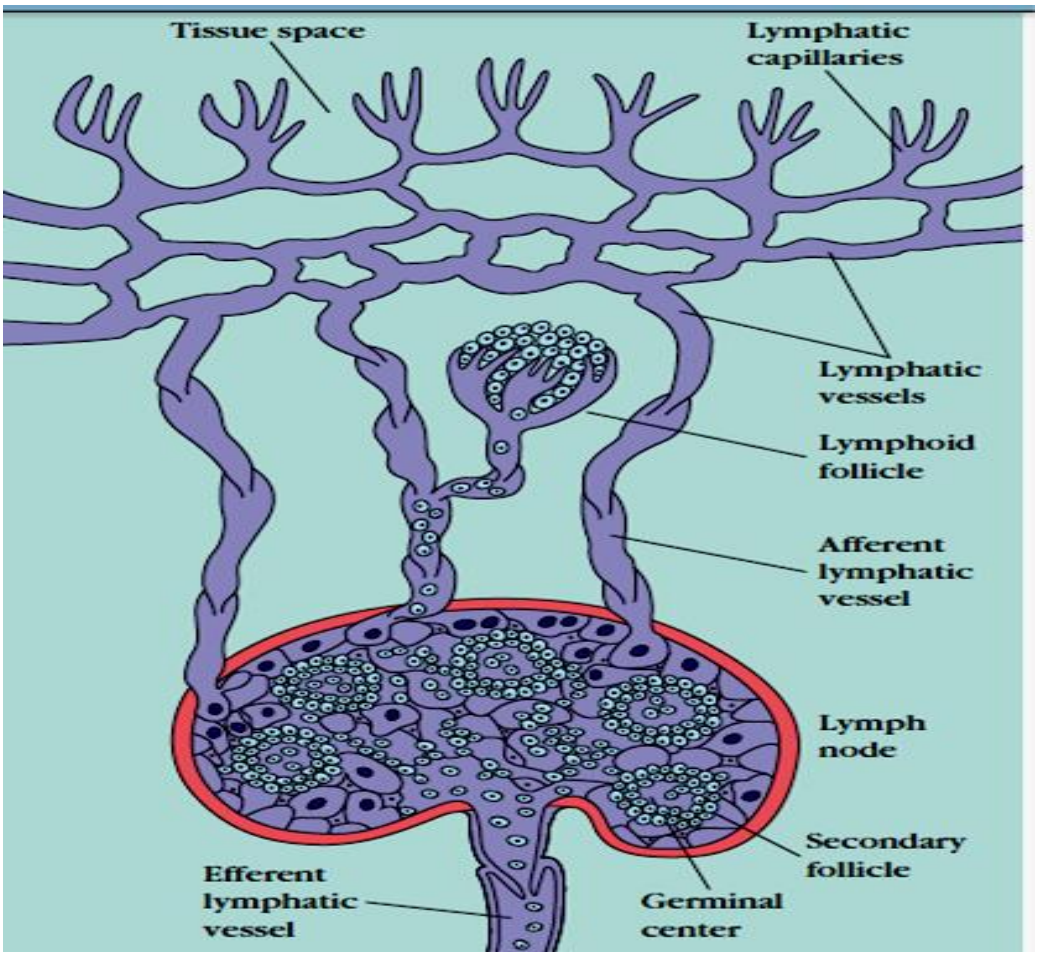
## Lymphatic System

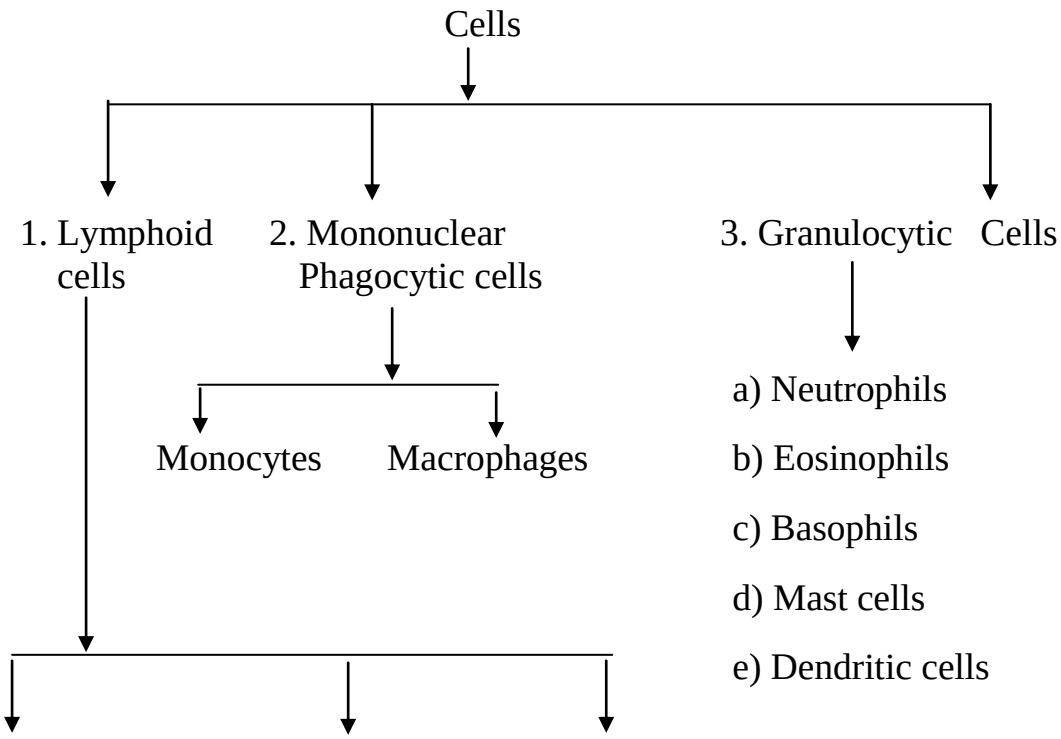
As blood circulates under pressure, its fluid component **(plasma)** leaks through the thin wall of the capillaries into the surrounding tissue. Much of this fluid, called **interstitial fluid,** returns to the blood through the capillary membranes. The remainder of the interstitial fluid, now called **lymph,** flows from the spaces in connective tissue into a network of tiny open lymphatic capillaries and then into a series of progressively larger collecting vessels called **lymphatic vessels.**




The largest lymphatic vessel, the **thoracic duct,** empties into the left subclavian vein near the heart. In this way, the lymphatic system captures fluid lost from the blood and returns it to the blood, thus ensuring steady-state levels of fluid within the circulatory system. The heart does not pump the lymph through the lymphatic system; instead the flow of lymph is achieved as the lymph vessels are squeezed by movements of the body's muscles. A series of one-way valves along the lymphatic vessels ensures that lymph flows only in one direction. When a foreign antigen gains entrance to the tissues, it is picked up by the lymphatic system (which drains all the tissues of the body) and is

carried to various organized lymphoid tissues such as lymph nodes, which trap the foreign antigen. As lymph passes from the tissues to lymphatic vessels, it becomes progressively enriched in lymphocytes. Thus, the lymphatic system also serves as a means of transporting lymphocytes and antigen from the connective tissues to organized lymphoid tissues where the lymphocytes may interact with the trapped antigen and undergo activation.

**B) Lymphoid cells (Cells of the lymphoreticular system)**




**B** Lymphocytes **T** Lymphocytes Natural Killer Cells (NK cells)

**1. Lymphoid cells: -**

Lymphocytes constitute 20%–40% of the body's white blood cells and 99% of the cells in the lymph. These lymphocytes continually circulate in the blood and lymph and are capable of migrating into the tissue spaces and lymphoid organs, thereby integrating the immune system to a high degree.

The lymphocytes can be broadly subdivided into three types of cells on the basis of function and cell-membrane components.

a) B cells
b) T cells
c) Natural killer cells (NK cells) / Null Cells

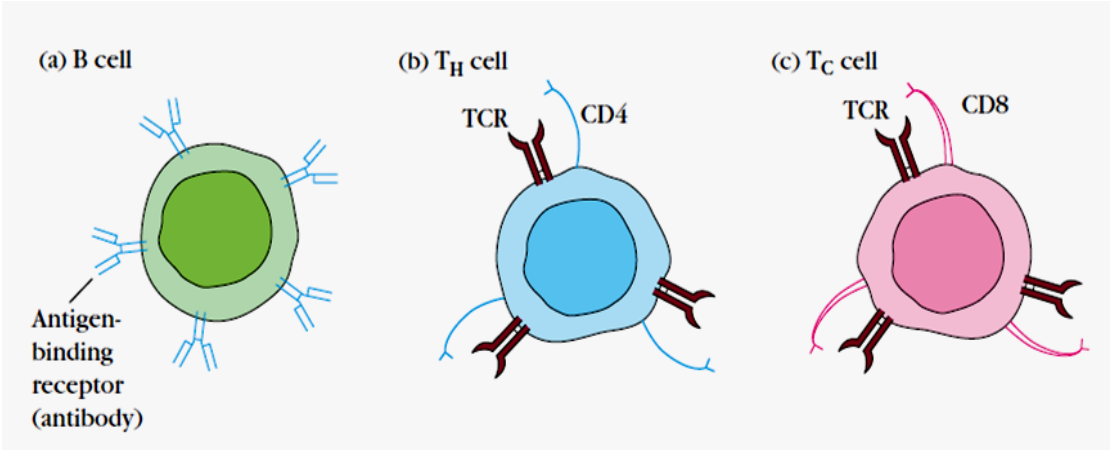
A number of surface antigens or markers have been identified on lymphocytes and other leucocytes by means of monoclonal antibodies. These markers reflect the stage of differentiation and functional properties of the cells. As they were given different designations by the investigators who prepared the antibodies, the same marker came to be known by different names **(T4, Leu3, and so on)**. Order was introduced at the **'International Workshops for Leucocyte Differentiation Antigens'** by comparing the specificities of different antisera. When a cluster of monoclonal antibodies was found to react with a particular antigen, it was defined as a separate marker and given a **CD (Cluster of Differentiation)** number. Over 150 CD markers have been identified so far.

