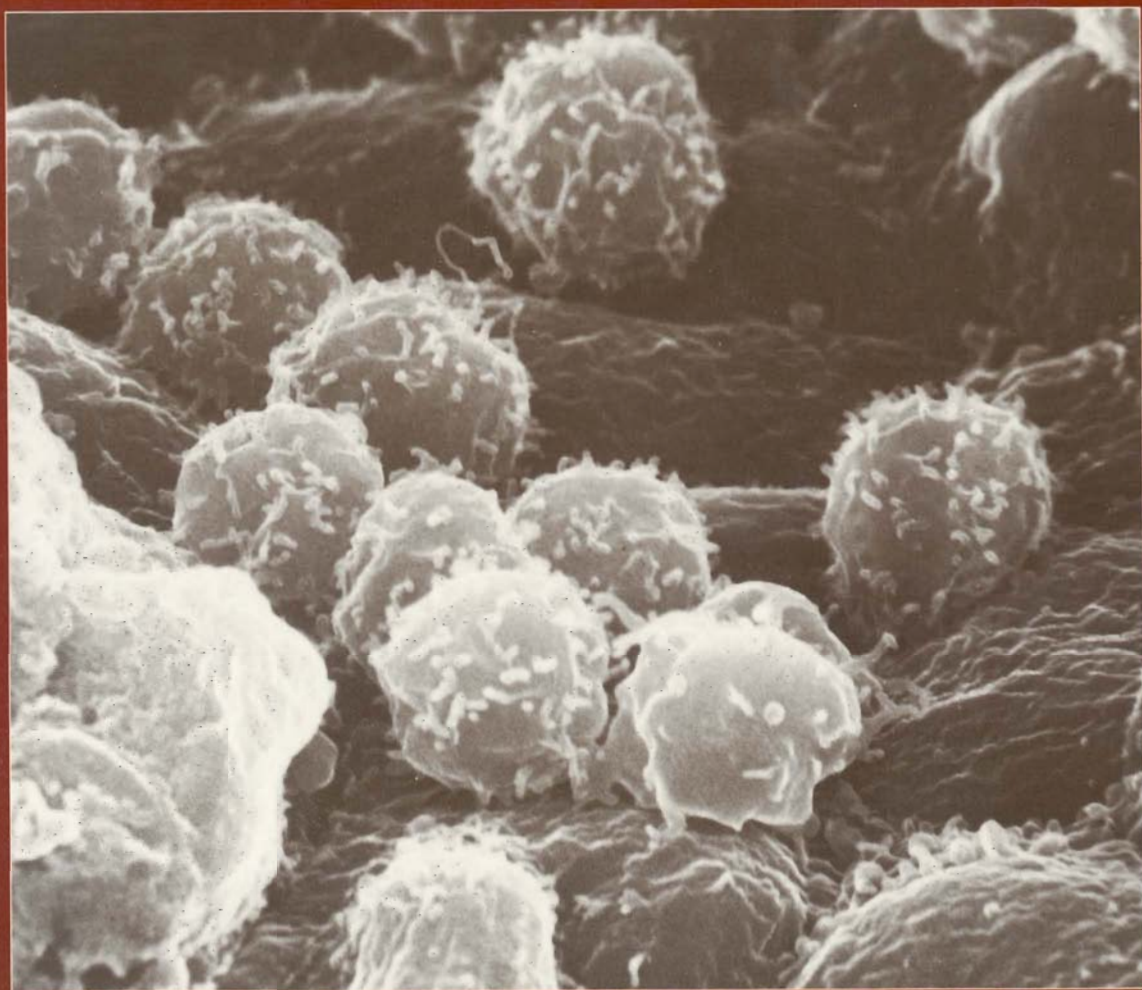


Immunophysiology



*The Role of Cells and Cytokines
in Immunity and Inflammation*

Edited by Joost J. Oppenheim & Ethan M. Shevach

Structure and Organization of the Lymphatic System

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The central and peripheral lymphatic tissues are composed of cells that are not permanently anchored to stromal or structural components as in organs such as the kidneys or the liver. Cells of the immune system move continuously, and this movement is essential to immunosurveillance and clonal selection. The cellular composition of lymphoid organs results from structural and molecular factors that control entry, compartmentalization, activation, and exit of circulating lymphoid cells. The unique connective tissue framework, vasculature, innervation, and tissue fluid dynamics of individual lymphoid organs represent specific functions, such as the development of immunocompetence or tolerance, antigen sampling from various internal or external environments, and compartmentalization or dissemination of immunoreactive cells. Morphokinetic development of lymphatic tissues depends upon gradients of diffusible or deposited factors and interactions among cells or between cells and the substratum. Receptor-ligand interactions regulate cell division, maturation, differentiation, secretion, membrane receptor expression, ion fluxes, cytoplasmic polarity, movement, and taxis. Recognition receptors on hematopoietic and lymphoid cells facilitate interactions with other cells and enable the latter to locate and enter tissues and fluid spaces in the intact animal. Traffic of T and B lymphocytes and mononuclear cells

through and within lymphatic tissues is vitally important for the intricate inductive and regulatory cellular interactions which must take place during the initiation and course of *in vivo* immune responses. Without circulation, migration, and recirculation, there would be little integration among lymphoid cells that monitor the environment, help or suppress immunity, and perform effector functions that are so vitally important to survival in the sea of microbes, toxins, parasites, and mutagens we call Earth.

