# IMMUNO TECHNOLOGY

## LECTURE 02: LYMPH NODES

Lymph nodes are the sites where immune responses are mounted to antigens in lymph. They are encapsulated beanshaped 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 encounter antigens that enter the tissue spaces. As lymph percolates through a node, any particulate antigen that is brought in with the lymph will be 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 encounter 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. Beneath 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 TH cells. Lymph nodes taken from neonatally thymectomized mice have unusually few cells in the paracortical region; 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 more sparsely 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 TH cells. The initial activation of B cells is also thought to take place within the T-cell-rich paracortex. Once activated, TH 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 TH 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 TH 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 pierce the capsule of a lymph node at numerous sites and empty lymph into the subcapsular sinus (see Figure 2-18b). 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.

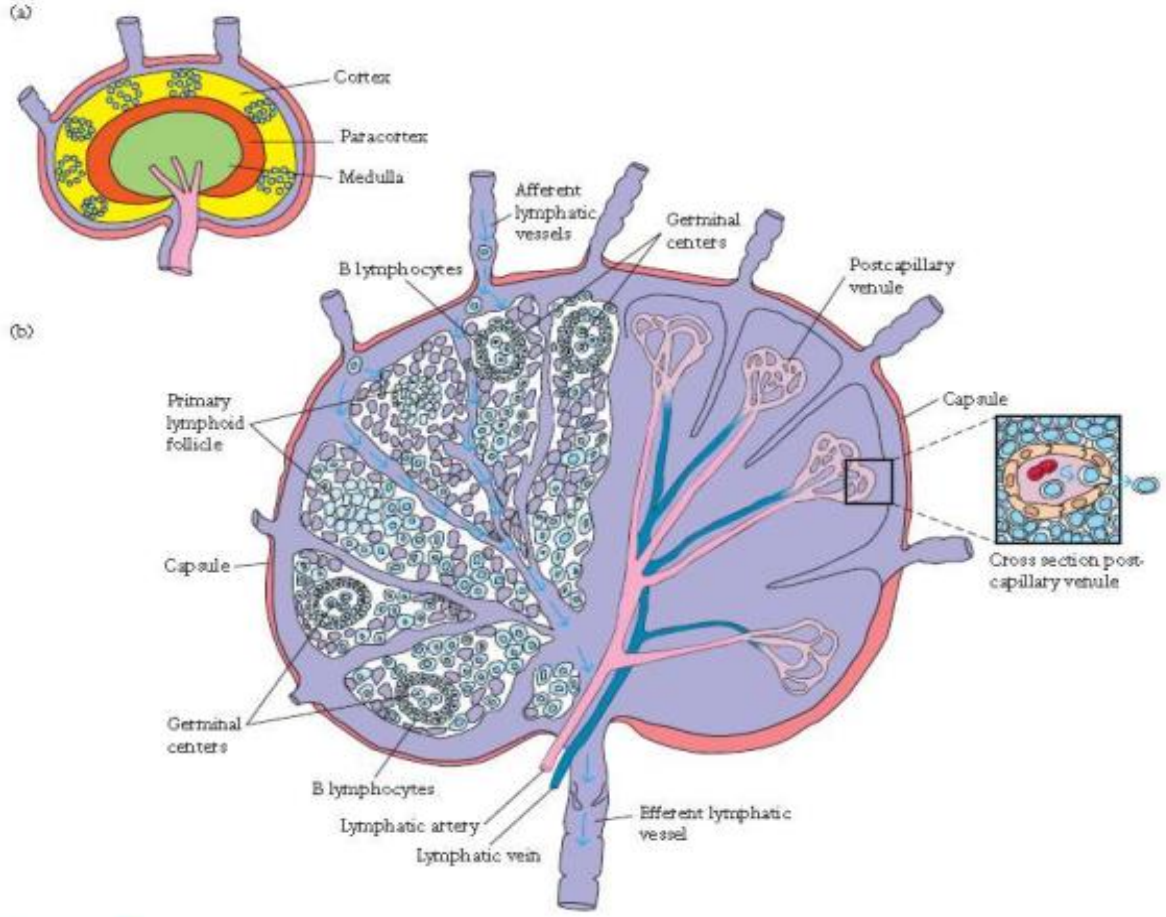



FIGURE 2-18 Structure of a lymph node. (a) The three layers of a lymph node support distinct microenvironments. (b) The left side depicts the arrangement of reticulum and lymphocytes within the various regions of a lymph node. Macrophages and dendritic cells, which trap antigen, are present in the cortex and paracortex. $T_H$ cells are