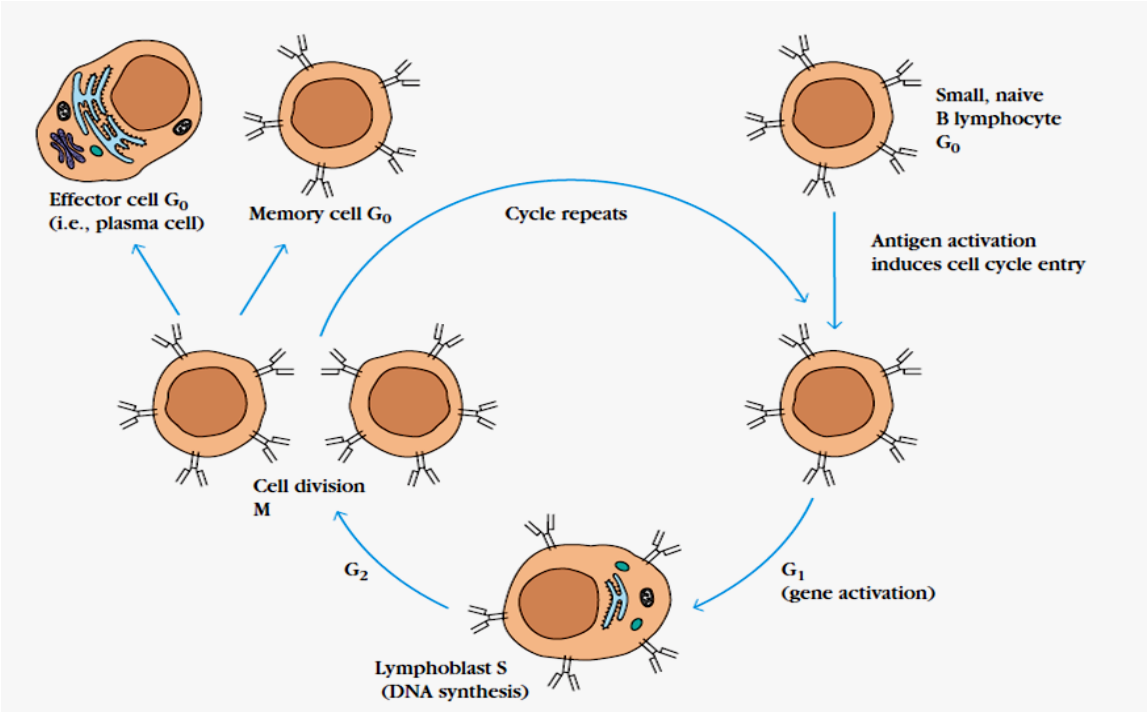
**a) B Lymphocytes**

B lymphocytes are small, motile, nonphagocytic cells, which cannot be distinguished morphologically B lymphocytes mature within the bone marrow; when they leave it, each expresses a unique antigen-binding receptor on its membrane. This antigen-binding or B-cell receptor is a membrane-bound **antibody molecule.**

When a naïve (immature / inexperienced) B cell (one that has not previously encountered antigen) first encounters the antigen that matches its membrane bound antibody, the binding of the antigen to the antibody causes the cell to divide rapidly; its progeny differentiate into **memory B cells** and **effector B cells** called **plasma cells.**

Memory B cells have a longer life span than naive cells, and they express the same membrane-bound antibody as their parent B cell. Plasma cells produce the antibody in a form that can be secreted and have little or no membrane-bound antibody. Although plasma cells live for only a few days, they secrete enormous amounts of antibody during this time.




The B lymphocyte derived its letter designation from its site of maturation, in the Bursa of Fabricius in birds; the name turned out to be apt, for Bone marrow is its major site of maturation in a number of mammalian species, including humans and mice. Mature B cells are definitively distinguished from other lymphocytes by their synthesis and display of membrane-bound immunoglobulin (antibody) molecules, which serve as receptors for antigen. Each of the approximately 1.5 X $10^5$ molecules of antibody on the membrane of single B cell has an identical binding site for antigen. Among the other molecules expressed on the membrane of mature B cells are the following:

- **B220** (a form of CD45) is frequently used as a marker for B cells and their precursors. However, unlike antibody, it is not expressed uniquely by B-lineage cells.
- **Class II MHC molecules** permit the B cell to function as an antigen-presenting cell (APC).
- **CR1** (CD35) and **CR2** (CD21) are receptors for certain complement products.
- **FcγRII** (CD32) is a receptor for IgG, a type of antibody.
- **B7-1** (CD80) and **B7-2** (CD86) are molecules that interact with CD28 and CTLA-4, important regulatory molecules on the surface of different types of T cells, including $T_H$ cells.
- **CD40** is a molecule that interacts with CD40 ligand on the surface of helper T cells. In most cases this interaction is critical for the survival of antigen stimulated B cells and for their development into antibody-secreting plasma cells or memory B cells.

Interaction between antigen and the membrane-bound antibody on a mature naive B cell, as well as interactions with T cells and macrophages, selectively induces the activation and differentiation of B-cell clones of corresponding specificity. In this process, the B cell divides repeatedly and differentiates over a 4- to 5-day period, generating a population of plasma cells and memory cells. Plasma cells, which have lower levels of membrane-bound antibody than B cells, synthesize and secrete antibody. All clonal progeny from a given B cell secrete antibody molecules with the same antigen-binding specificity. Plasma cells are terminally differentiated cells, and many die in 1 or 2 weeks.

## b) T LYMPHOCYTES

T lymphocytes are small, motile, nonphagocytic cells,which cannot be distinguished morphologically T lymphocytes also arise in the bone marrow. Unlike B cells, which mature within the bone marrow, T cells migrate to the thymus gland to mature. During its maturation within the thymus, the T cell comes to express a unique antigen-binding molecule, called the **T-cell receptor,** on its membrane. Unlike membrane-bound antibodies on B cells, which can recognize antigen alone, T-cell receptors can recognize only antigen that is bound to cell-membrane proteins called **major histocompatibility complex (MHC) molecules.** MHC molecules termed “antigen presentation,” are polymorphic glycoproteins found on cell membranes.

There are two major types of MHC molecules: Class I MHC molecules, and Class II MHC molecules. When a naive T cell encounters antigen combined with a MHC molecule on a cell, the T cell proliferates and differentiates into memory T cells and various effector T cells. There are two well-defined subpopulations of T cells: **T helper ($T_H$)** and **T cytotoxic ($T_C$) cells.** Although a third type of T cell, called a T suppressor ($T_S$) cell, T helper and T cytotoxic cells can be distinguished from one another by the presence of either **CD4** or **CD8** membrane glycoproteins on their surfaces. T cells displaying CD4 generally function as $T_H$ cells, whereas those displaying CD8 generally function as $T_C$ cells.

After a $T_H$ cell recognizes and interacts with an antigen MHC class II molecule complex, the cell is activated it becomes an effector cell that secretes various growth factors known collectively as **cytokines.** The secreted cytokines play an important role in activating B cells, $T_C$ cells, macrophages, and various other cells that participate in the immune response.

Differences in the pattern of cytokines produced by activated $T_H$ cells result in different types of immune response. Under the influence of $T_H$ derived cytokines, a $T_C$ cell that recognizes an antigen–MHC class I molecule complex proliferates and differentiates into an effector cell called a **cytotoxic T lymphocyte (CTL).** In contrast to the TC cell, the CTL generally does not secrete many cytokines and instead exhibits cell-killing or cytotoxic activity. The CTL has a vital function in monitoring the cells of the body and eliminating any that display antigen, such as virus-infected cells, tumor cells, and cells of a foreign tissue graft. Cells that display foreign antigen complexed with a class I MHC molecule are called *altered self-cells*; these are targets of CTLs.

T lymphocytes derive their name from their site of maturation in the thymus. Like B lymphocytes, these cells have membrane receptors for antigen. Most T cells recognize antigen only when it is bound to a self-molecule encoded by genes within the major histocompatibility complex (MHC).

B cell is capable of binding soluble antigen, whereas the T cell is restricted to binding antigen displayed on self-cells. To be recognized by most T cells, this antigen must be displayed together with MHC molecules on the surface of antigen-presenting cells or on virus-infected cells, cancer cells, and grafts. Like B cells, T cells express distinctive membrane molecules. All T-cell subpopulations express the T-cell receptor, a complex of polypeptides that includes CD3; and most can be distinguished by the presence of one or the other of two membrane molecules, CD4 and CD8. T cells that express the membrane glycoprotein molecule CD4 are restricted to recognizing antigen bound to class

II MHC molecules, whereas T cells expressing CD8, a dimeric membrane glycoprotein, are restricted to recognition of antigen bound to class I MHC molecules.

In general, expression of CD4 and of CD8 also defines two major functional subpopulations of T lymphocytes. $CD4^+$ T cells generally function as T helper ($T_H$) cells and are class-II restricted; CD8+ T cells generally function as T cytotoxic ($T_C$) cells and are class-I restricted. Thus the ratio of $T_H$ to $T_C$ cells in a sample can be approximated by assaying the number of $CD4^+$ and $CD8^+$ T cells. This ratio is approximately 2:1 in normal human peripheral blood, but it may be significantly altered by immunodeficiency diseases, autoimmune diseases, and other disorders. Some $CD4^+$ cells can act as killer cells. Also, some $T_C$ cells have been shown to secrete a variety of cytokines and exert an effect on other cells comparable to that exerted by $T_H$ cells.

T helper ($T_H$) cells as being $CD4^+$ and class-II restricted and of T cytotoxic cells ($T_C$) as being $CD8^+$ and class-I restricted $T_H$ cells are activated by recognition of an antigen–class II MHC complex on an antigen-presenting cell. After activation, the $T_H$ cell begins to divide and gives rise to a clone of effector cells, each specific for the same antigen–class II MHC complex. These $T_H$ cells secrete various cytokines, which play a central role in the activation of B cells, T cells, and other cells that participate in the immune response.

$T_C$ cells are activated when they interact with an antigen–class I MHC complex on the surface of an altered self-cell (e.g., a virus-infected cell or a tumor cell) in the presence of appropriate cytokines. This activation, which results in proliferation, causes the $T_C$ cell to differentiate into an effector cell called a C**ytotoxic T Lymphocyte (CTL).** In contrast to $T_H$ cells, most CTLs secrete few cytokines. Instead, CTLs acquire the ability to recognize and eliminate altered self-cells.

Another subpopulation of T lymphocytes—called **T suppressor ($T_S$) cells**—has been postulated. It is clear that some T cells help to suppress the humoral and the cell-mediated branches of the immune system,

### c) Natural Killer cells (NK cells) / Null Cells

The natural killer cells contain a small population of large, granular lymphocytes that display cytotoxic activity against a wide range of tumor cells in the absence of any previous immunization with the tumor. They are called **'Null Cells'.** Because of their morphology, they are also known as **'Large Granular lymphocytes' (LGL).** They are nearly double the size of the small

lymphocytes, with indented nuclei and abundant cytoplasm containing several azurophilic granules, composed of mitochondria, ribosomes, endoplasmic reticulum and Golgi apparatus.

LGL (Null Cells) are a heterogenous group of cells with differences in their functional and surface marker features. The most important member of this group is the ***Natural Killer Cell*** **(NK).** Others are the ***Antibody Dependent Cellular Cytotoxic*** **(ADCC)** lymphocytes and the ***Lymphokine Activated Killer*** **(LAK)** cells.

NK cells were subsequently shown to play an important role in host defense both against tumor cells and against cells infected with some viruses. These cells, which constitute 5%–10% of lymphocytes in human peripheral blood, do not express the membrane molecules and receptors that distinguish T- and B-cell lineages.

Although NK cells do not have T-cell receptors or immunoglobulin incorporated in their plasma membranes, they can recognize potential target cells in two different ways. In some cases, an NK cell employs NK cell receptors to distinguish abnormalities, notably a reduction in the display of class I MHC molecules and the unusual profile of surface antigens displayed by some tumor cells and cells infected by some viruses. Another way in which NK cells recognize potential target cells depends upon the fact that some tumor cells and cells infected by certain viruses display antigens against which the immune system has made an antibody response, so that antitumor or antiviral antibodies are bound to their surfaces. Because NK cells express CD16, a membrane receptor for the carboxyl-terminal end of the IgG molecule, called the Fc region, they can attach to these antibodies and subsequently destroy the targeted cells. This is an example of a process known as A**ntibody-Dependent Cell mediated Cytotoxicity (ADCC).**

Lymphokine Activated Killer (LAK) cells are NK lymphocytes treated with **Interleukin – 2 (IL – 2),** which are cytotoxic to a wide range of tumor cells without affecting normal cells. LAK cells have shown promise in the treatment of some tumors such as renal cell carcinoma. IL – 2 also acts as a growth factor for NK cells.