The Lymphocyte

Structure and Function

Small lymphocytes are incredibly heterogeneous with respect to antigen specificity and function. These cells are nearly the most compact cells in the body. Small lymphocytes measure 5–8 μm in diameter and have a round compact nucleus surrounded by sparse cytoplasm. Because each T or B cell is committed to a single antigenic epitope, there must be a vast number of morphologically identical but immunologically diverse small lymphocytes to comprise the immune repertoire. Stem cells for T and B cells, long-lived T and B memory cells, and specific effector and immunoregulatory T cells are small lymphocytes (Fig. 2-1).

Medium and large lymphoblasts develop

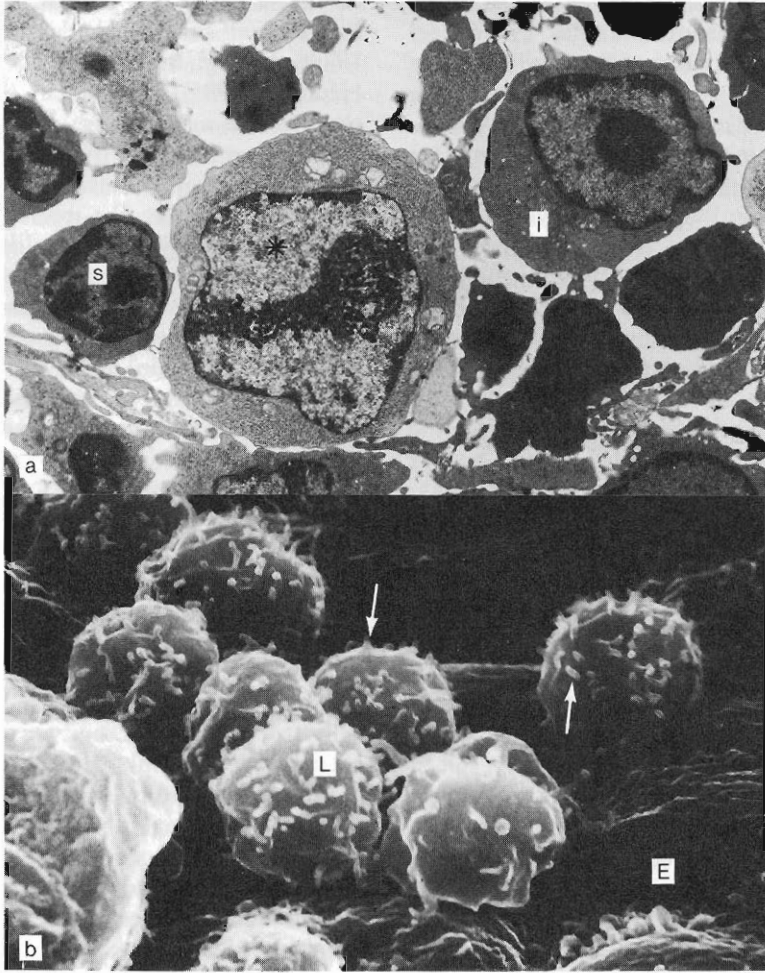


Figure 2-1. Lymphocyte size, shape, and surface morphology correlate with activity or function. (a) Three T cell morphologies are shown in this electron micrograph of a lymph node that had been stimulated by allogeneic lymphocytes. The large cell is a T lymphoblast (*), recognized as a metabolically active cell by a large vesicular nucleus, an organized nucleolus, and polyribosomes in abundant cytoplasm. To the left of the lymphoblast is a small lymphocyte (s), which has a condensed nucleus and little cytoplasm. This is what a "mature," recirculating T cell looks like. The intermediate-sized (i) lymphocyte to the right of the lymphoblast is peanut agglutinin positive (PNA⁺) and interleukin-2 (IL-2) receptor positive and does not recirculate. Although highly motile, this cell makes one pass in the circulation before migrating into tissue to complete maturation. (b) Circulating small lymphocytes (L) have surfaces studded with microvilli (arrows), as shown in this scanning electron micrograph of lymphocytes adhering to the endothelium of a high endothelial venule in a lymph node. Microvilli enable lymphocytes to make initial contact with membrane receptors on venule endothelial cells (E) by penetrating the repulsive, negatively charged glycocalyx on luminal surfaces (Anderson and Anderson, 1976). Microvilli are redistributed and eliminated during emigration from the blood into lymphatic tissues (van Ewijk et al., 1975).

from small lymphocytes after stimulation by mitogens, antigens, or cytokines. Their morphology reflects a series of metabolic changes that prepare the cell for division or secretion. They are 10–30 μm in diameter with a large nucleus, prominent nucleolus, and abundant cytoplasm containing polyribosomes and rough endoplasmic reticulum. Lymphoblasts actively produce DNA copies in the nucleus, ribosomal RNA in the nucleolus, and messenger RNA and proteins in the cytoplasm. They secrete lymphokines or are preparing for division. Lymphoblasts are sessile while cycling but become highly mobile following division. These cells express receptors for the lymphokine IL-2 and bind the lectin peanut agglutinin (PNA), which identifies "immature" T and B cells through characteristic surface carbohydrate composition. The presence of rough endoplasmic reticulum in the cytoplasm of B lymphoblasts indicates that they will become plasma cells.

