Chapter 2
COMPONENTS OF THE IMMUNE SYSTEM

The immune system consists of various components, namely organs, tissues, and cells which belong to it according to functional criteria (performance of organism defense) as well as anatomic-physiological principle of their organization (organ-circulatory principle). According to this point of view, the immune system is composed of the central/primary organs (bone marrow and thymus), peripheral/secondary organs (spleen, lymph nodes, Peyer's patches, and diffuse lymphatic tissue in different organs) and, finally, circulatory pathways of immunocompetent cells.

- **Lymphoid system** is part of the immune system. Interactions with other systems of organism are important for it (with blood cells and vessels of cardio-vascular system as well as with integumentary tissues such as mucous membranes and skin). These systems are the close partners of the lymphocytic immunity system.
- **Organ-circulatory principle of organization.** A healthy adult organism contains 10^{13} lymphocytes, or approximately each tenth cell is a lymphocyte. The anatomical-physiological principle of the immune system arrangement is organ-circulatory. It means that lymphocytes do not permanently reside in lymphoid organs, but intensively re-circulate between lymphoid organs and non-lymphoid tissue through lymphatic vessels and blood. Hence each lymph node admits ~10^9 lymphocytes per hour. The mechanism of lymphocyte migration is determined by specific interaction between certain surface molecules on lymphocytes and endothelial cells of blood vessel walls (such molecules are termed adhesins, selectins, integrins, homing receptors). In fact, each organ has a specific spectrum of both lymphocytes and their partners in immune response.
- **Composition of the immune system.** The following organs and tissues compose the immune system (Fig. 2-1).
  - **Bone marrow** is populated by Hematopoietic Stem Cells (HSC).
  - **Encapsulated organs**: thymus, spleen, lymph nodes.
  - **Non-encapsulated lymphoid tissue**:

– Mucosal-Associated Lymphoid Tissue (MALT). Including:
  - Gut-Associated Lymphoid Tissue (GALT) — tonsils, appendix, Peyer's patches, and intraepithelial lymphocytes of the gastrointestinal tract mucous membrane.
  - Bronchus-Associated Lymphoid Tissue (BALT) and intraepithelial lymphocytes of the respiratory system.
  - Vulvovaginal-Associated Lymphoid Tissue (VALT) and its mucosal intraepithelial lymphocytes.
  - Nose-Associated Lymphoid Tissue (NALT) and its mucosal intraepithelial lymphocytes.
– The liver also has a special role in the immune system. It contains lymphocyte subsets and other cells which monitor portal vein blood carrying substances absorbed in the intestine.
– Skin-Associated Lymphoid System (SALT) — disseminated intraepithelial lymphocytes, regional lymph nodes, and lymphatic drainage vessels.

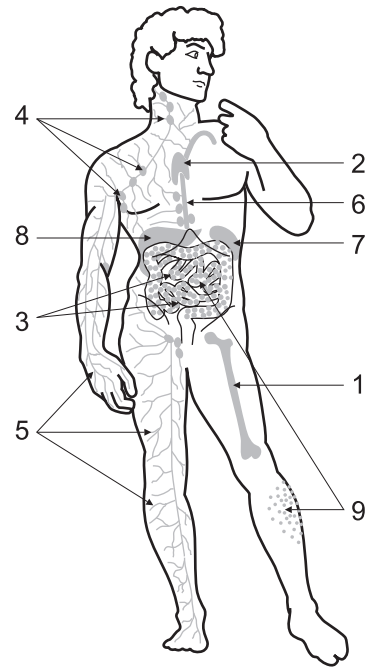

Fig. 2-1. Components of the immune (lymphoid) system. 1 — hematopoietic bone marrow; 2 — thymus; 3 — mucosal-associated non-encapsulated lymphoid tissue; 4 — lymph nodes; 5 — lymph drainage vessels of integumentary tissues (afferent lymphatic vessels); 6 —thoracic lymph duct (it joins systemic circulation via superior vena cava); 7 — spleen; 8 — liver; 9 — intraepithelial lymphocytes.

- **Peripheral blood** is the communication route of the immune system.

▸ Central and peripheral organs of the immune system.