## 2. Mononuclear Phagocytic cells

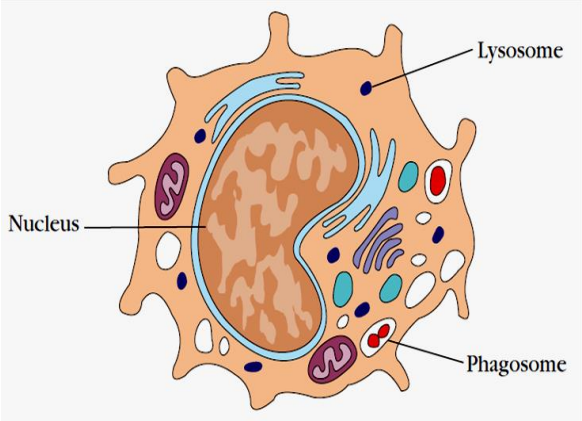
The mononuclear phagocytic system consists of

**a) Monocytes (**circulating in the blood)

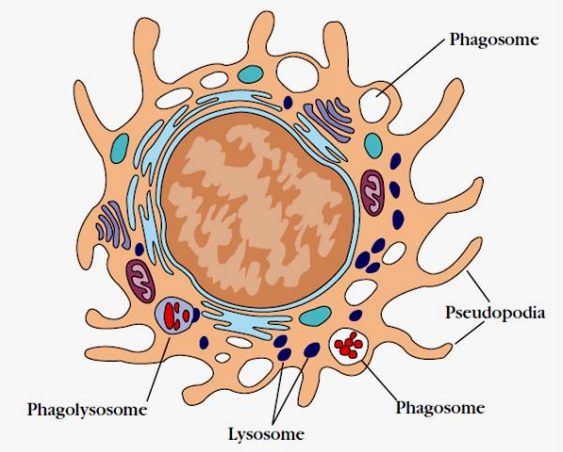
**b) Macrophages (**circulating in the tissues)

During hematopoiesis in the bone marrow, granulocyte-monocyte progenitor cells differentiate into promonocytes, which leave the bone marrow and enter the blood, where they further differentiate into mature monocytes.

Monocytes circulate in the bloodstream for about 8 h, during which they enlarge; they then migrate into the tissues and differentiate into specific tissue macrophages or into dendritic cells.




**Monocyte**




**Macrophage**

Differentiation of a monocyte into a tissue macrophage involves a number of changes: The cell enlarges five to tenfold; its intracellular organelles increase in both number and complexity; and it acquires increased phagocytic ability, produces higher levels of hydrolytic enzymes, and begins to secrete a variety of soluble factors. Macrophages are dispersed throughout the body. Some take up residence in particular tissues, becoming fixed macrophages, whereas others remain motile and are called free or wandering macrophages. Free macrophages travel by amoeboid movement throughout the tissues.

Macrophage like cells serve different functions in different tissues and are named according to their tissue location:

- **Kupffer cells** in the liver
- **Histiocytes** in connective tissues
- **Alveolar macrophages** in the lung
- **Osteoclasts** in bone
- **Mesangial cells** in the kidney
- **Microglial cells** in the brain

Although normally in a resting state, macrophages are activated by a variety of stimuli in the course of an immune response. Phagocytosis of particulate antigens serves as an initial activating stimulus. However, macrophage activity can be further enhanced by cytokines secreted by activated $T_H$ cells, by mediators of the inflammatory response, and by components of bacterial cell walls. One of the most potent activators of macrophages is interferon gamma (IFN- γ) secreted by activated $T_H$ cells.

Activated macrophages are more effective than resting ones in eliminating potential pathogens, because they exhibit greater phagocytic activity, an increased ability to kill ingested microbes, increased secretion of inflammatory mediators, and an increased ability to activate T cells. In addition, activated macrophages, but not resting ones, secrete various cytotoxic proteins that help them eliminate a broad range of pathogens, including virus-infected cells, tumor cells, and intracellular bacteria. Activated macrophages also express higher levels of class II MHC molecules, allowing them to function more effectively as antigen-presenting cells. Thus, macrophages and $T_H$ cells facilitate each other's activation during the immune response.

## ANTIMICROBIAL AND CYTOTOXIC ACTIVITIES

A number of antimicrobial and cytotoxic substances produced by activated macrophages can destroy phagocytosed microorganisms. Many of the mediators of cytotoxicity listed as follows are reactive forms of oxygen.

| Oxygen-dependent killing | Oxygen-independent killing |
|---|---|
| Reactive oxygen intermediates | Defensins |
| $O^{\bullet}{}_2^-$ (superoxide anion) | Tumor necrosis factor α |
| $OH^{\bullet}$ (hydroxyl radicals) | (macrophage only) |
| $H_2O_2$ (hydrogen peroxide) | Lysozyme |
| $ClO^-$ (hypochlorite anion) | Hydrolytic enzymes |
| Reactive nitrogen intermediates | |
| NO (nitric oxide) | |
| $NO_2$ (nitrogen dioxide) | |
| $HNO_2$ (nitrous acid) | |
| Others | |
| $NH_2CL$ (monochloramine) | |

## ANTIGEN PROCESSING AND PRESENTATION

Most of the antigen ingested by macrophages is degraded and eliminated. Phagocytosed antigen is digested within the endocytic processing pathway into peptides that associate with class II MHC molecules; these peptide–class II MHC complexes then move to the macrophage membrane. Activation of macrophages induces increased expression of both class II MHC molecules and the co-stimulatory B7 family of membrane molecules, thereby rendering the macrophages more effective in activating $T_H$ cells. This processing and presentation of antigen are critical to $T_H$ cell activation, a central event in the development of both humoral and cell-mediated immune responses.

## SECRETION OF FACTORS

A number of important proteins central to development of immune responses are secreted by activated macrophages. These include a collection of cytokines, such as **interleukin 1 (IL-1),** TNF- α and **interleukin 6 (IL-6),** that promote inflammatory responses. Typically, each of these agents has a variety of effects. For example, IL-1 activates lymphocytes; and IL-1, IL-6 and TNF-α promote fever by affecting the thermoregulatory center in the hypothalamus.

| Factor | Function |
|---|---|
| Interleukin 1 (IL-1) | Promotes inflammatory responses and fever |
| Interleukin 6 (IL-6)<br>TNF-α | Promote innate immunity and elimination of pathogens |
| Complement proteins | Promote inflammatory response and elimination of pathogens |
| Hydrolytic enzymes | Promote inflammatory response |
| Interferon alpha (IFN-α) | Activates cellular genes, resulting in the production of proteins that confer an antiviral state on the cell |
| Tumor necrosis factor (TNF-α) | Kills tumor cells |
| GM-CSF<br>G-CSF<br>M-CSF | Promote inducible hematopoiesis |

## 3. Granulocytic cells

On the basis of cellular morphology and cytoplasmic staining characteristics, the **granulocytes** are classified as follows

a) Neutrophils

b) Eosinophils

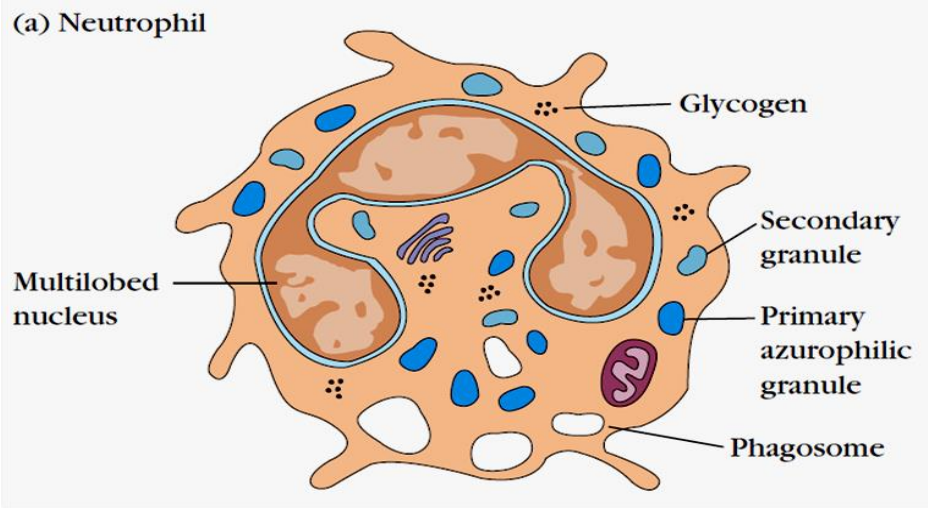
c) Basophils

d) Mast cells

e) Dendritic cells

### a) Neutrophils

The **neutrophil** has a multilobed nucleus and a granulated cytoplasm that stains with both acid and basic dyes; it is often called a polymorphonuclear leukocyte (PMN) for its multilobed nucleus and constitutes 50%–70%. These are phagocytic.




Neutrophils are produced by hematopoiesis in the bone marrow. They are released into the peripheral blood and circulate for 7–10 h before migrating into the tissues, where they have a life span of only a few days. In response to many types of infections, the bone marrow releases more than the usual number of neutrophils and these cells generally are the first to arrive at a site of inflammation. The resulting transient increase in the number of circulating neutrophils, called **leukocytosis,** is used medically as an indication of infection.

Movement of circulating neutrophils into tissues, called **extravasation,** takes several steps: the cell first adheres to the vascular endothelium, then penetrates the gap between adjacent endothelial cells lining the vessel wall, and finally penetrates the vascular basement membrane, moving out into the tissue spaces.

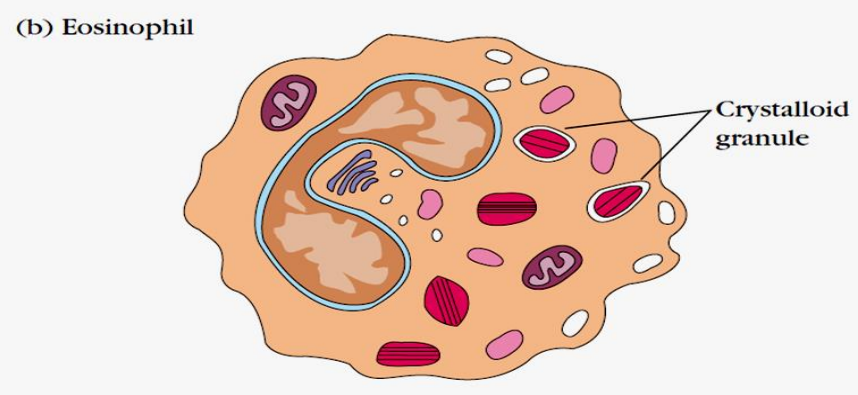
A number of substances generated in an inflammatory reaction serve as **chemotactic factors** that promote accumulation of neutrophils at an inflammatory site. Among these chemotactic factors are some of the complement components, components of the blood-clotting system, and several cytokines secreted by activated $T_H$ cells and macrophages.

Like macrophages, neutrophils are active phagocytic cells. Phagocytosis by neutrophils is similar to that described for macrophages, except that the lytic enzymes and bactericidal substances in neutrophils are contained within primary and secondary granules. The larger, denser primary granules are a type of lysosome containing peroxidase, lysozyme, and various hydrolytic enzymes. The smaller secondary granules contain collagenase, lactoferrin, and lysozyme. Both primary and secondary granules fuse with phagosomes, whose contents are then digested and eliminated much as they are in macrophages.

Neutrophils also employ both oxygen-dependent and oxygen-independent pathways to generate antimicrobial substances. Neutrophils are in fact much more likely than macrophages to kill ingested microorganisms. Neutrophils exhibit a larger respiratory burst than macrophages and consequently are able to generate more reactive oxygen intermediates and reactive nitrogen intermediates. In addition, neutrophils express higher levels of defensins than macrophages do.