Plasma cells represent the end stage of B cell differentiation. Although these cells are found rarely in blood and lymph, they are one of the dominant populations present in spleen and lymph nodes. Plasma cells range from 8 to 20 μm in diameter, exhibit an eccentric nucleus with condensed chromatin, and are easily identified in electron micrographs by the extensively developed rough endoplasmic reticulum used for manufacturing antibody. Differentiated plasma cells do not recirculate and remain at the same location for the rest of their existence. Although mature plasma cells are a prime source of antibody synthesis and secretion, they lack the surface immunoglobulin markers and motility of precursor B cells.

Motility

Lymphocytes migrating in vitro or in vivo exhibit cycles of motility alternating with resting stages of variable length (Lewis, 1931). Migration by small lymphocytes is executed with great difficulty because of the high nuclear:cytoplasmic ratio. Unlike other inflammatory cells, lymphocytes require a three-dimensional substrate support for effective locomotion, moving in a loose connective tis-

sue meshwork, in a fibrin gel, or upon and between cell surfaces (Anderson and Warren, 1979; Chang et al., 1979; Ratner and Heppner, 1985). Attachment of lymphocyte processes to a surface results in cytoplasmic polarization followed by a wave of cytoskeletal contraction, which propagates from front to rear along the midline axis, giving moving lymphocytes a wormlike appearance. Small T and B lymphocytes exhibit different rates of mobility. T cells locomote in vitro at 12 $\mu\text{m}/\text{min}$ whereas small B cells move more slowly, at 6 $\mu\text{m}/\text{min}$. Both rates are extremely slow as compared to professional "inflammatory" cells such as the neutrophils, which move 60 $\mu\text{m}/\text{min}$ (Anderson et al., 1982).

Chemotaxis

Directed migration of leukocytes along a chemical gradient is generally accepted as playing a role in cellular traffic during development of the lymphatic system, in recruitment of recirculating and nonrecirculating cells from the blood, and in promoting the accumulation of leukocytes at sites of inflammation and tissue injury. Lymphocytes were regarded as being incapable of chemotaxis because they are nonadherent, exhibit erratic motility in vitro, and do not respond to chemotactic factors selective for neutrophils and monocytes. Recent evidence from several laboratories suggests that small lymphocytes respond to gradients of biological materials containing various cytokines (Parrott and Wilkinson, 1981) such as acute-phase serum from endotoxin-stimulated rats and culture supernatants from activated macrophages, B cells (Anderson and Warren, 1979, 1980), thymic epithelial cells (Ben Slimane et al., 1983), mixed lymphocyte reactions, and mitogen-stimulated lymphocytes (Ward et al., 1971; Larsen et al., 1989). Mixed populations of T and B lymphocytes exhibit inefficient chemotaxis in vitro because individual cells are often producers as well as responders to chemotactic factors. Furthermore, the state of activation of lymphocytes affects their responsiveness and specificity for respective chemotactic factors. Purified interleukin 1 (IL-1) is chemotactic for small lymphocytes

(Miossec et al., 1984). Intermediate-sized lymphocytes respond to gradients of pure IL-2 (Kornfeld et al., 1985), lysophosphorylcholine, C5a-des-Arg, and leukotriene 4, etc. Small lymphocytes do not respond to gradients of pure IL-2, and intermediate-sized lymphocytes do not respond to pure IL-1 gradients. Increased frequency and magnitude of migration, stimulated by a gradient but not resulting in net orientation of the cells toward the gradient, is called *chemikinesis*. Neutrophil lysates stimulate this kind of migration in T cells (Anderson and Warren, 1980). Chemotaxis is gradient directed; the cells orient and migrate toward the gradient source (Zigmond and Hirsch, 1973). Therefore, whether a T or B cell exhibits chemotactic or chemikinesis depends upon the factor used to stimulate movement. In lymphatic tissues and inflammatory foci, chemotaxis may facilitate cell recruitment and interactions, whereas chemikinesis may contribute to cell mixing.