concentrated in the paracortex; B cells are located primarily in the cortex, within follicles and germinal centers. The medulla is populated largely by antibody-producing plasma cells. Lymphocytes circulating in the lymph are carried into the node by afferent lymphatic vessels; they either enter the reticular matrix of the node or pass through it and leave by the efferent lymphatic vessel. The right side of (b) depicts the lymphatic artery and vein and the postcapillary venules. Lymphocytes in the circulation can pass into the node from the postcapillary venules by a process called extravasation (*inset*).

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 are thought to facilitate this increased migration.

## SPLEEN

The spleen plays a major role in mounting 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.

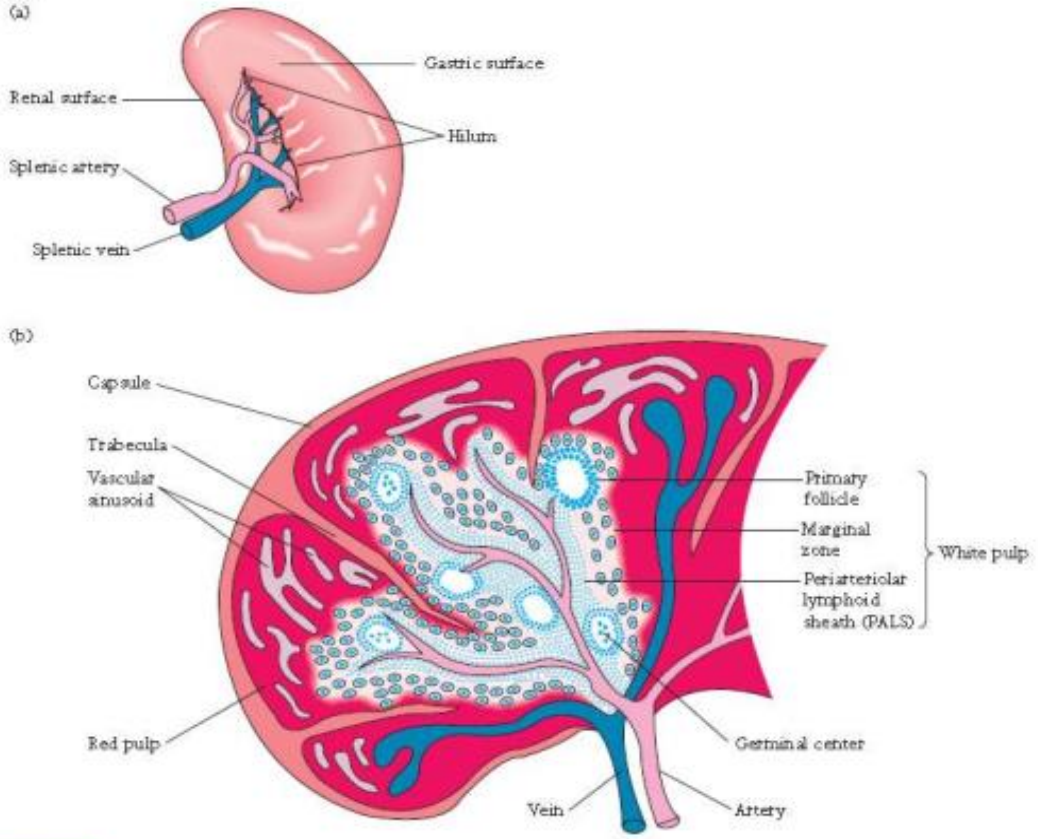



FIGURE 2-19 Structure of the spleen. (a) The spleen, which is about 5 inches long in adults, is the largest secondary lymphoid organ. It is specialized for trapping blood-borne antigens. (b) Diagrammatic cross section of the spleen. The splenic artery pierces the capsule and divides into progressively smaller arterioles, ending in vascular sinusoids that drain back into the splenic vein. The erythrocyte-filled red pulp surrounds the sinusoids. The white pulp forms a sleeve, the periarteriolar lymphoid sheath (PALS), around the arterioles; this sheath contains numerous T cells. Closely associated with the PALS is the marginal zone, an area rich in B cells that contains lymphoid follicles that can develop into secondary follicles containing germinal centers.