- **Central (primary) organs.** The red bone marrow and thymus are central organs of the immune system, and it is there where lymphopoiesis (lymphocytic differentiation) from HSC to mature naïve lymphocytes proceeds. B-cells development initially occurs in the fetal liver; after birth it shifts to the bone marrow.
  – Differentiation of the common lymphoid progenitors continues when the cells are “resettled” from the bone marrow to other organs and tis-

sues, namely: T-lymphocyte precursors — to the thymus and gut mucosa; precursors of B1-subset of B-lymphocytes — to abdominal and pleural cavities.

– Erythropoiesis (generation of erythrocytes), myelopoiesis (generation of neutrophils, monocytes, eosinophils, basophils), and megakaryocytopoiesis (generates platelets) proceed to completion in the bone marrow. DC, NK- and B2-lymphocyte differentiation is initiated in the bone marrow too. Having proceeded with their maturation steps in peripheral lymphoid organs and during their differentiation into plasma cells B2-lymphocytes subside *in situ* or migrate in different sites (for instance in loose connective tissue, bone marrow, etc.) where they secrete large quantities of antibodies during a time period of several days up to many years.

- **Peripheral (secondary) organs.** In peripheral lymphoid organs (spleen, lymph nodes, non-encapsulated lymphoid tissue), mature naïve lymphocytes encounter antigen-presenting cells. When antigen binds an epitope-specific receptor, the lymphocyte follows a pathway of further differentiation as part of a specific immune response. In other words, it undergoes proliferation and production of effector molecules (cytokines, perforins, granzymes etc.). The final stage of lymphocyte differentiation that is performed at the periphery is called **immunogenesis**. Immunogenesis results in the generation of immune (effector) lymphocyte clones. They recognize antigen and perform destruction of both the antigen and the peripheral tissues where the antigen was present.

- **Cells of the immune system.** Immune system cells comprise the subsets of different origin: mesenchymal, ecto- and entodermal.
  - **Cells of mesenchymal origin — immunocytes** — include all lymphoid cell types such as T-, B-, and NK. Lymphocytes closely interact with various leukocytes, namely monocytes/macrophages, neutrophils, eosinophils, basophils, and also DCs, mast cells and vascular endothelial cells. Even erythrocytes contribute to the immune response — they transport immune complexes "antigen–antibody–complement" into the liver and spleen for phagocytosis and destruction.
  - **Epithelium.** Some lymphoid organs (thymus and certain non-encapsulated lymphoid tissue) comprise epithelial cells of ectodermic and entodermic origin.
- **Humoral factors.** In addition to cells, "immunologic matter" is represented by soluble molecules — humoral factors. These are products of B-lymphocytes such as **antibodies** (immunoglobulins), and **cytokines** (soluble mediators of cellular interactions).

Thymus

Maturation of most T-lymphocytes ("T" abbreviation originated from "Thymus") occurs in the thymus. The thymus gland has two lobes. Each lobe contains lobules separated by trabeculae. In each thymic lobule (Fig. 2-2), two zones are distinguishable: cortical — at periphery, and medullar — in the center. The organ has stromal-cell framework **(epithelial cells, interdigitating DCs**, and **macrophages),** which contributes to maturation of **thymocytes** (thymic lymphocytes). DCs are predominantly located in the transitional zone between cortex and medulla. Macrophages are present in both zones.

- **Cortex Thymic Epithelial Cells** (cTECs), also called "nurse cells", by means of their cytoplasmic extensions enclose and "embrace and lull" thymic lymphocytes. These cells not only physically interact with the developing thymocytes, but also produce the following cytokines: IL-1, IL-3, IL-6, IL-7, LIF, GM-CSF, and express adhesion molecules LFA-3