Concept of Lymphocyte Recirculation

All connective tissues of the body contain afferent lymphatic vessels that drain centripetally toward lymph nodes (Fig. 2-2). After passing through a series of lymph nodes, lymph is collected into major efferent vessels which connect with the great veins near the right atrium of the heart. The efferent lymphatic that drains the lower half of the body and the abdominal viscera including the spleen is the thoracic duct. Lymph from the upper body is returned to the blood via the right innominate duct. The output of small lymphocytes in thoracic duct lymph (TDL) is enormous but is rapidly depletable if drained away. When TDL are labeled in vitro and returned to the blood, they can be recovered in efferent lymph. Through studies like this, Gowans (1959) established the concept that long-lived small lymphocytes are repositories of immunologic memory and continually recirculate from blood to lymph across lymphatic tissues, to be returned to the blood again as they complete each cycle.

T and B lymphocytes exist in two populations with regard to their ability to recircu-

late. The observation that large lymphocytes labeled with [^3H]thymidine from recent cell division did not return to the efferent lymph when reinfused, but that resting cells labeled with [^3H]uridine did return, defined nonrecirculating and recirculating populations, respectively. Lymphocytes that do not recirculate have membrane glycoproteins that are incompletely sialated. They avidly bind the lectin peanut agglutinin (PNA^{high}), express IL-2 receptors, and are no longer small. Nonrecirculating lymphoblasts and intermediate B and T cells, which are generated following antigenic stimulation, immigrate into the spleen, mucosal lamina propria, and sites of inflammation after a single pass through the circulation. They undergo additional cycles of division after lodging, whereupon they mature and differentiate into either plasma cells or T effector cells.

Long-lived recirculating lymphocytes enter and leave specific areas of peripheral lymphatic tissues. The magnitude of recirculation is sufficient to replenish the circulating pool 10 times in 24 hours, or enough cells to populate completely a new 20-mg lymph node in the same time. The transit time for recirculating lymphocytes represents a summation of entrances and exits of cells that take different lengths of time to travel across specific lymphoid organs. For example, it takes less than 2 hours for lymphocytes to enter and leave mucosal lymphatic tissues, whereas the transit time is 6 hours for the spleen and between 18 and 24 hours for peripheral lymph nodes (Sprent, 1972).

Regardless of the rarity of a clonal precursor for a given antigen, recirculation guarantees that enough antigen-specific lymphocytes will pass through lymphatic tissue containing the antigen to initiate a response within 2 hours. Once in these locations, a recirculating lymphocyte may leave in the efferent lymph unless it encounters an antigen-presenting cell displaying the appropriate antigen-la complex. Antigen-specific lymphocytes will become activated, stop migrating, and remain in situ to divide, differentiate, or secrete. About 86% of the cells in efferent lymph of a "resting" primary lymph node arrived there by blood-to-lymph recirculation,