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 (Figure 2-19). 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 interdigitating 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 Tcell- rich PALS. Here interdigitating dendritic cells capture antigen and present it combined with class II MHC molecules to TH cells. Once activated, these TH cells can then activate B cells. The activated B cells, together with some TH 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. The effects of splenectomy on the immune response depends on the age at which the spleen is removed. In children, splenectomy often leads to an increased incidence of bacterial sepsis caused primarily by *Streptococcus pneumoniae, Neisseria meningitidis,* and *Haemophilus influenzae.* Splenectomy in adults has less adverse effects, although it leads to some increase in blood-borne bacterial infections **(bacteremia).**

## MUCOSAL-ASSOCIATED LYMPHOID TISSUE

The mucous membranes lining the digestive, respiratory, and urogenital systems have a combined surface area of about 400 m2 (nearly the size of a basketball court) and are the major sites of entry for most pathogens. These vulnerable membrane surfaces are defended by a group of organized lymphoid tissues mentioned earlier and known collectively as **mucosal-associated lymphoid tissue (MALT).**

Structurally, these tissues range from loose, barely 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 attested to 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 (Figure 2-20). All three tonsil groups are nodular structures consisting of a meshwork of reticular cells and fibers interspersed 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.

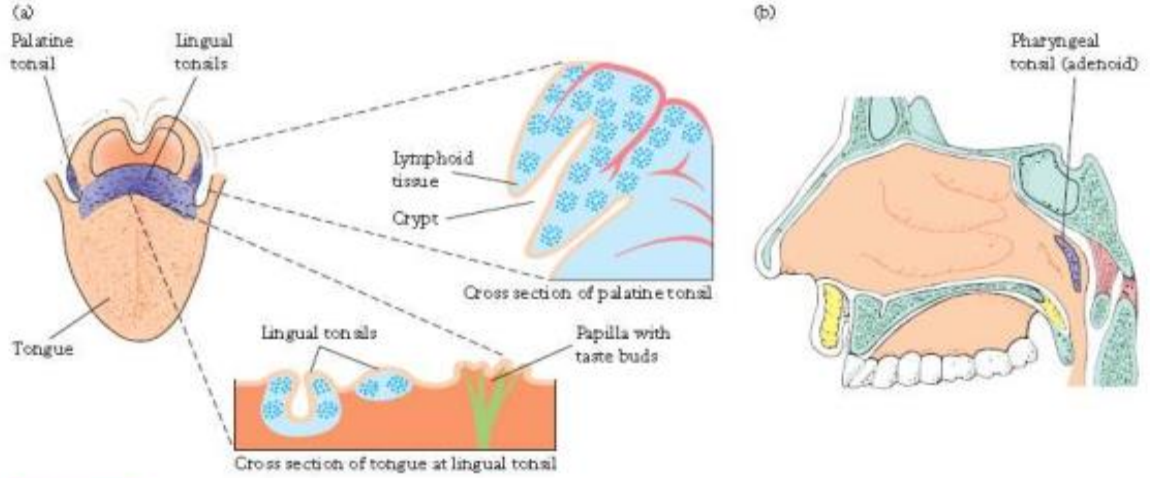



FIGURE 2-20 Three types of tonsils. (a) The position and internal features of the palatine and lingual tonsils; (b) a view of the position of the nasopharyngeal tonsils (adenoids). [*Part b adapted from J. Klein, 1982, Immunology, The Science of Self-Nonself Discrimination, © 1982 by John Wiley and Sons, Inc.*]

As shown in Figures 2-21 and 2-22, lymphoid cells are found in various regions within this tissue. The outer mucosal epithe lial 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.