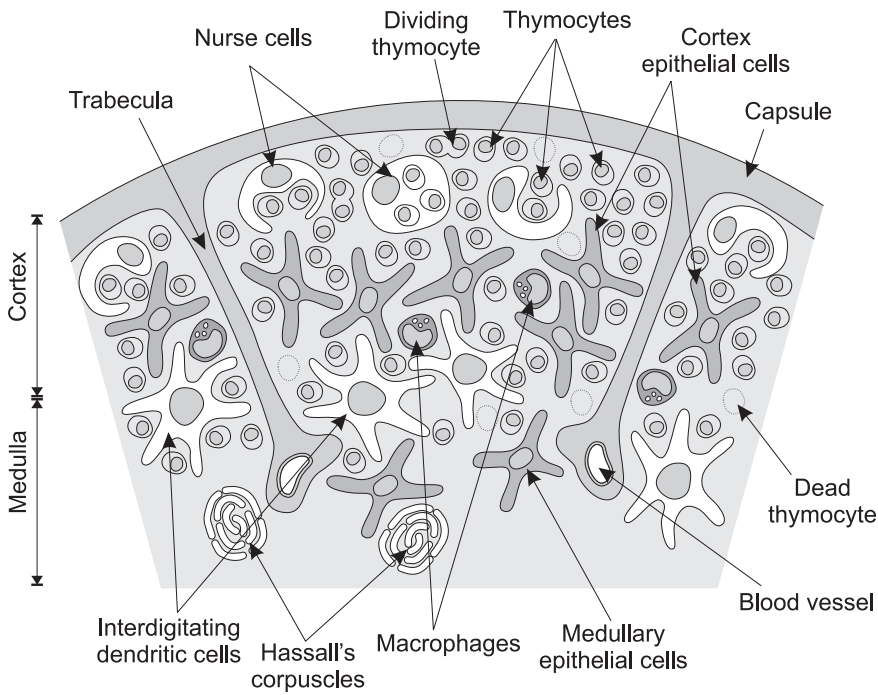

Fig. 2-2. Scheme of thymic lobule.

and ICAM-1, complementary to adhesion molecules on thymocyte surface (CD2 and LFA-1). cTECs express MHC class I and II molecules and participate in positive selection of thymocytes presenting them autopeptides. Another type of endothelial cells resides in medulla and is called **medullary Thymic Epithelial Cells** (mTECs). They express about 80% of all proteins (even those which are ordinarily being expressed only by specialized cell types). This unique property is controlled by a transcription factor AIRE (AutoImmune REgulator). mTECs present derived from them peptides in the context of MHC class I and II molecules as autoantigens to developing thymocytes. Those thymocytes that bind these peptides with high affinity undergo apoptosis. Thus, elimination of autoreactive clones of thymocytes (negative selection) occurs. In the medullar zone there are concentric layers of epithelial cells called **Hassall's corpuscles** (thymus gland corpuscles). They are sites of compact accumulation of degenerating epithelial cells.

- **Thymocytes** originate from bone marrow hemopoietic stem cells (HSC). CD7, CD2, CD34 antigens and the cytoplasmic form of CD3 are expressed by progenitor T-cells even before entering the thymus.
  - **Blood-thymus barrier.** The thymus is intensively vascularized. Both capillary and venule walls constitute a blood-thymus barrier protecting the thymus from circulating macromolecules. Mature T lymphocytes can leave the thymus either through efferent lymphatic vessels that are present in each thymic lobule and carry lymph away to the lymph nodes of mediastinum or by means of extravasation across the wall of postcapillary high-endothelial venules in the cortex-medullary region, and/or through the walls of ordinary circulatory capillaries.
- **Age-related changes.** At birth the thymus has already been entirely formed. It is densely populated by thymocytes during childhood up to puberty. Following puberty, the thymus slowly begins to shrink, becomes replaced by fat (thymic involution) and eventually loses its capacity to produce new T-lymphocytes. Thymectomy in adults does not result in serious immunity disorders, as long as an essential pool of peripheral T-lymphocytes has been sufficiently generated between birth and adolescence.