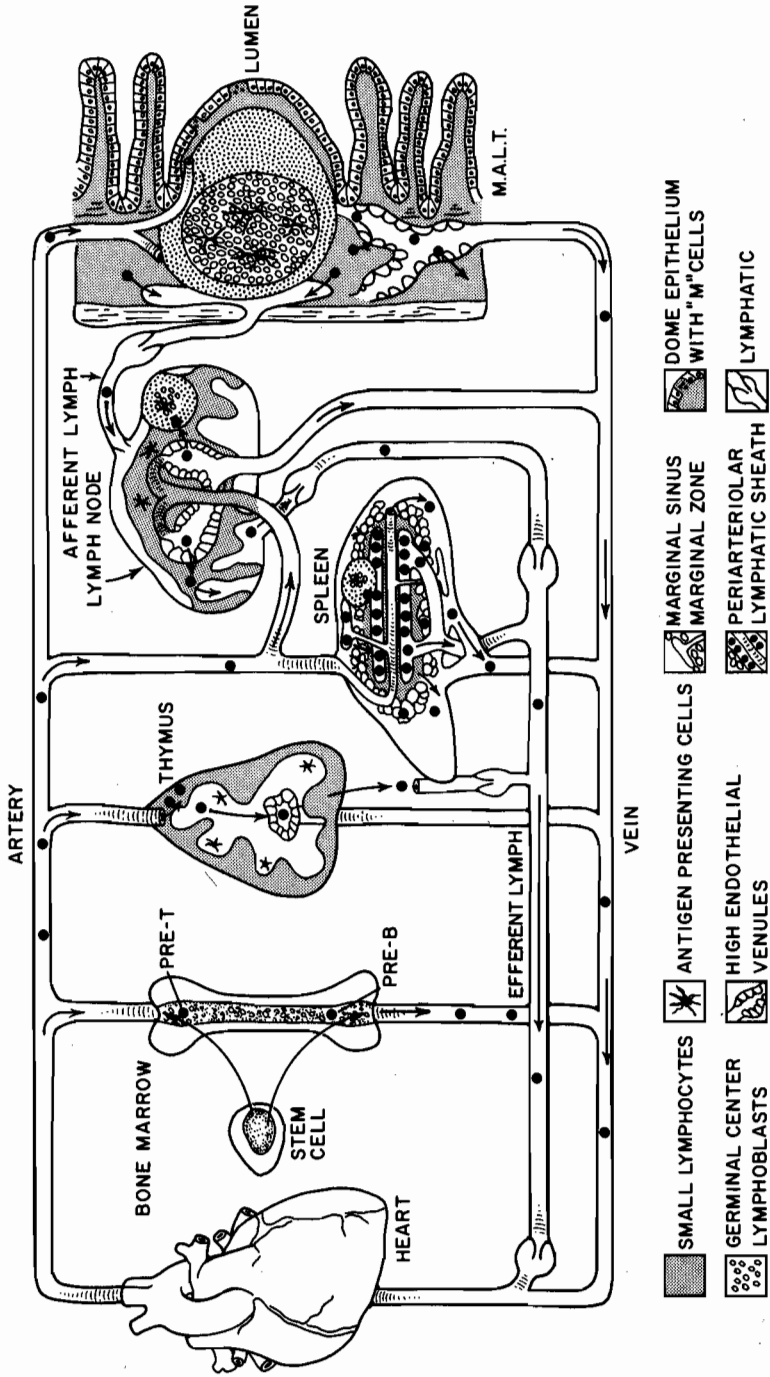


Figure 2-2. Diagram of the principal organs of the lymphatic system with their respective connections to blood and lymph vasculature. Use the patterns in the squares to identify structures while following the circuits taken by recirculating and lodging lymphocytes. Precursors of T and B cells are released from the bone marrow into the blood. The pre-T cells lodge in the thymus, where less than 5% of their progeny will mature into small recirculating T cells. Pre-B cells may require temporary residence in the spleen before they can enter follicles or join the recirculating pool. T and B cells enter lymph nodes and mucosa-associated lymphatic tissues (MALT) by selectively crossing high endothelial venules in the deep cortex and interfollicular areas, respectively. Migration through the spleen across the marginal sinus, through the marginal zone, into the periafferent lymphatic sheath does not require recognition receptors as in lymph nodes. Lymphocytes leave lymph nodes, MALT, and spleen via efferent lymphatics that carry them back to the blood. Lymphocytes that encounter antigen, presented on the appropriate accessory cell, will become nonmotile as they prepare to divide. The daughter cells leave in efferent lymph to seed other tissues and do not recirculate.

4% passed through from the afferent lymph, and the remainder were generated from division in the tissue. Lymph nodes whose afferent lymph drains from other lymph nodes may receive as much as 50% of their lymphocytes from the upstream node. Lymphocytes emigrate into nonlymphoid tissues like skin or muscle at a much slower rate and follow circuitous paths through connective tissues around nearly all cells until they ultimately enter afferent lymphatics draining toward lymph nodes. The ability of lymphocytes to monitor the surfaces all normal and "transformed" cells of the body is fundamental to immunosurveillance.

Cooperative interactions during immune induction to thymus-dependent antigens require that antigen and specific B and T cells meet in the same place at the same time. Differential migratory properties of T and B cells through and within tissues of the lymphatic system may facilitate lymphocyte cooperation because fast crawling helper T cells will overtake sessile antigen-responsive B cells wherever they are. Cooperation is enhanced by release of chemotactic factors for T cells by either macrophages or B cells (Anderson and Warren, 1980). The processes controlling lymphocyte recirculation are a topic of much interest. It is not known whether recirculation is maintained by a continuous but unknown inflammatory stimulus or whether the process is genetically programmed as are cycles of morphokinesis and expression of surface molecules during development of embryos. The tempo of recirculation and possibly the distribution of functional subsets among trafficking lymphoid cells in lymphatic tissues may be affected by local release of cytokines, prostaglandins, and other mediators that cause an increase or decrease in blood flow, vascular permeability, and rates of lymphocyte emigration from the blood (Hall and Morris, 1965; Cahill et al., 1976; Anderson, 1985). Andrews et al. (1980) proposed that the endothelial cells of high endothelial venules (HEV) secrete a highly sulfated molecule that influences recirculation. Hendriks and Eastermans (1983) have shown that surgical ablation of the afferent lymphatic supply to a lymph node results in gradual diminution and

loss of recirculation through that node, suggesting that afferent lymph supplies cell types or factors that are essential for the "activity" of HEV in supporting lymphocyte recirculation. Neuropeptides and noradrenergic stimulation from unmyelinated nerve fibers that ramify throughout vascular and nonvascular lymphatic tissues may play a role also in controlling lymphocyte recirculation, especially with regard to blood flow distribution (Anderson and Anderson, 1975; Ottaway, 1984; Felten et al., 1985). Norepinephrine- and acetylcholine-releasing nerve fibers are found in all central and peripheral lymphatic tissues. Vasoactive intestinal peptide, neuropeptide Y, Met-enkephalin, cholecystokinin-8, and neurotensin immunoreactivity is present in the thymus and spleen. The greatest variety of neuropeptides is present in, and active upon, mucosal lymphatic tissues. Noradrenergic and peptidergic innervation of lymphoid compartments in central and peripheral lymphatic tissues suggests that there are important links between the nervous system and elements of the immune system (Goetzl, 1985; Jankovic et al., 1987).