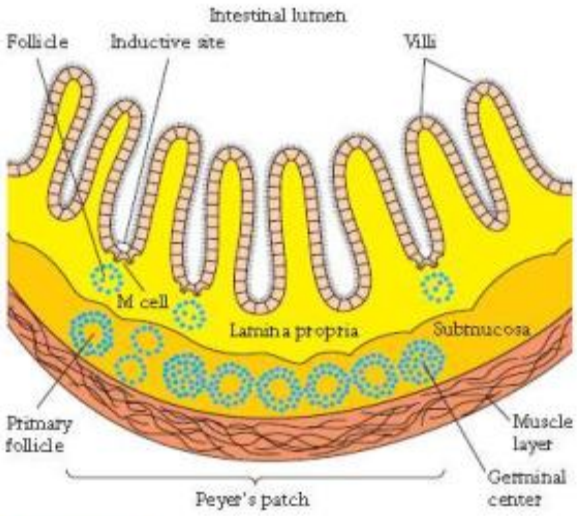



FIGURE 2-21 Cross-sectional diagram of the mucous membrane lining the intestine showing a nodule of lymphoid follicles that constitutes a Peyer's patch in the submucosa. The intestinal lamina propria contains loose clusters of lymphoid cells and diffuse follicles.

The lamina propria, which lies under the epithelial layer, contains large numbers of B cells, plasma cells, activated TH 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.

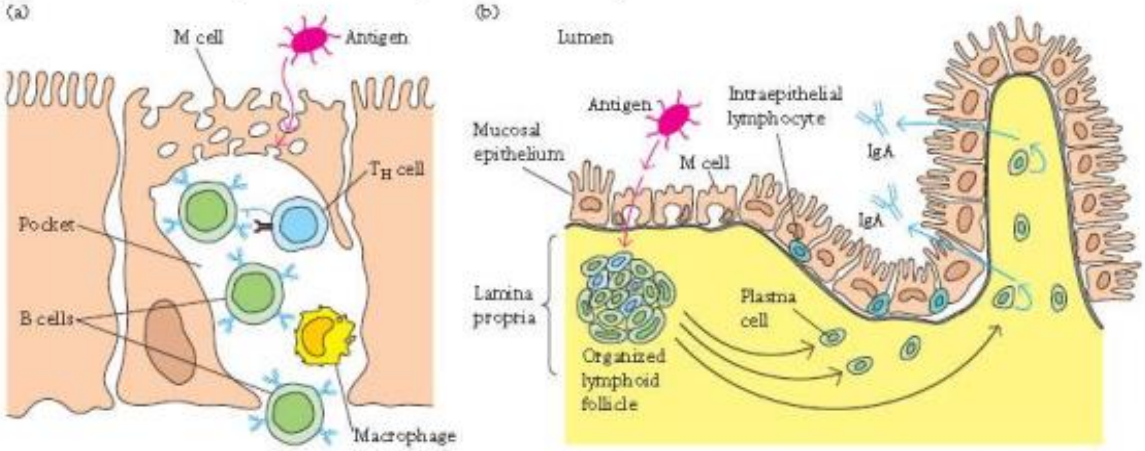



FIGURE 2.22 Structure of M cells and production of IgA at inductive sites. (a) M cells, located in mucous membranes, endocytose antigen from the lumen of the digestive, respiratory, and urogenital tracts. The antigen is transported across the cell and released into the large basolateral pocket. (b) Antigen transported across the epithelial layer by M cells at an inductive site activates B cells in the underlying lymphoid follicles. The activated B cells differentiate into IgA-producing plasma cells, which migrate along the submucosa. The outer mucosal epithelial layer contains intraepithelial lymphocytes, of which many are $CD8^+$ T cells that express γδ TCRs with limited receptor diversity for antigen.