Lymph nodes

Lymph nodes (Fig. 2-3) are encapsulated bean-shaped organs of 0.5 to 1.5 cm in length (in inflammation-free state). They are located symmetri-

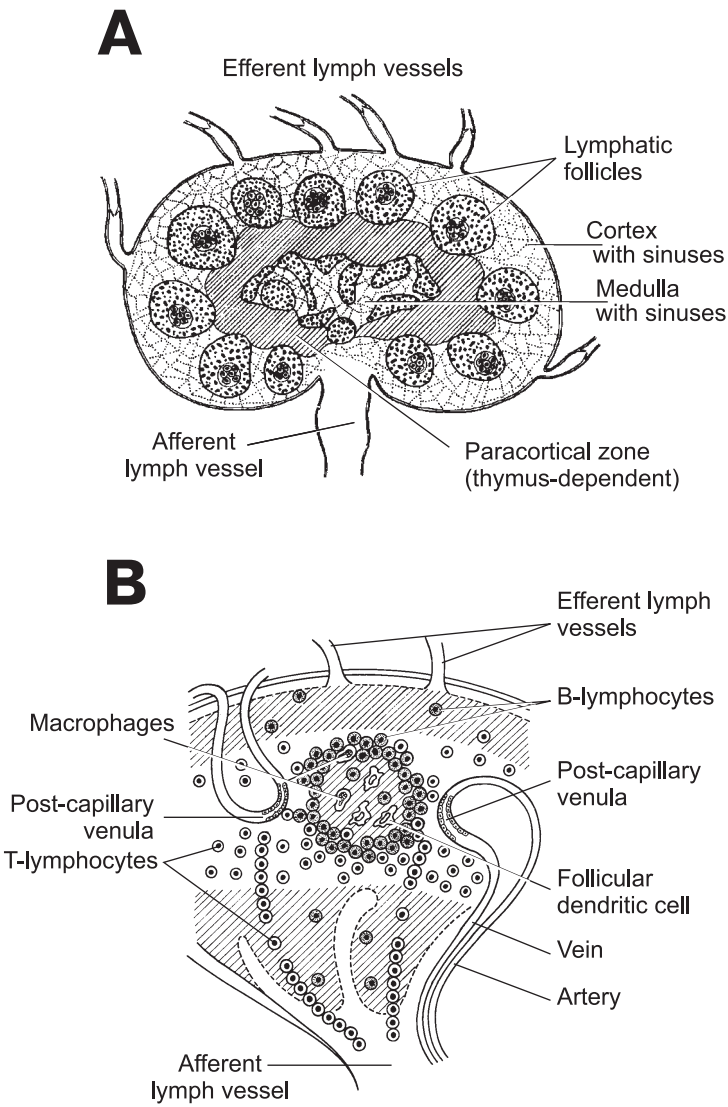

Fig. 2-3. Structure of a lymph node. A. Cortical, paracortical and medullar regions. Lymphoid follicles are located in the cortical region. T-cell paracortical zone is shaded. The medulla contains medullary cords and sinuses. **B.** T- and B-lymphocyte distribution within a lymph node. The T-cell zone is light. The B-cell zone is shaded. T-lymphocytes enter the parenchyma from the post-capillary venules and interact with the follicular dendritic cells and B-lymphocytes.

cally at various places in the body and drain interstitial fluid, or lymph, via afferent lymphatic vessels (several for each node). Hence lymph nodes are "customs barriers" for all "regional" antigens. One single efferent vessel carrying lymph to the thoracic lymphatic duct comes out of the hilum (anatomic gate of the node) parallel to the artery and vein. Lymph node parenchyma is composed of T- and B-cell zones, and medullary cords.

- **B-cell zone.** Cortical substance is divided into radial sectors by connective tissue trabeculae and contains lymphoid follicles (B-cell zone or B-cell follicles). Follicular stroma consists of follicular dendritic cells (FDCs) forming a specific microenvironment, where unique processes that happen only in B-lymphocytes occur, namely somatic hypermutation of immunoglobulin variable gene segments and immunoglobulin class switching. Clonal selection of B-lymphocytes producing antibodies with increasing affinity ("affinity maturation") is performed there too. Lymphoid follicles undergo three stages of development. *Primary follicle* is a small follicle composed of naïve B-lymphocytes. After B-lymphocytes are stimulated with an antigen and enter immunogenesis (it happens approximately a week after active immunization), *a germinal center* appears, and the primary follicle gets transformed into a *secondary follicle*. The *germinal center* consists of intensively proliferating B-lymphocytes. When immunogenesis is completed a lymphoid follicle becomes significantly reduced in size.
- **T-cell zone.** T-lymphocytes and interdigitating DCs of bone marrow origin (they differ from FDC) which present antigens to T-lymphocytes are located in the paracortical (T-cell) zone of the lymph node. Lymphocytes migrate from the blood to the lymph node across the walls of post-capillary high endothelial venules.
- **Medulla is composed of cords of lymphatic tissue (medullary cords) and spaces between them (medullary sinuses).** It is located under the paracortical zone. During active immune response a multitude of plasma cells are accumulated in the cords. The lymph flows from cortical sinuses into medullary sinuses, and finally into an efferent lymphatic vessel.