A number of studies indicate that there may be organ-specific segregation in patterns of lymphocyte recirculation regulated at the level of recognition receptors because populations of cells have been identified that are restricted to migration circuits through peripheral, mucosal, or chronic inflammatory tissues (Cahill et al., 1977; Reynolds et al., 1982). Circulating lymphocytes "see" only the luminal surfaces of vascular endothelium; yet they are capable of locating sites for emigration in specific organs and sites of inflammation. This concept suggests that endothelial cells either differentiate in unknown ways to reflect the local milieu, or they transport and express on their luminal surfaces something that reflects the local environment. Rotrosen et al. (1987) have produced data that support the latter hypothesis. Endothelial cells, exposed to the chemotactic peptide fMet-Leu-Phe on their abluminal sides, transport and display the peptide on their luminal surfaces. This observation suggests an important mechanism for producing organ specificity in leukocyte traffic if it can be generalized

to chemotaxins selective for subsets of lymphoid cells that exhibit selective traffic patterns as it has for neutrophils.

Tissues of the Immune System

The lymphatic tissues in mammals can be classified as either central or peripheral according to whether they generate functional precursor lymphocytes or provide microenvironments for interaction of lymphoid cells with antigen and accessory cells. The central lymphoid organs consist of bone marrow and thymus, where stem cells give rise to a diverse progeny of lymphocytes through processes that are believed to be independent of antigenic stimulation. The peripheral lymphatic tissues include lymph nodes, spleen, and mucosal-associated lymphatic tissues in the respiratory and gastrointestinal tract, where lymphocyte development is antigen dependent.

Central Lymphoid Organs

Bone Marrow

DEVELOPMENT

The hematopoietic stem cell that gives rise to all formed elements of the blood exhibits a peripatetic life cycle by moving to multiple sites during embryogenesis. The earliest location for the primordial stem cell is in the dorsal mesentery of the embryo near the primitive aorta (Martin et al., 1979; Dieterlen-Lievre and Martin, 1981). The stem cell migrates through loose connective tissues to the yolk sac, where its progeny rapidly divide and develop into precursors of blood cells and endothelial cells (Peault et al., 1983).

The location of hematopoiesis shifts from the yolk sac to the developing liver, where clonal hematopoietic colonies expand in close proximity to endoderm-derived hepatocytes and sinus lining cells (Rossant et al., 1986). The liver remains the major site of fetal hematopoiesis until shortly before birth, when stem cells travel by blood to the spleen which supports hematopoiesis until vascular invasion of cartilaginous bones begins the process of ossification and formation of mar-

row cavity. Population of the developing marrow may be facilitated by chemotactic and growth factors released from bone matrix (Mundy et al., 1978) and from the cells forming and remodeling it (Gordon et al., 1987). The bone marrow remains the primary site of hematopoiesis in adults until death, although some stem cells in abdominal mesenteries can be induced to form hematopoietic colonies by cytokines released during inflammation (Potter et al., 1985).

ADULT BONE MARROW

The bone marrow is the site of origin of all T and B cells, mononuclear phagocytes, platelets, erythrocytes, and other leukocytes in the adult (Fig. 2-3). The aggregate volume and weight of the bone marrow surpass those of the liver. The marrow is divided into wedge-shaped hematopoietic compartments filled with proliferating and differentiating blood cells in connective tissue matrices bordered by venous sinuses. Monoclonal antibody-detected phenotypes and reactions for enzymes expressed at stages of differentiation show progressive hematopoietic cell maturation from immaturity in areas near bone to full differentiation at the vascular interface. The radial venous sinuses that merge with a central longitudinal vein occupy a major portion of mouse bone marrow. Blood cells complete maturation immediately adjacent to the dilated vascular channels into which they will emigrate.

The microenvironment of the bone marrow is produced by a unique endothelium and connective tissue stroma combined with locally deposited cytokines that regulate compartmentalization, proliferation, and differentiation of hematopoietic stem cells (Dorschkind, 1986). Adventitial cells lining the interstitial side of venous sinuses extend cytoplasmic processes into the hematopoietic compartment, making contact with numerous cells. Stromal cells are essential for regulation of hematopoietic cell development (Dexter, 1982; Keating et al., 1982; Marshall et al., 1984). In situ hybridization with probes for allotypic markers proved that stromal cells are capable of transferring the hematopoietic microenvironment of the donor after allogeneic bone marrow transplantation