## Cutaneous-Associated Lymphoid Tissue

The skin is an important anatomic barrier to the external environment, and its large surface area makes this tissue important in nonspecific (innate) defenses. The epidermal (outer) layer of the skin is composed largely of specialized epithelial cells called keratinocytes. These cells secrete a number of cytokines that may function to induce a local inflammatory reaction. In addition, keratinocytes can be induced to express class II MHC molecules and may function as antigen-presenting cells. Scattered among the epithelial-cell matrix of the epidermis are Langerhans cells, a type of dendritic cell, which internalize antigen by phagocytosis or endocytosis. The Langerhans cells then migrate from the epidermis to regional lymph nodes, where they differentiate into interdigitating dendritic cells. These cells express high levels of class II MHC molecules and function as potent activators of naive TH cells. The epidermis also contains so-called *intraepidermal lymphocytes.* These are similar to the intraepithelial lymphocytes of MALT in that most of them are CD8_ T cells, many of which express _ T-cell receptors, which have limited diversity for antigen. These intraepidermal T cells are well situated to encounter antigens that enter through the skin and some immunologists believe that they may play a role in combating antigens that enter through the skin. The underlying dermal layer of the skin contains scattered CD4_ and CD8_ T cells and macrophages.Most of these dermal T cells were either previously activated cells or are memory cells.

# IMMUNO TECHNOLOGY

## 7. Types of immune cells, B-lymphocytes,T-lymphocytes macrophages, dendritic cells

### T LYMPHOCYTES

T lymphocytes derive their name from their site of maturation in the *t*hymus. Like B lymphocytes, these cells have membrane receptors for antigen. Although the antigenbinding T-cell receptor is structurally distinct from immunoglobulin, it does share some common structural features with the immunoglobulin molecule, most notably in the structure of its antigen-binding site. Unlike the membrane- bound antibody on B cells, though, the T-cell receptor (TCR) does not recognize free antigen. Instead the TCR recognizes only antigen that is bound to particular classes of self-molecules.Most T cells recognize antigen only when it is bound to a self-molecule encoded by genes within the major histocompatibility complex (MHC). Thus, as explained in Chapter 1, a fundamental difference between the humoral and cell-mediated branches of the immune system is that the 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.

The T-cell system has developed to eliminate these altered self-cells,which pose a threat to the normal functioning of the body. 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. In addition, most mature T cells express the following membrane molecules: _ **CD28,** a receptor for the co-stimulatory B7 family of molecules present on B cells and other antigen presenting cells _ **CD45,** a signal-transduction molecule 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. Thus the expression of CD4 versus CD8 corresponds to the MHC restriction of the T cell. 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 (TH) cells and are class-II restricted; CD8_ T cells generally function as T cytotoxic (TC) cells and are class-I restricted. Thus the ratio of TH to TC 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.

The classification of CD4_ class II–restricted cells as TH cells and CD8_ class I–restricted cells as TC cells is not absolute. Some CD4_ cells can act as killer cells. Also, some TC cells have been shown to secrete a variety of cytokines and exert an effect on other cells comparable to that exerted by TH cells. The distinction between TH and TC cells, then, is not always clear; there can be ambiguous functional activities. However, because these

ambiguities are the exception and not the rule, the generalization of T helper (TH) cells as being CD4_ and class-II restricted and of T cytotoxic cells (TC) as being CD8_ and class-I restricted is assumed throughout this text, unless otherwise specified. TH cells are activated by recognition of an antigen–class II MHC complex on an antigen-presenting cell. After activation, the TH cell begins to divide and gives rise to a clone of effector cells, each specific for the same antigen–class II MHC complex. These TH 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.

Changes in the pattern of cytokines produced by TH cells can change the type of immune response that develops among other leukocytes. The **TH1 response** produces a cytokine profile that supports inflammation and activates mainly certain T cells and macrophages, whereas the **TH2 response** activates mainly B cells and immune responses that depend upon antibodies. TC 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 TC cell to differentiate into an effector cell called a **cytotoxic T lymphocyte (CTL).** In contrast to TH 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 (TS) 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, but the actual isolation and cloning of normal TS cells is a matter of controversy and dispute among immunologists. For this reason, it is uncertain whether TS cells do indeed constitute a separate functional subpopulation of T cells. Some immunologists believe that the suppression mediated by T cells observed in some systems is simply the consequence of activities of TH or TC subpopulations whose end results are suppressive.