Spleen

The spleen is a relatively large unpaired organ that weighs about 150 g. The spleen is supplied only with blood and not with lymph, and is a lymphocytic "customs" for "systemic" (blood-borne) antigens. Splenic lymphoid tissue composes **white pulp**. Splenic lymphocytes (mainly T-cells) surround arte-

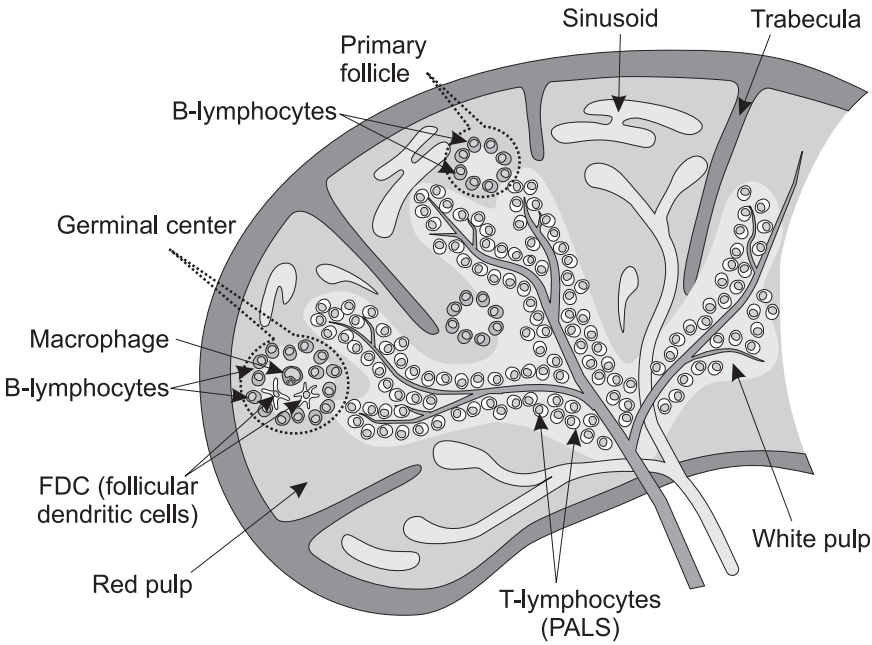

Fig. 2-4. Spleen. The accumulation of T-lymphocytes (light cells) around branches coming out of the trabecular artery, constitute the T-cell zone. Lymphoid follicle is where B-lymphocytes (dark cells), macrophages and follicular dendritic cells are residing.

rioles forming a cuff or so-called PeriArteriolar Lymphoid Sheaths (PALS) (Fig. 2-4). B-cell follicles are located peripheral to the PALS. Splenic arterioles drain into the sinusoids (part of the **red pulp**). Sinusoids join together to form venules which coalesce into the spleen vein routing blood to the hepatic portal vein. Marginal zone of the spleen is located at the border between white and red pulp. The marginal zone contains special population of B-cells (marginal zone B-cells) and APCs (DCs and specialized metallophillic macrophages).

Liver

The liver performs very important immune functions due to the following:

- The liver is a major organ of lymphopoiesis at embryonic stage and to some degree at the adult stage.
- Liver allografts are rarely rejected.

- Portacaval shunt surgery completely abrogates tolerance to orally administered antigens.
- The liver synthesizes acute-phase proteins (C-reactive protein and mannose-binding lectin) as well as the complement system proteins.
- The liver hosts different lymphocyte subsets including a unique one combining T- and NK-cell features (NKT-cells).