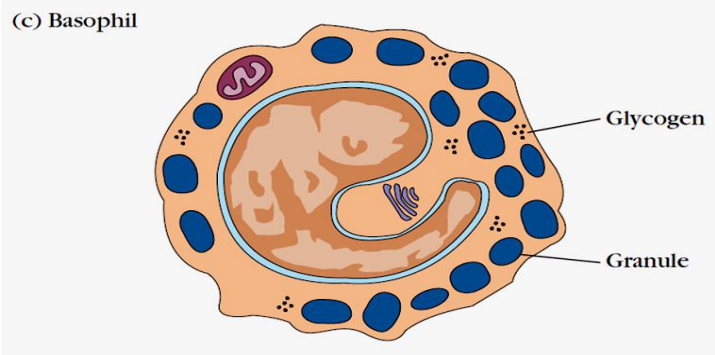
### b) Eosinophils

The **eosinophil** has a bilobed nucleus and a granulated cytoplasm that stains with the acid dye eosin red (hence its name). Eosinophils, like neutrophils, are motile phagocytic cells that can migrate from the blood into the tissue spaces and constitute 1%–3%. Their phagocytic role is significantly less important than that of neutrophils, and it is thought that they play a role in the defense against parasitic organisms. The secreted contents of eosinophilic granules may damage the parasite membrane.




**c) Basophils**

The **basophil** has a lobed nucleus and heavily granulated cytoplasm that stains with the basic dye methylene blue. Basophils are not phagocytic and constitute less than1%.



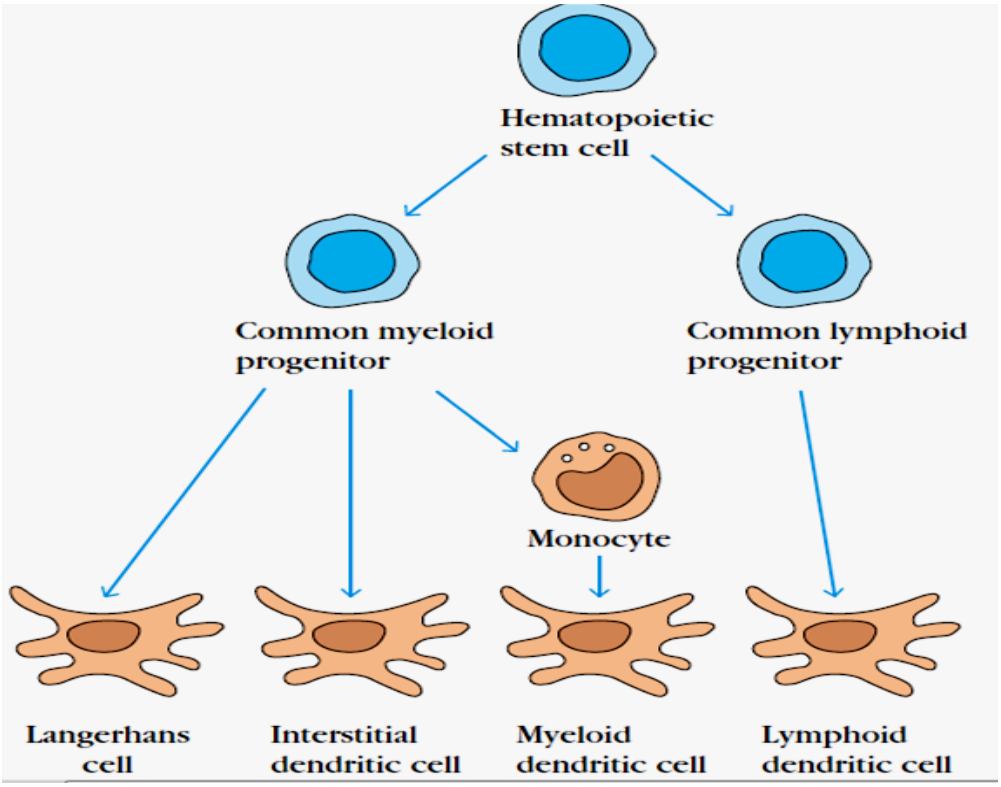

**d) Mast cells**

Mast-cell precursors, which are formed in the bone marrow by hematopoiesis, are released into the blood as undifferentiated cells; they do not differentiate until they leave the blood and enter the tissues. Mast cells can be found in a wide variety of tissues, including the skin, connective tissues of various organs, and mucosal epithelial tissue of the respiratory, genitourinary, and digestive tracts. Like circulating basophils, these cells have large numbers of cytoplasmic granules that contain histamine and other pharmacologically active substances. Mast cells, together with blood basophils, play an important role in the development of allergies.

**e) Dendritic cells**

The **dendritic cell (DC)** acquired its name because it is covered with long membrane extensions that resemble the dendrites of nerve cells. Dendritic cells can be difficult to isolate because the conventional procedures for cell isolation

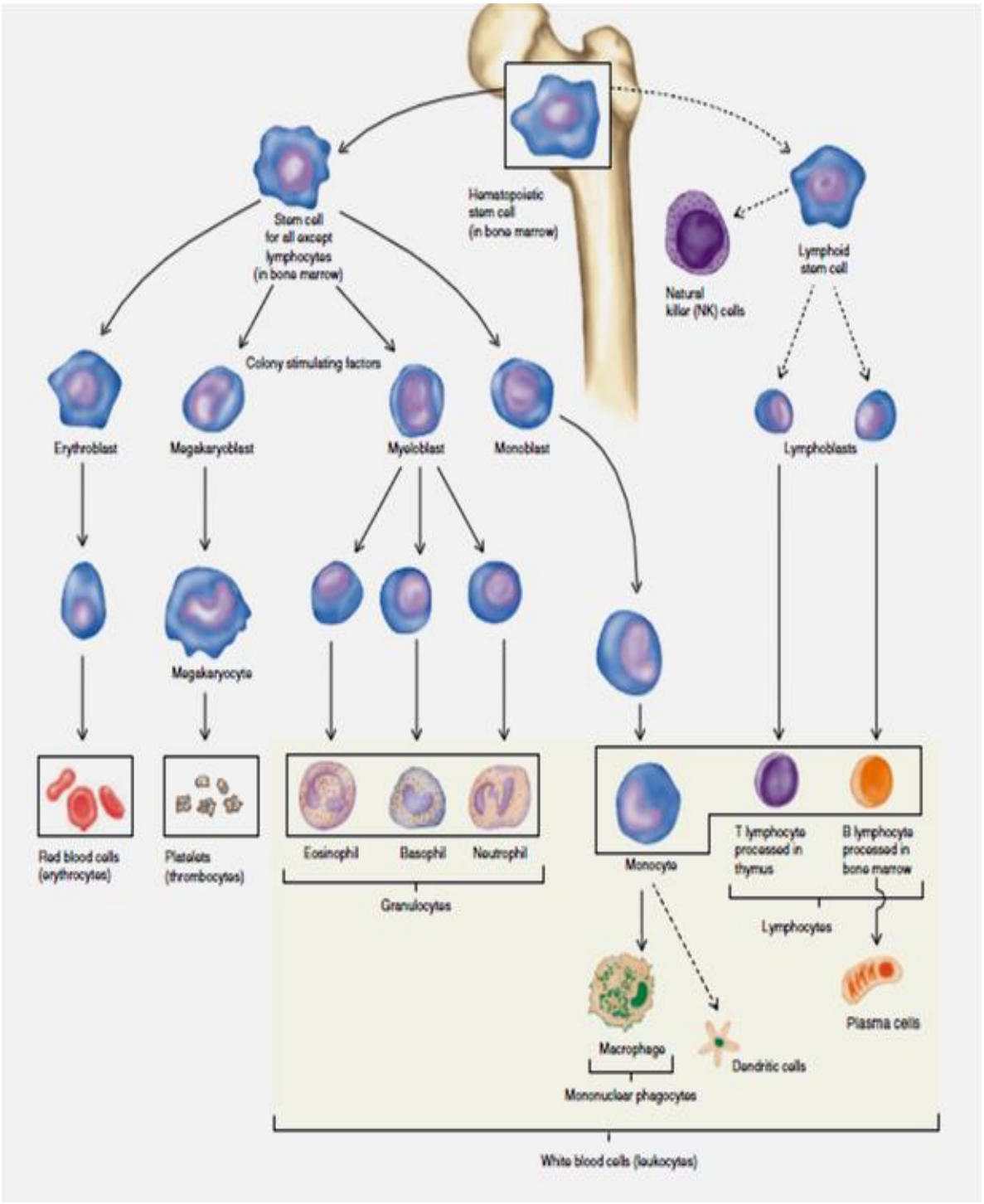
tend to damage their long extensions. There are many types of dendritic cells, although most mature dendritic cells have the same major function, the presentation of antigen to $T_H$ cells. Four types of dendritic cells are known: Langerhans cells, interstitial dendritic cells, myeloid cells, and lymphoid dendritic cells. Each arises from hematopoietic stem cells via different pathways and in different locations.




Above figure shows that they descend through both the myeloid and lymphoid lineages. Despite their differences, they all constitutively express high levels of both class II MHC molecules and members of the co-stimulatory B7 family. For this reason, they are more potent antigen-presenting cells than macrophages and B cells, both of which need to be activated before they can function as antigen-presenting cells (APCs). Immature or precursor forms of each of these types of dendritic cells acquire antigen by phagocytosis or endocytosis; the antigen is processed, and mature dendritic cells present it to $T_H$ cells. Following microbial invasion or during inflammation, mature and immature forms of Langerhans cells and interstitial dendritic cells migrate into draining lymph nodes, where they make the critical presentation of antigen to $T_H$ cells that is required for the initiation of responses by those key cells.

Another type of dendritic cell, the **follicular dendritic cell**, does not arise in bone marrow and has a different function from the antigen-presenting dendritic cells described above. Follicular dendritic cells do not express class II MHC molecules and therefore do not function as antigen presenting cells for

$T_H$-cell activation. These dendritic cells were named for their exclusive location in organized structures of the lymph node called lymph follicles, which are rich in B cells. Although they do not express class II molecules, follicular dendritic cells express high levels of membrane receptors for antibody, which allows the binding of antigen-antibody complexes. The interaction of B cells with this bound antigen can have important effects on B cell responses.

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# Production of antibodies (Imunoglobulins)

## *Activation of B Lymphocytes: Clonal Selection, Expansion, and Antibody Production*

The immunologic activation of most B cells requires a series of events-

**1. Clonal selection and binding of antigen.**

In this case, a precommitted B cell of a particular clonal specificity picks up the antigen on its Ig receptors and processes it into small peptide determinants. The antigen is then bound to the MHC II receptors on the B cell. The MHC/Ag receptor on the B cell is bound by a $T_H$ cell.

**2. Instruction by chemical mediators.**

The B cell receives developmental signals from macrophages and T cells (interleukin- 2 and interleukin-6) and various other growth factors, such as IL-4 and IL-5.

**3.** The combination of these stimuli on the membrane receptors causes a signal to be transmitted internally to the B-cell nucleus.