**B cells**

About 5-15% of the circulating lymphoid pool are B cells defined by the presence of surface immunoglobulin. These are constitutively produced and are inserted into the cell surface membrane where they act as specific antigen receptors. These receptors can be detected on the cell surt1ce using fluorochromelabelled antibodies specific for immunoglobulin.

Immunofluorescence staining shows a ‘ring-like’ pattern over the B cell. Divalent antibodies to surface immunoglobulin bind to and crosslink the surface receptors, producing ‘patches’ of immunoglobulin on the cell surface.

**Capping:** On warming up, most of these complexes are actively swept along the cell surface and are seen as a ‘cap’ over one pole of the cell. This is the structural peculiarity of B cells. Capping is followed by internalization and degradation of the Ig. Capping may also be seen with other surface glycoproteins and is not exclusive to B cells.

Cell surface immunoglobulin and signalling molecules form the ‘B-cell receptor’ complex The majority of human B cells in peripheral blood express two immunoglobulin isotypes on

their surface, IgM and IgD. On any B cell, the antigen-binding sites of these isotypes are identical. Fewer than 10% of the B cells in the circulation express IgG, IgA or IgE, although these are present in larger numbers in specific locations of the body, for example, IgA-bearing cells in the intestinal mucosa.

Immunoglobulin associated with other molecules on the B-cell surface forms the 'B-cell antigen receptor complex' (BCR). These 'accessory' molecules consist of disulphide-bonded heterodimers of Iga (CD79a) and Igb3 (CD79b). The heterodimers interact with the transmembrane segments of the immunoglobulin receptor, and like the separate molecular components of the TCRjCD3 complex, are involved in cellular activation.

**Other B-cell Markers and Subsets**

The majority of B cells carry MHC class II antigens, which are important for cooperative (cognate) interactions with T cells. These class II molecules consist of I-A or I-E in the mouse and HLA-DP, DQ and DR antigens in man. Complement receptors for C3b (CD35) and C3d (CD21) are commonly found on B cells and are associated with activation and, possibly, 'homing' of the cells. CD19 j CD21 interactions with complement associated with antigen plays a role in antigen-induced B-cell activation via the antigen-binding antibody receptor. Fc receptors for exogenous IgG (Fc_RII, CD32) are also present and play a role in negative signaling to the B cell. The main markers currently used are CD19, CD20 and CD22 to identify human B cells. Other human B cell markers are CD72 to CD78. The CD72 molecule has also been described for murine B cells (Lyb-2) together with B220, a high molecular weight (220 kDa) isoform of CD45 (Lyb-5). CD40 is an important molecule on B cells which is involved in cognate interactions between T and B cells.

**CD5+ B Lymphocytes are a Distinctive Cell Subset**

Many of the first B cells that appear during ontogeny express CD5, a marker originally found on T cells. These cells (termed B-1 cells) are found predominantly in the peritoneal cavity in mice and there is some evidence for a separate differentiation pathway trom 'conventional' B cells (B-2 cells). They express their immunoglobulins from unmutated or minimally mutated germline genes. CD5+ B cells produce mostly IgM, but also some. IgG and IgA. These so-called natural antibodies are of low avidity but, unusually, they are polyreactive and are found at high concentration in the adult serum. CD5+ cells may respond well to TI (T-independent) antigens. They may also be involved in antigen processing and antigen presentation to T cells, and probably play a role in both tolerance and antibody responses.

**Functions Proposed for Natural Antibodies Include The Following**

**1.** The first line of defense against microorganisms;

**2. C**learance of damaged self components; and 'idiotype network' interactions within the immune system.

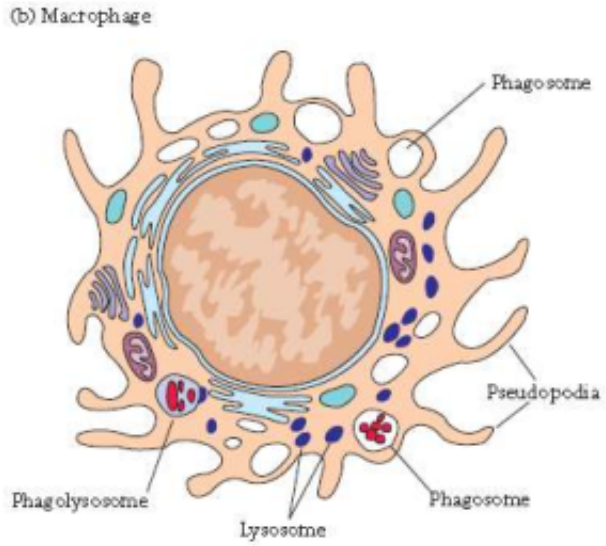
**3.** Characteristically, these antibodies also react against autoantigens including DNA, Fc of IgG, phospholipids and cytoskeletal components.