Cellular composition of the liver

- **Hepatocytes** form the liver parenchyma and express very few MHC class I molecules. A healthy liver is free of the MHC class II molecules, which may be expressed during liver diseases.
- **Kupffer cells** are liver macrophages; they constitute nearly 15% of total liver cells and 80% of total body macrophages. Their density is higher in periportal regions.
- **Endothelial cells** of liver sinusoids have no basal membrane.
- **The lymphoid system** of the liver contains, besides lymphocytes, an anatomic substrate of lymph circulation — **Disse's spaces**. These spaces lie between liver sinusoids and hepatocytes. Liver lymph contributes about 15–20% of the total thoracic duct lymph volume.
- **Stellate cells** (Ito cells) are located in Disse spaces. They contain fat vacuoles with vitamin A, α-actin and desmin, characteristic of smooth muscle cells, and may acquire myofibroblast-like phenotype.

Lymphoid tissue of mucous and skin

Non-encapsulated lymphoid tissue of mucous membranes is mainly represented by Waldeyer's (Pirogov's) lymphoid pharyngeal ring; Peyer's patches of the small intestine; lymphoid follicles of the appendix; lymphoid tissues of the stomach, bowel, bronchi, bronchioles, and urogenital organs.

- **Peyer's patches** (Fig. 2-5) is a group of lymphatic follicles found in the *lamina propria* of the small intestine (ileum) extending into its submucosa. B-lymphocytes comprise 50–70% and T-lymphocytes — 10-30% of Peyer's patch cell population. The majority of B-lymphocytes are located in germinal centers of secondary follicles. T-cell zone surrounds follicle and underlie so-called membranous (M) cells of intestinal epithelium. These epithelial cells lack microvilli that are present on other cells of mucosal epithelium. M-cells are the "entrance gate" of Peyer's patch and absorb antigens from intestinal lumen. Major function of Peyer's patches is to maintain immunogenesis of B-lymphocytes and their differentiation

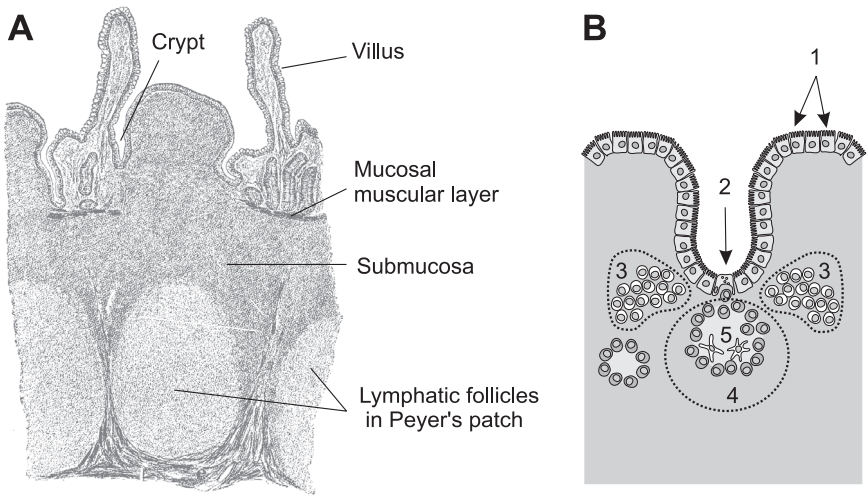

Fig. 2-5. **Peyer's patch in gut wall. A.** An overview. **B.** A significantly simplified scheme. 1 — enterocytes (gut epithelium); 2 — M-cells; 3 — T-cell zone; 4 — B-cell zone; 5 — follicle. The scale between structures is not observed.

into plasma cells producing antibodies — secretory immunoglobulins A (IgA) and E (IgE). IgA production in the mucous membrane of the gut accounts for about 70% of total immunoglobulin production in a human organism or nearly 3g daily per adult. Over 90% of synthesized IgA is transported through the mucous membrane into the gut lumen.

- **Intraepithelial lymphocytes.** In addition to being found in organized lymphoid tissue of mucous membranes, T-lymphocytes are disseminated among epithelial cells. A specific molecule (HML-1 or CD103) is being expressed on their surface and mediates their adhesion to enterocytes. $\gamma\delta$T/CD8$\alpha\alpha^+$ cells comprise about 10–50% of intraepithelial lymphocytes.