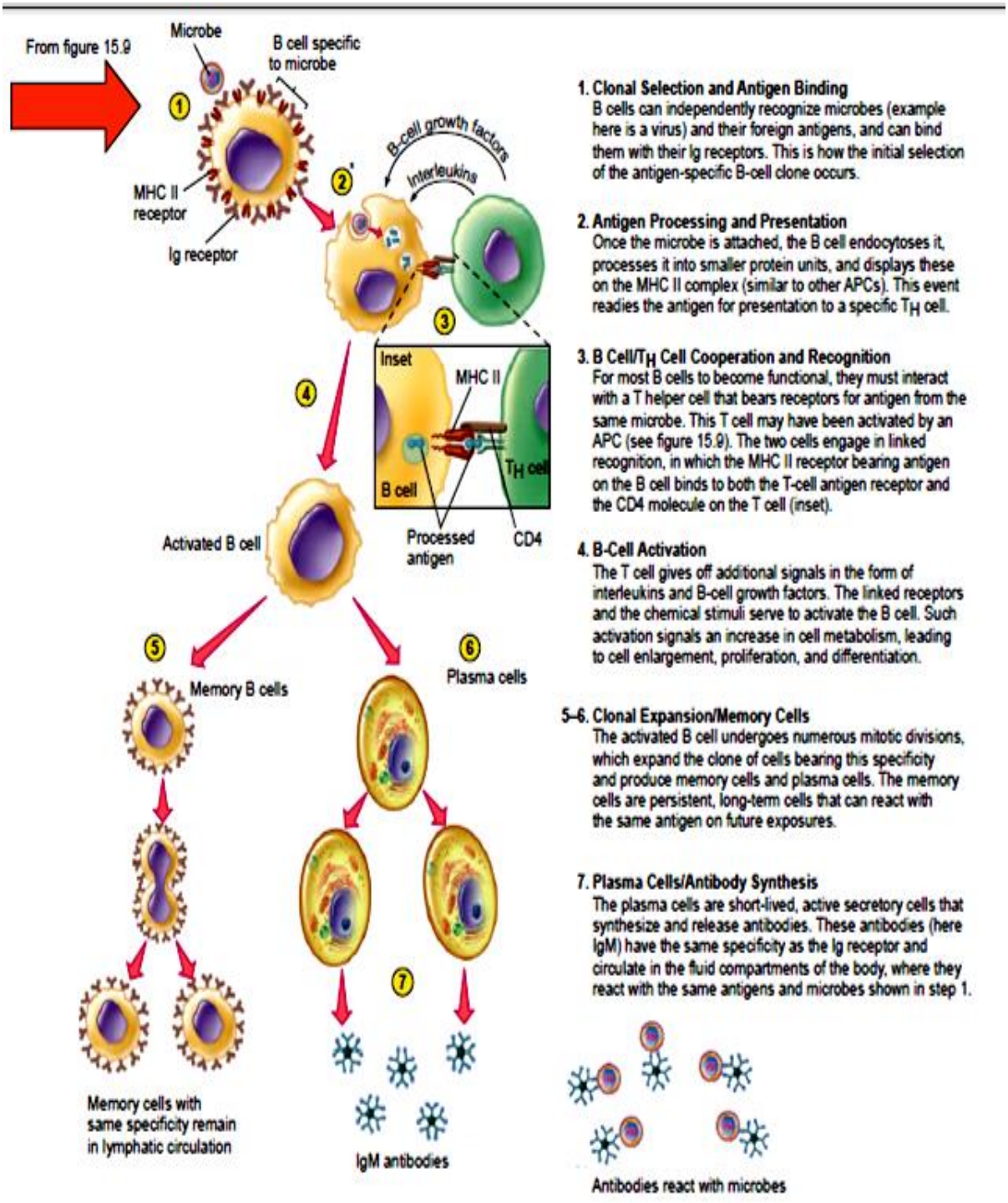
**4.** These events trigger B-cell activation. An activated B cell called a *lymphoblast* undergoes an increase in size and DNA and protein synthesis, in preparation for entering the cell cycle and mitosis.

**5–6. Clonal expansion.**

A stimulated B cell multiplies through successive mitotic divisions and produces a large population of genetically identical daughter cells. Some cells that stop short of becoming fully differentiated are **memory cells,** which remain for long periods to react with that same antigen at a later time. This reaction also expands the clone size, so that subsequent exposure to that antigen provides more cells with that specificity. This expansion of the clone size is one factor in the increased memory response. The most numerous progeny are large, specialized, terminally differentiated B cells called **plasma cells.**

**7. Antibody production and secretion.**

The primary action of plasma cells is to secrete into the surrounding tissues copious amounts of antibodies with the same specificity as the original receptor. Although an individual plasma cell can produce around 2,000 antibodies per second, production does not continue indefinitely. The plasma cells do not survive for long and deteriorate after they have synthesized antibodies.



**Regulation of antibody production (genetic control) / Diversity of Antibodies**

One unique property of antibodies is their remarkable diversity. According to current estimates each human can synthesize more than $10^{11}$ (100 billion) different kinds of antibodies. How is this diversity generated? The answer is threefold:

- rearrangement of antibody gene segments,
- somatic mutations,
- and generation of different codons during antibody gene splicing.

I Immunoglobulin genes are split or interrupted genes with many gene segments.

Embryonic B cells contain a small number of gene segments, close together on the same chromosome, that determine the constant (C) region of the light chains. Separated from them, but on the same chromosome, is a larger cluster of segments that determines the variable (V) region of the light chains. During B-cell differentiation, one segment for the constant region is joined by a process of recombination mediated by specific proteins to one segment for the variable region. This splicing produces a complete light-chain antibody gene. A similar splicing mechanism also occurs to join the constant and variable segments of the heavy chains. Because the light-chain genes actually consist of three parts, and the heavy-chain genes consist of four, the formation of a finished antibody molecule is slightly more complicated.

Germ line DNA for the light chain gene contains multiple coding sequences called V and J (joining) regions. During the differentiation of a B cell, a deletion (which is variable in length) occurs that joins one V gene segment with one J segment. This DNA joining process is termed combinatorial joining because it can create many combinations of the V and J regions. When the light-chain gene is transcribed, transcription continues through the DNA region that encodes for the constant portion of the gene. RNA splicing subsequently joins the VJ and C regions, creating mRNA.

Combinatorial joining in the formation of a heavy-chain gene occurs by means of DNA splicing of the heavy-chain counterparts of V and J along with a third set of D (diversity) sequences. Initially, all heavy chains have the type of constant region. This corresponds to antibody class IgM. Another DNA splice

joins the VDJ region with a different constant region that can subsequently change the class of antibody produced by the B cell.

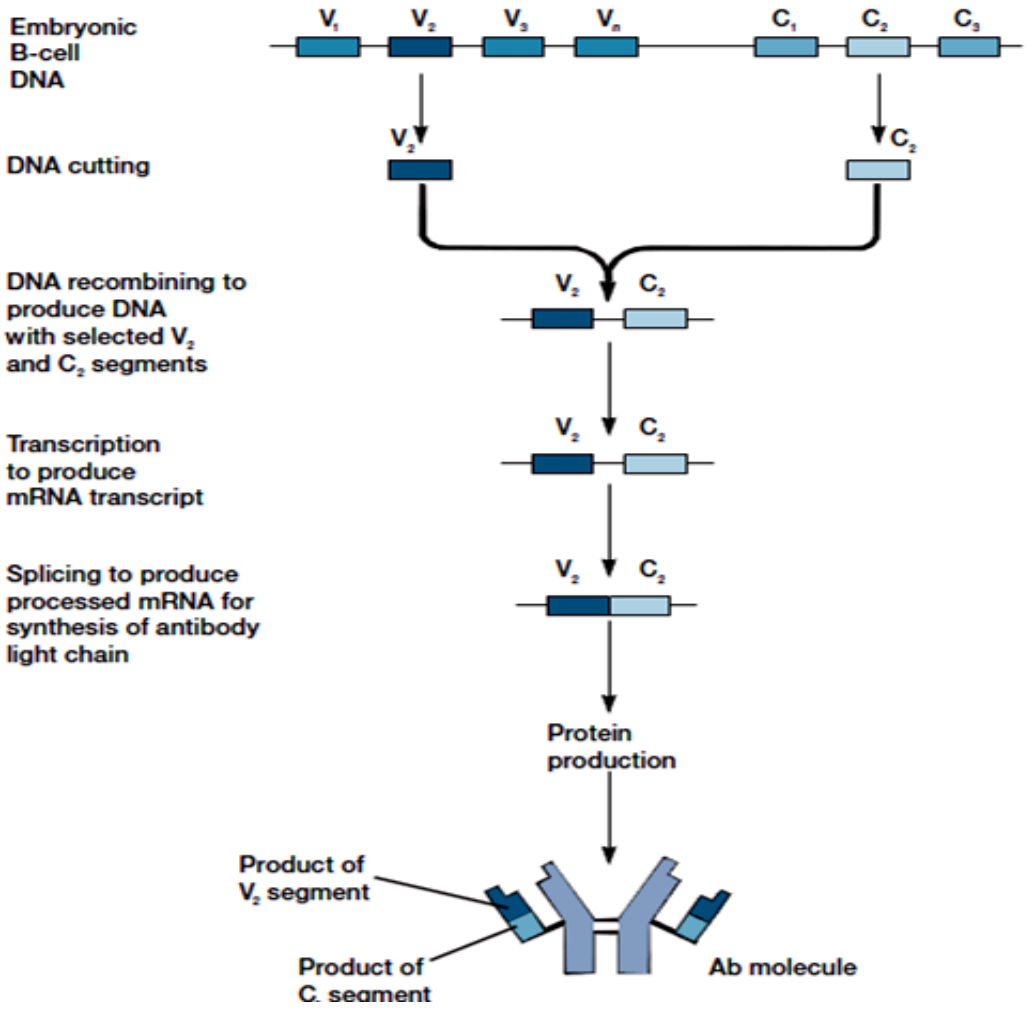
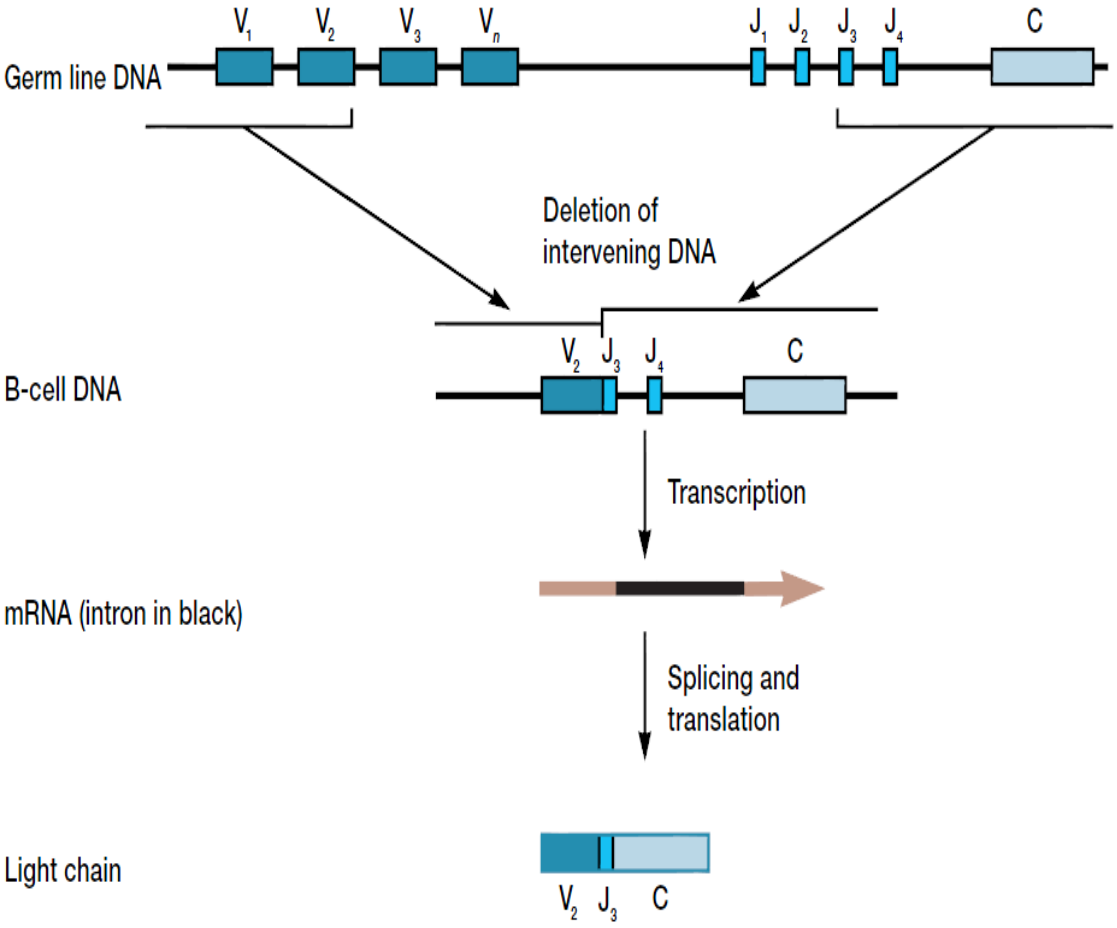



**Fig. Gene Shuffling and Antibody Diversity.** Antibody diversity is partly the result of the shuffling of gene sequences that code for both heavy and light chains. This drawing shows the shuffling, cutting, and splicing process used to produce an assembled light chain of the antibody molecule.