Since CD5 has been shown to be expressed by B2 cells when they are activated appropriately, there is still some controversy as to whether CD5 represents an activation antigen on B cells. The current theories therefore support the notion for there being two different kinds of CD5+ B cells. Although the function of CDS is unknown on human B cells, it is associated with the BCR and may be involved in the regulation ofB-cell activation. B-cell differentiation leads to the formation of plasma cells and memory cells: Following B-cell activation, many B-cell blasts mature into APCs, which progress *in vivo* to terminally differentiated plasma cells. Some B blasts do not develop rough endoplasmic reticulum cisternae. These cells are found in germinal centres and are named follicle centre cells or centrocytes. Under light microscopy, the cytoplasm of the plasma cells is basophilic; this is due to the large amount ofRNA being utilized for antibody synthesis in the rough endoplasmic reticulum. At the ultrastructural level, the rough endoplasmic reticulum can often be seen in parallel arrays. Plasma cells are infrequent in the blood, comprising less than 0.1% of circulating lymphocytes. They are normally restricted to the secondary lymphoid organs and tissues but are also abundant in the bone marrow. Antibodies produced by a single plasma cell are of one specificity and immunoglobulin class. Immunoglobulins can be visualized in the plasma cell cytoplasm by staining with fluorochrome-labelled specific antibodies. Many plasma cells have a short lifespan, surviving for a few days and dying by apoptosis whereas a subset of plasma cells with a long lifespan (months) has been recently described in the bone marrow.

**PHAGOCYTOSIS**

Macrophages are capable of ingesting and digesting exogenous antigens, such as whole microorganisms and insoluble particles, and endogenous matter, such as injured or dead host cells, cellular debris, and activated clotting factors. In the first step in phagocytosis, macrophages are attracted by and move toward a variety of substances generated in an immune response; this process is called **chemotaxis.** The next step in phagocytosis is adherence of the antigen to the macrophage cell membrane. Complex antigens, such as whole bacterial cells or viral particles, tend to adhere well and are readily phagocytosed; isolated proteins and encapsulated bacteria tend to adhere poorly and are less readily phagocytosed. Adherence induces membrane protrusions, called **pseudopodia,** to extend around the attached material (Figure 2-9a). Fusion of the pseudopodia encloses the material within a membrane-bounded structure called a **phagosome,** which then enters the endocytic processing pathway (Figure 2-9b). In this pathway, a phagosome moves toward the cell interior, where it fuses with a **lysosome** to form a **phagolysosome.** Lysosomes contain lysozyme and a variety of other hydrolytic enzymes that digest the ingested material. The digested contents of the phagolysosome are then eliminated in a process called **exocytosis** (see Figure 2-9b). The macrophage membrane has receptors for certain classes of antibody. If an antigen (e.g., a bacterium) is coated with the appropriate antibody, the complex of antigen and antibody binds to antibody receptors on the macrophage membrane more readily than antigen alone and phagocytosis is enhanced. In one study, for example, the rate of phagocytosis of an antigen was 4000-fold higher in the presence of specific antibody to the antigen than in its absence. Thus, antibody functions as an **opsonin,** a molecule that binds to

both antigen and macrophage and enhances phagocytosis. The process by which particulate antigens are rendered more susceptible to phagocytosis is called **opsonization.**




## ANTIGEN PROCESSING AND PRESENTATION

Although most of the antigen ingested by macrophages is degraded and eliminated, experiments with radiolabeled antigens have demonstrated the presence of antigen peptides on the macrophage membrane. As depicted in Figure 2-9b, 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 TH cells. This processing and presentation of antigen, examined in detail in Chapter 7, are critical to TH-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 (Table 2-7). These include a collection of cytokines, such as **interleukin 1 (IL-1),** TNF-_ 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-_ promote fever by affecting the thermoregulatory center in the hypothalamus. Activated macrophages secrete a variety of factors involved in the development of an inflammatory response.