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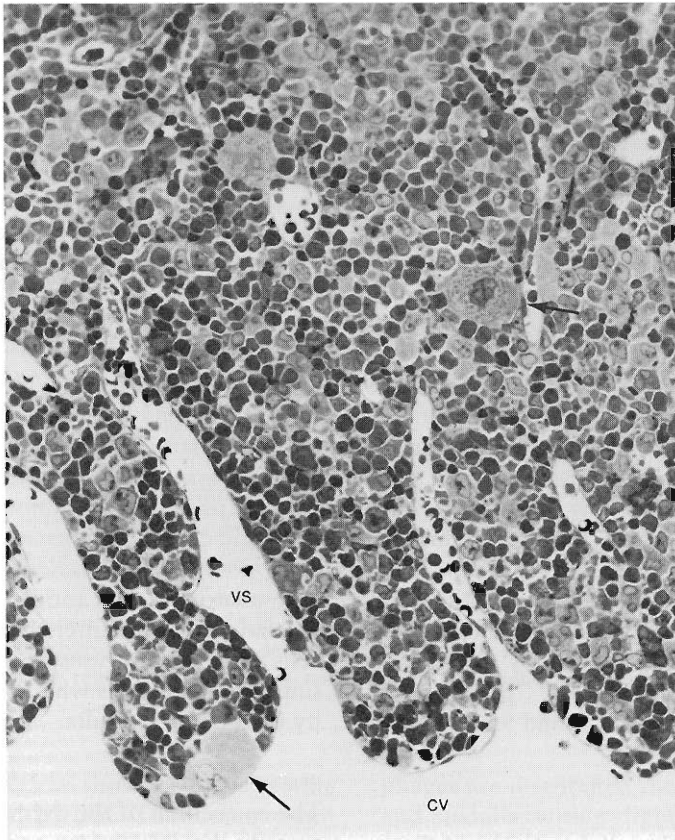


Figure 2-3. Murine bone marrow resembles an estuarial bog. Hematopoietic colonies are crammed together in "deltas" of tissue divided by venous sinuses (vs) branching from the central vein (cv). The nuclei of hematopoietic cells are denser (more mature) near the sinus endothelium and less dense farther away. Megakaryocytes (arrows) are usually located next to venous sinuses so they can extrude platelets into the blood stream.

(Simmons et al., 1987). Marrow stroma assists hematopoiesis through production of a glycosaminoglycan extracellular matrix that binds and distributes growth factors such as granulocyte/macrophage colony-stimulating factor (GM-CSF) (Gordon et al., 1987).

The vascular system of the marrow is anatomically closed, with little leakage. Mature cells are transported from interstitium to venous sinus through cytoplasmic apertures in the endothelium (Weiss, 1972). Location of immature cells at sites far removed from the venous sinuses requires that maturing cells acquire sufficient motility to approach the blood vascular interface. Accidental release of cycling hematopoietic cells is prevented because motility is suppressed during cell division. Tidal blood flow through dilated venous

sinuses gently supports peripheralization of hematopoietic cells that leave marrow compartments to lodge in other environments as soon as they are able. The marrow monitors and controls release of hematopoietic cells to the periphery by mechanisms that are still unknown.

Noradrenergic nerve fibers penetrate into the bone marrow with arteries and follow them deep into regions within the cellular marrow adjacent to the central venous sinus. A few fine varicosities branch from the perivascular plexus to supply aggregates of hematopoietic cells that lay within areas of marrow close to venous sinuses. Little is known of the function of bone marrow innervation outside of its assumed role in controlling blood flow (Felten et al., 1985).

B CELL DEVELOPMENT IN BONE MARROW

The bone marrow generates cells with immunologic diversity for the lymphatic system. B cell development begins in the liver and continues in the bone marrow as maturation progresses. During maturation from the primordial stem cell, B cell progenitors contain cytoplasmic IgM. These cells do not express this protein on their surfaces, although they share another surface antigen with mature B cells (Kincade, 1981). B cells are susceptible to tolerance induction by antigen or anti-immunoglobulins when they are in these early stages of differentiation (Nossal, 1979). Shortly before birth, lymphocytes with detectable surface IgM appear in bone marrow; this is followed by that of other cells bearing both IgM and IgD on their surfaces. Antigen specificity of precursor B cells is determined by germline genes encoding the variable and hypervariable regions of the immunoglobulin light and heavy chains expressed on the membranes of "precursor" B cells, as discussed by Potter and Smith-Gill in Chapter 7.

Thymus

DEVELOPMENT

This lymphoepithelial organ develops from ectoderm derived from the third branchial cleft and endoderm of the third branchial pouch including mesenchymal components derived from cells of the cephalic neural crest which all migrate from the neck to the anterior mediastinum (Bockman and Kirby, 1984). The rudimentary thymus enlarges in successive waves as lymphocytes and hematopoietic cells emigrate from embryonal blood vessels to infiltrate spaces between plump epithelial cells. Proliferation and entry of lymphoid precursors distend interepithelial spaces, resulting in densely packed clusters of lymphocytes in a desmosome-linked epithelial cell reticulum (Clark, 1973). Ultimately, the thymus becomes an encapsulated, lobulated organ with a cortex that is densely populated with lymphocytes and medulla that appears more epithelial because of a relative paucity of lymphoid cells (Fig. 2-4).