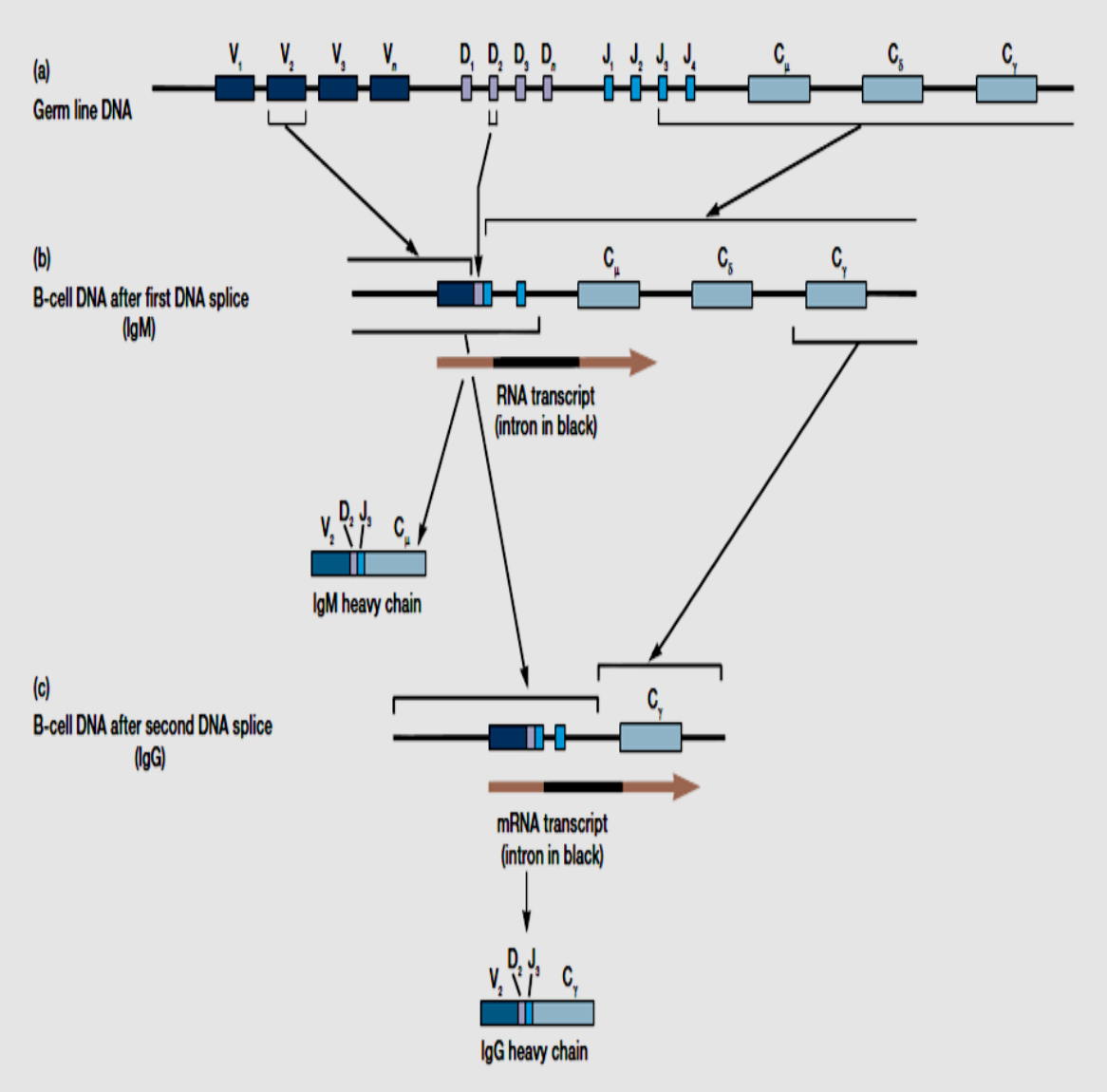



The amount of antibody diversity in the mouse that can be generated by combinatorial joining is shown in **table below.**

| | |
|---|---|
| λ light chains | V regions = 2<br>J regions = 3<br>Combinations = $2 \times 3 = 6$ |
| κ light chains | $V_\kappa$ regions = 250–350<br>$J_\kappa$ regions = 4<br>Combinations = $250 \times 4 = 1{,}000$<br>$= 350 \times 4 = 1{,}400$ |
| Heavy chains | $V_H$ = 250–1,000<br>D = 10–30<br>$J_H = 4$<br>Combinations = $250 \times 10 \times 4 = 10{,}000$<br>$= 1{,}000 \times 30 \times 4 = 120{,}000$ |
| Diversity of antibodies | κ-containing: $1{,}000 \times 10{,}000 = 10^7$<br>$1{,}400 \times 120{,}000 = 2 \times 10^8$<br>λ-containing: $6 \times 10{,}000 = 6 \times 10^4$<br>$6 \times 120{,}000 = 7 \times 10^5$ |

In this animal the κ light chains can be formed from any combination of about 250 to 350 $V_k$ and 4 $J_k$ regions giving a maximum of approximately 1,400 different k chains. The λ chains have their own $V_\lambda$ and $J_\lambda$ regions but smaller in number than their κ counterparts (6 different λ chains).

The heavy chains have approximately 250 to 1,000 $V_H$, 10 to 30 D, and 4 $J_H$ regions, giving a maximum of 120,000 different combinations. Because any light chain can combine with any heavy chain, there will be a maximum of 2 $10^8$ possible κ chain antibody types.




**The Formation of a Gene for the Heavy Chain of an Antibody Molecule.**

## Specificity of Antibodies / clonal selection theory

As noted previously, combinatorial joining, somatic mutations, and variations in the splicing process generate the great variety of antibodies produced by mature B cells. From a large, diverse B cell pool, specific cells are stimulated by antigens to reproduce and form a B-cell clone whose cells contain the same genetic information. This is known as the **clonal selection theory,** a hypothesis to explain immunologic specificity and memory.

The existence of a B-cell **clone** (a population of cells derived asexually from a single parent) that can respond to an antigen by producing the correct antibody is the first tenet of this theory. The lymphoid system is thus considered to contain many B-cell clones, each clone able to recognize a specific antigen. The antigen selects the appropriate B cell to form a clone (hence the phrase "clonal selection"), and the cells with other antigen specificities are unaffected.

According to the second tenet, each B-cell is genetically programmed to respond to its own distinctive antigen before the antigen is introduced. The particular antibody an individual B cell can produce is integrated into the plasma membrane of that B cell and acts as a specific surface receptor for the corresponding antigen molecule. The reaction of the antibody and antigen initiates the differentiation and multiplication of the B cell to form two different cell populations: plasma cells and **memory B cells.**

Plasma cells are literally protein factories that produce about 2,000 antibodies per second in their brief five- to seven-day life span. Memory B cells can initiate the antibody-mediated immune response upon detecting the particular antigen specific for their antibody molecules. These memory cells circulate more actively from blood to lymph and live much longer (years or even decades) than plasma cells. Memory cells are responsible for the immune system's rapid secondary antibody response to the same antigen. Finally, memory B cells and plasma cells are usually not produced unless the B cell has interacted with, and received cytokine signals from, activated helper T cells.

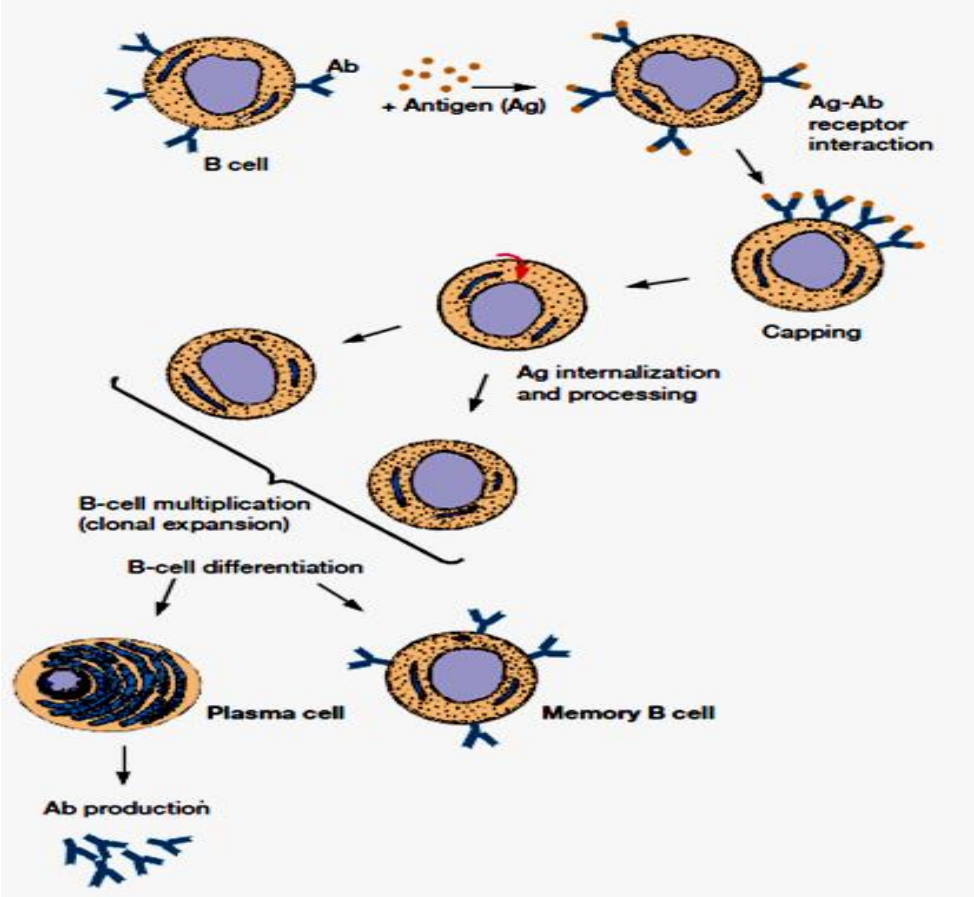



**Fig. Clonal Selection.**

The immune system can respond specifically to a myriad of possible antigens, whether they are individual molecules or are attached to pathogens and abnormal cells such as cancer cells. B cells or B lymphocytes constantly roam the body, particularly the blood and lymphoid tissues. Each B cell synthesizes only one of the millions of possible antibodies and displays this antibody on its surface. When the antigen meets a B cell having a surface antibody of the proper specificity (top left), it complexes with the antibody and capping occurs. (Capping is the regional aggregation of antibodies on the surface of the cell following Ag-Ab interaction.) The antigen is then internalized; the B cell swells and begins to divide rapidly, producing a B-cell clone. The activated B-cell clone differentiates into plasma cells and memory cells. Plasma cells form the specific antibody that immediately attacks the antigen that provoked its formation. Memory B cells persist in the body and boost the immune system's readiness to eliminate the same antigen if it presents itself in the future. Most antibody responses also require signals from T helper cells.