**TABLE 2-7** Some factors secreted by activated macrophages

| Factor | Function |
|---|---|
| Interleukin 1 (IL-1) | Promotes inflammatory responses and fever |
| Interleukin 6 (IL-6), 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, G-CSF, M-CSF | Promote inducible hematopoiesis |

The **complement proteins** are a group of proteins that assist in eliminating foreign pathogens and in promoting the ensuing inflammatory reaction. The major site of synthesis of complement proteins is the liver, although these proteins are also produced in macrophages. The hydrolytic enzymes contained within the lysosomes of macrophages also can be secreted when the cells are activated. The buildup of these enzymes within the tissues contributes to the inflammatory response and can, in some cases, contribute to extensive tissue damage. Activated macrophages also secrete soluble factors, such as TNF-_, that can kill a variety of cells. The secretion of these cytotoxic factors has been shown to contribute to tumor destruction by macrophages. Finally, as mentioned earlier, activated macrophages secrete a number of cytokines that stimulate inducible hematopoiesis.

**TABLE 2-6** Mediators of antimicrobial and cytotoxic activity of macrophages and neutrophils

| Oxygen-dependent killing | Oxygen-independent killing |
|---|---|
| Reactive oxygen intermediates | Defensins |
| $O_2^{\cdot-}$ (superoxide anion) | Tumor necrosis factor α (macrophage only) |
| $OH^{\cdot}$ (hydroxyl radicals) | Lysozyme |
| $H_2O_2$ (hydrogen peroxide) | Hydrolytic enzymes |
| $ClO^-$ (hypochlorite anion) | |
| Reactive nitrogen intermediates | |
| NO (nitric oxide) | |
| $NO_2$ (nitrogen dioxide) | |
| $HNO_2$ (nitrous acid) | |
| Others | |
| $NH_2CL$ (monochloramine) | |

# IMMUNO TECHNOLOGY

## 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. The development of isolation techniques that employ enzymes and gentler dispersion has facilitated isolation of these cells for study in vitro. There are many types of dendritic cells, although most mature dendritic cells have the same major function, the presentation of antigen to TH 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. Figure 2-11 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.

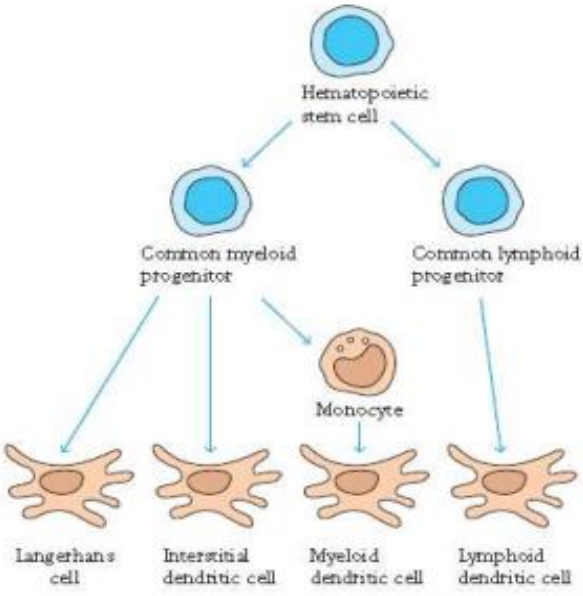



FIGURE 2-11 Dendritic cells arise from both the myeloid and lymphoid lineages. The myeloid pathway that gives rise to the monocyte/macrophage cell type also gives rise to dendritic cells. Some dendritic cells also arise from the lymphoid lineage. These considerations do not apply to follicular dendritic cells, which are not derived from bone marrow.

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 TH 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 TH cells that is required for the initiation of responses by those key cells.

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Another type of dendritic cell, the **follicular dendritic cell** (Figure 2-12), 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 antigenpresenting cells for TH-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.