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# Factors influencing antibody production

*1.* ***Genetic factor:*** The immune response is under genetic control. The differences in immune response to the same antigen shown by different individuals in a species is determined by genetic differences. The terms 'responded and 'non-responder' are used for individuals who are or are not capable of responding to a particular antigen. The Ir genes control this property.

***2. Age:*** The embryo is immunologically immature. The capacity to produce antibodies starts only with the development and differentiation of lymphoid organs. The age at which embryos acquire immunological competence varies with different species. When the potential immunocompetent cell comes into contact with its specific antigen during embryonic life, the response is elimination of the cell or induction of tolerance. This is believed to be the basis for the nonantigenicity of self antigens. During embryonic life, the developing lymphoid cells come into contact with all the tissue antigens of the body released by cellular breakdown, so that all clones of cells that have specificity towards self antigens are eliminated.

Immunocompetence is not complete at birth, but continues to develop as the infant grows. The infant has to depend on itself for antibody production from 3-6 months of age, by which time the maternal antibodies disappear. But full competence is acquired only by about the age of 4 years.

***3. Nutritional status:*** Malnutrition affects the immune response adversely, though serum components necessary for immunity are conserved selectively till nutritional deficiency becomes marked. Protein calorie malnutrition suppresses both humoral and cellular immune responses, the latter more severely. Deficiencies of aminoacids (tryptophan. phenylalanine. methionine. glycine, isoleucine) and vitamins (vitamin A, and B group factors riboflavine, pyridoxine. pantothenic acid, folic acid) have been shown to cause decrease in antibody synthesis.

***4. Route of administration:*** The humoral immune response is better following parenteral administration of antigen than through oral or nasal routes. Large particulate antigens, such as bacteria or erythrocytes are more effective when injected intravenously whereas soluble antigens are more effective when injected into tissues. The route of administration may also influence the type of antibody produced. For production of IgA antibodies, the oral or nasal route is most suitable. Inhalation of pollen antigens induces IgE synthesis whereas the same antigens given parenterally lead to IgG antibodies. With some antigens the route of administration determines whether tolerance or antibody response results. Injection of protein antigens into the mesenteric vein or intrathymically usually induces tolerance. Sulzberger (1929) and Chase (1959) showed that guinea pigs can be rendered specifically tolerant if certain antigens are fed before a parenteral challenge (Sulzberger-Chase phenomenon). Application of simple chemicals to the

skin usually leads to cellular immune response (delayed hypersensitivity) rather than antibody formation.

***5. Size and number of doses:*** Antibody response is, to an extent, dose dependent. An antigen is effective only above a minimum critical dose. Further increase in dose enhances the intensity of the antibody response. But beyond a level, increase in the dose of antigen does not improve the antibody response, but may even inhibit it and induce tolerance. Mice injected with 0.5/µg of pncumococcal capsular polysaccharide produce specific antibodies, but those injected with 50/µg of the antigen not only fail to form antibody, but may not respond even to subsequent doses of the same antigen. The massive antigenic stimulus appears to swamp the antibody producing system and paralyse it. This phenomenon was designated 'immunological paralysis' by Felton (1949).

The increased antibody response to a secondary antigenic stimulus has already been noticed. With repeated antigen injections the antibody response increases progressively. But after a certain stage, no further increase occurs. The term 'anamnestic reaction" was originally applied to the production in response to an antigenic stimulus of a heterologous but related antibody that the host had earlier produced. For instance, a person who had been immunized earlier against typhoid bacilli may sometimes produce antityphoid antibodies in response to infection with some other bacterium. This may cause confusion in the serological diagnosis of typhoid fever, but anamnestic reaction can be differentiated from a true secondary response as it is transient. The term anamnestic reaction has been employed by some to refer to the secondary response as well, so that some confusion attaches to this usage.

***6. Multiple antigens:*** When two or more antigens are administered simultaneously, the effects may vary. Antibodies may be produced against the different antigens just as though they had been given separately, or antibody response to one or other of the antigens may be enhanced, or the response to one or more of them may be diminished (antigenic competition). When two bacterial vaccines (for example, typhoid and cholera vaccines) are given in a mixed form, the antibody response to each is not influenced by the other. When toxoids are given along with bacterial vaccines (for example triple vaccine containing diphtheria and tetanus toxoids along with *Bord. petussis* vaccine DPT) the response to the toxoid is potentiated. When diphtheria and tetanus toxoids are given together, with one in excess, the response to the other is inhibited. When triple antigen is given to a person- who had earlier received a primary dose of diphtheria toxoid, the response to tetanus and pertussis antigens will be diminished. Such antigenic competition is important from a practical point of view in immunization with polyvalent antigens. For optimal effect, the nature and relative proportions of the different antigens in -a mixture should be carefully adjusted.

***7. Adjuvants:*** The term adjuvant refers to any substance that enhances the immunogenicity of an antigen. Adjuvants may confer immunogenicity on nonantigenic substances, increase the concentration and persistence of the circulating antibody, induce or enhance the degree of cellular immunity and lead to the production of 'adjuvant diseases' such as allergic disseminated encephalomyelitis. A variety of substances exhibit adjuvant activity. Some, such as aluminium hydroxide or phosphate and incorporation of protein antigens in the water phase of a water-in-oil emulsion (Freund's incomplete adjuvant), delay the release of antigen from the site of injection and prolong the antigenic stimulus. Others such as silica particles, beryllium sulphate and endotoxins are toxic to macrophages and induce the liberation of lysosomal enzymes, though how this potentiates the immune response is not known. The most potent adjuvant is Freund's complete adjuvant, which is the incomplete adjuvant along with a suspension of killed tubercle bacilli. Besides increasing humoral immune response, it induces a high degree of cellular immunity (delayed hypersensitivity) as well. As it produces a local granuloma it is unsuitable for human use. The adjuvants commonly used with human vaccines are aluminium hydroxide or phosphate, endotoxin and mineral oils.

***8. Immunosuppressive agents:*** These inhibit the immune response. They are useful in certain situations like transplantation, when it becomes necessary to prevent graft rejection. Immunosuppressive agents commonly employed are X-irradiation, radiomirnetic drugs, corticosteroids, antimetabolites and antilymphocyte serum.

Sub lethal whole body irradiation suppresses antibody response. When antigenic stimulus follows 24 hours after irradiation, antibody production does not occur, whereas if the antigen is administered 2-3 days before irradiation, the antibody response is actually enhanced. The primary response is more radiosensitive than the secondary response.

Radiomirnetic drugs are agents with an action resembling that of X-rays. They belong in general to the class of alkylating agents *(e.g.* cyclophosphamide, nitrogen mustard). In man, cyclophosphamide given for 3 days after the antigen, completely suppresses the antibody response. It is much less effective when given before the antigen.

Corticostcroids cause depletion of lymphocytes from the blood and lymphoid organs. They also stabilize the membranes of cells and lysosomes, inhibiting histamine release and the inflammatory response. They suppress antibody formation in the rat and rabbit, but are much less effective in the guinea pig, monkey and man. Therapeutic doses have little effect on the antibody formation in man. They inhibit the induction and manifestations of delayed hyper-sensitivity in man.

Antimetabolites are substances that interfere with the synthesis of DNA, RNA or both and thus inhibit cell division and differentiation necessary for humoral and cellular immune responses. They include folic acid antagonists (methotrexate) and analogues of purine (6-mercaptopurine, azathioprine), cytosine (cytosine arabinoside) and uracil (5-Fluorouracil). Many antimetabolites find clinical application in the prevention of graft rejection.

The drug most widely used now for immunosuppression is cyclosporine. It is a cyclic polypeptide which is not cytotoxic for lymphocytes and has no antimitotic activity. It selectively inhibits helper T cell activity.

Antilymphocyte serum (ALS) is a heterologous antiserum raised against lymphocytes or thymocytes. ALS for human use is raised in horses. Antibodies to other antigens such as erythrocytes are removed by selective absorption. While all other immunosuppressive agents have undesirable side effects, ALS is devoid of any action other than on lymphocytes. The antibody class active in ALS is IgG, the IgM antibody being inactive. ALS acts primarily against T lymphocytes and therefore specifically on cell mediated immunity. Humoral antibody response to thymus dependent antigens may be inhibited, but the response to thymus independent antigens is unaffected and may even be enhanced. ALS acts only against lymphocytes in circulation and not cells in lymphoid organs. As ALS is a foreign protein its effect is decreased on repeated administration, which may lead to serum sickness and other hypersensitivity reactions. ALS is used in the prevention of graft rejection.

***9. Effect of antibody.*** The humoral immune response to an antigen can be suppressed specifically by passive administration of the homologous antibody. The action appears to be by a feedback mechanism. The primary response is more susceptible to inhibition than the secondary response. The antibody may also combine with the antigen and prevent its availability for the immunocompetent cells. The inhibitory effect of a passively administered antibody on the humoral immune response has been applied in the prevention of Rh sensitisation in Rh-negative women carrying Rh-positive fetus. This is achieved by administration of anti-Rh globulin immediately following delivery (within 72 hours).

This effect is also relevant in the practice of combined immunisation as in diphtheria and tetanus. In such cases, the toxoid and antitoxin should be given at separate sites. Absorbed toxoid should be used as the inhibitory effect is much less than with fluid toxoid.

# Monoclonal antibodies

## Monoclonal antibodies (Hybridomas)

The limitations of antiserum as a source of antibodies have been overcome with the development of hybridoma techniques to manipulate and culture various mammalian cells that synthesize antibodies in vitro. Each cell and its progeny normally produce a **monoclonal antibody (MAb)** of a single specificity.

The methodology of one of these techniques is illustrated in **figure.** Animals (usually mice or rats) are immunized with antigens as discussed previously. Once the animals are producing a large quantity of antibodies, their spleens are removed and antibody producing B and plasma cells (lymphocytes) isolated. These lymphocytes are then fused with myeloma cells by the addition of polyethylene glycol, which promotes membrane fusion. **Myeloma cells** are cancerous plasma cells that can readily be cultivated; mutant myeloma cells incapable of producing immunoglobulins are used. These fused cells, derived from lymphocytes and myeloma cells, are called **hybridomas** (they are hybrids of the two cells).

The fusion mixture is then transferred to a culture medium containing a combination of **H**ypoxanthine, **A**minopterin, and **T**hymidine (HAT). Aminopterin is a poison that blocks a specific metabolic pathway in cells. Myeloma cells lack an enzyme that allows their growth in the presence of aminopterin. However, the pathway is bypassed in lymphoid cells provided with the intermediate metabolites hypoxanthine and thymidine. As a result the hybridomas grow in the HAT medium but the myeloma cells die because they have a metabolic defect and cannot employ the bypass or salvage pathway. When the culture is initially established using the HAT medium, it contains lymphocytes, myeloma cells, and hybridomas. The unfused lymphoid cells die naturally in culture within a week or two, and the myeloma cells die in the HAT as just described. In contrast, the fused cells survive because they have the immortality of the myeloma and the metabolic bypass of the lymphoid cells. Hybridomas that have the antibody-producing capacity of the original lymphoid cells are randomly placed in culture wells. The wells are individually tested for production of the desired antibody, and, if positive, the cells within the well provide clones of immortal cells, all producing the same monoclonal antibody.

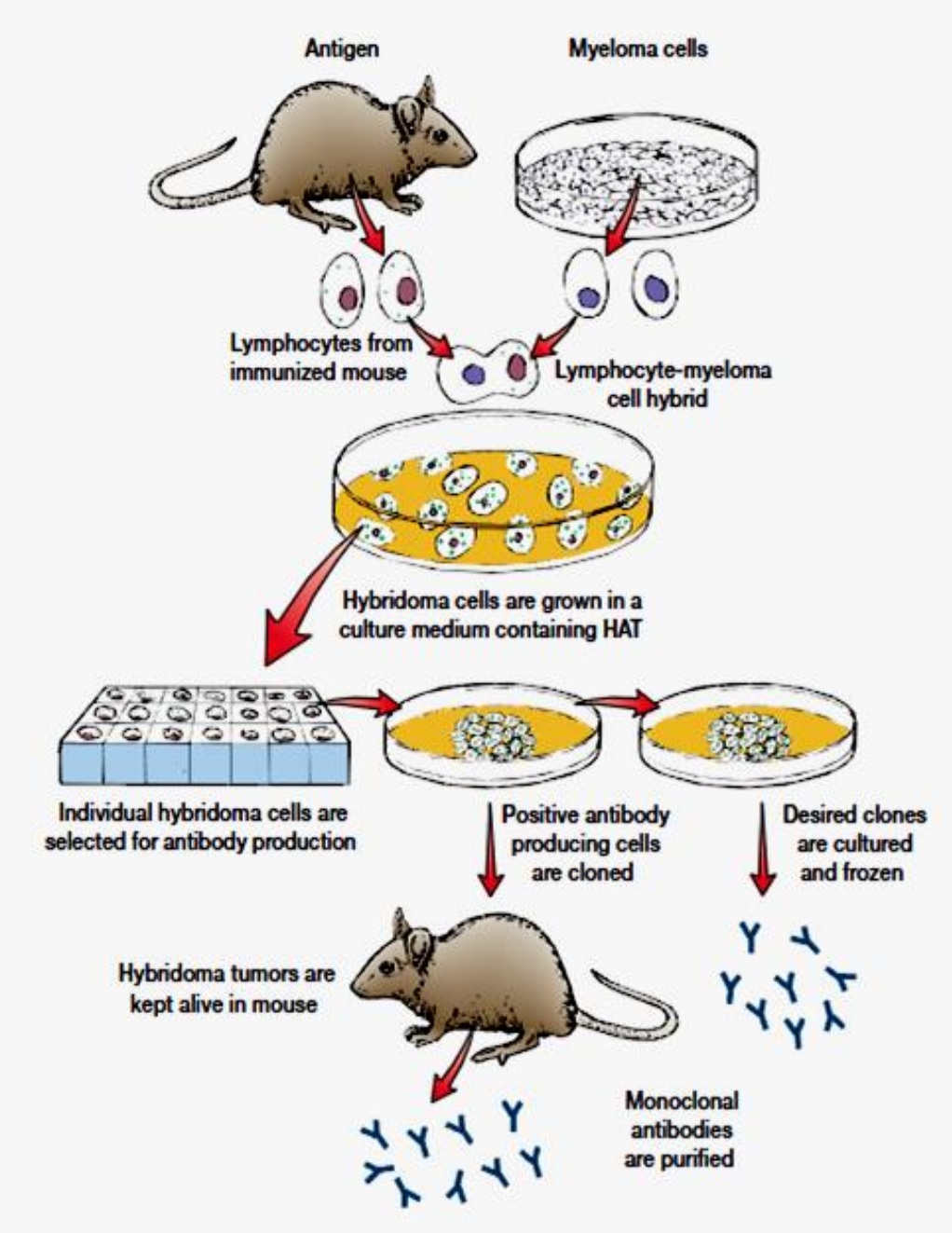


Fig. **Technique for the Production of Monoclonal Antibodies.**

Lymphocytes are fused with special mutant myeloma cells, yielding hybridomas. Each of them secretes a single, "monoclonal" antibody. Once the hybridoma secreting the desired antigen is identified, it is cloned to generate many antibody-secreting cells that yield the huge quantity of a single antibody needed in medicine or science. Some hybridoma cells may be stored frozen and later cloned for antibody production or kept alive in laboratory animals. Monoclonal antibodies currently have many applications. For example, they are routinely used in the typing of tissue, in the identification and epidemiological

study of infectious microorganisms, in the identification of tumor and other surface antigens, in the classification of leukemias, and in the identification of functional populations of different types of T cells. Anticipated future uses include (1) passive immunizations against infectious agents and toxic drugs, (2) tissue and organ graft protection, (3) stimulation of tumor rejection and elimination, (4) manipulation of the immune response, (5) preparation of more specific and sensitive diagnostic procedures, and (6) delivery of antitumor agents (immunotoxins) to tumor cells.

| Drug Names (Chemical* and Trade) | | Used in Therapy for |
|---|---|---|
| **Cancer Drugs** | | |
| Trastuzumab | Herceptin | Breast cancer |
| Retuximab | Rituxan | Non-Hodgkin's lymphoma |
| Bevacizumab | Avastin | Colorectal cancer/lung cancer |
| Gemtuzumab | Mylotarg | Acute myelogenous leukemia |
| **Others** | | |
| Omalizumab | Xolair | Asthma |
| Infliximab | Remicade | Crohn's disease |
| Palivizumab | Synagis | Respiratory syncytial virus (RSV) |

- **Introduction to stem cells and stem cell therapy.**

A development that promises to transform transplantation medicine is the use of stem cells, master cells that are capable of generating any of the myriad cell types that make up the body. The most interest is centered on embryonic stem cells (ESCs). These cells can be isolated from the very earliest stage of an embryo, usually from discarded embryos created for attempts at in vitro fertilization. The ESCs are *pluripotent,* meaning they are capable of generating many different types of tissue cells.

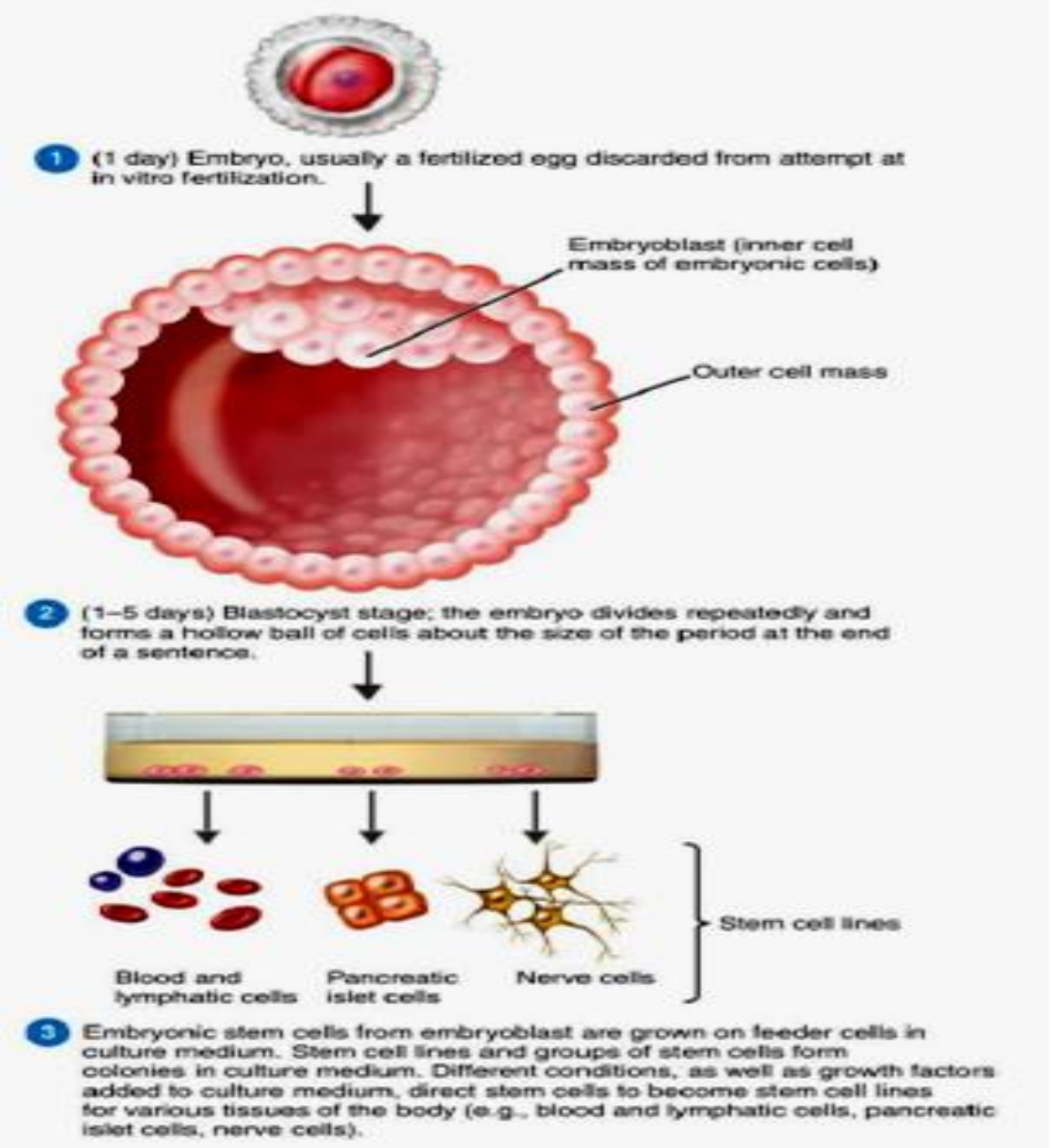


Figure shows how these cells are harvested from the blastocyst stage, a hollow ball of 100 to 150 undifferentiated cells that is reached a few days after the egg is fertilized. When cultured, ESCs can be trained to produce different cell lines, such as muscle, nerve, or blood cells. In the medical community there is great interest in using ESCs in therapy. For example, theoretically these cells could be used to regenerate damaged heart tissue or the failing insulin - producing cells in the pancreas that lead to diabetes. Damaged cartilage in the joints of rheumatoid arthritis patients might be replaced. Neurological

conditions such as Parkinson disease or trauma-caused paralysis might also be candidates for treatment.

There is even the prospect of growing complete new organs. In some instances the original donor might be the recipient, ensuring a genetic match of the tissues. Fortunately, human ESCs seem to express few MHC class I antigens and no class II antigens. This eases the problem of immune rejection but does not solve it. In any case, researchers want to create pluripotent cells that genetically match the patient or otherwise evade immune rejection.

Because ESCs are derived from embryos, even at their microscopic stage, many people object to their use. Possible alternatives are *adult stem cells (ASes)*, which exist in some tissues such as the blood or skin. These produce only a very few different cell types, mostly of the tissue type of origin, and are difficult to cultivate. A promising new avenue of research is to genetically reprogram ASCs by using viruses to insert genes into skin cells, or other adult cells, to convert them into *induced pluripotent stem cells (iPS).* Other nonembryonic sources of stem cells are cord blood cells, considered ASCs, harvested from umbilical cords. These are primarily *hematopoietic stem cells (HSCs ),* which are progenitors of blood and lymphatic (immune system) cells. Bone marrow transplants are a form of stem cell transplantation, mostly HSCs.

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