Chemotactic peptides elaborated by thymic epithelial cells and/or mononuclear cells are believed to initiate immigration of circulat-

ing lymphocytes to the developing thymus (Champion et al, 1986 and Savagner et al., 1986). Population of the thymus with lymphoid cells occurs in three discrete 24- to 36-hour waves separated by refractory periods (Jotereau and Le Douarin, 1982; Jotereau et al., 1987). Temporally expressed receptiveness or periodic release of chemotactic factors by thymic epithelial cells regulates population of the thymus.

The development and maturation of T cells are under the influence of the epithelial and mesenchymal stroma. The epithelial cell stroma exhibits regional specialization with regard to structure and expression of antigenic markers. The mesenchymal stroma consists primarily of mononuclear phagocytes, dendritic cells, and unusual cell types like the rhabdomyocyte; these cells appear to represent anatomic and antigenic stereotypes of normal host cells. Interaction of precursor T cells with these various stromal elements presumably determines whether immunoreactivity or tolerance results.

EPITHELIAL STROMA

The epithelium of the thymus undergoes progressive keratinization and maturation like the skin. The epithelial reticulum of outer cortex, inner cortex, corticomedullary junction, and medulla is distinguishable by monoclonal antibodies that bind cytoplasmic keratins of different molecular weight (Laster et al., 1986; Nicolas et al., 1986; von Gaudecker et al., 1986). Subcapsular thymic epithelium contains less mature keratin; the most mature keratin is found in Hassal's corpuscles in the medulla. Lectin binding patterns in the thymus also indicate corticomedullary maturation (Farr and Anderson, 1985). Taken together, the epithelium of the thymus may turn over continuously like the epithelium of the skin. The Hassal's corpuscles, which are filled with whorls of keratinized epithelium, leukocytes, and debris, may be internal sites where exfoliated epithelium is endocytosed, degraded, and carried away by leukocytes.

Thymic epithelial cells express major histocompatibility antigens in three basic patterns. The subcapsular epithelial layer is class II negative. The remaining cortex, including the thymic nurse cells (Wekerle et al., 1980), is strongly class II positive in a reticular pat-

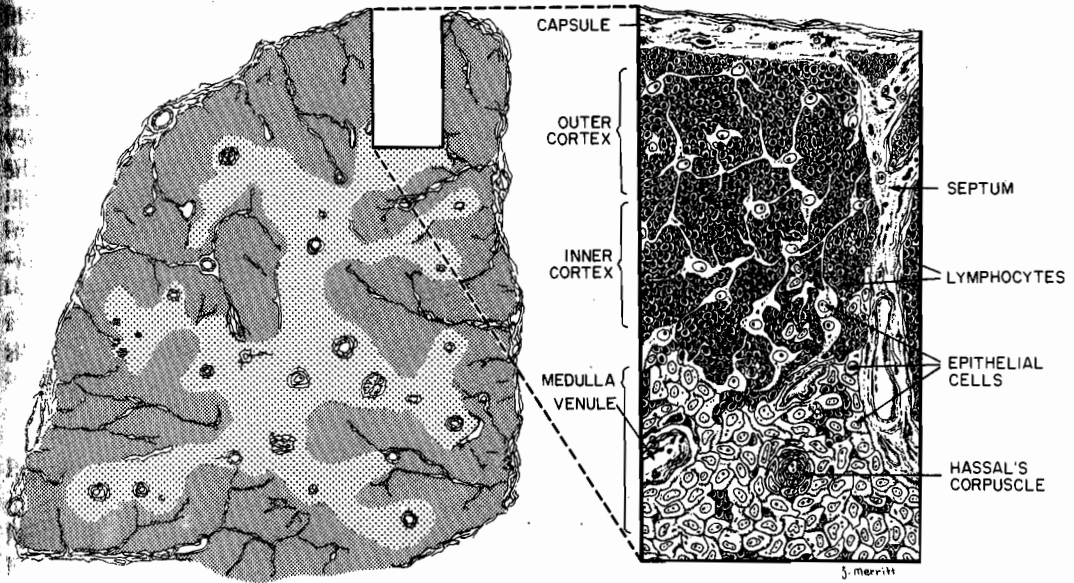


Figure 2-4. Diagram of a thymus lobule, indicating the relative density of lymphocytes in the cortex (fine pattern) and medulla (coarse pattern). The detailed drawing to the right magnifies the epithelial cell reticulum and identifies the regions of the cortex and medulla discussed in the text. (Modified after Weiss, 1972.)

tern that extends from the outer cortex to the corticomedullary border. The inner membrane of thymic nurse cells is strongly positive for class I as well (de Waal Malefijt et al., 1986). The medulla is densely class I and II positive, but it is uncertain whether class II is expressed on the spatulate epithelial cells because treatments that deplete bone marrow-derived dendritic cells render the medulla class II negative. Dendritic cells of the donor's class I and II type are present in the medulla by three weeks following bone marrow reconstitution, whereas the cortical reticulum retains that of the host. All cells in the thymus express major histocompatibility complex (MHC) class I antigens, but the highest expression is on nonepithelial cells. Although self/nonself education and acquisition of haplotype restriction of developing T cells require intrathymic expressed MHC antigens, it is not known whether MHC⁺ epithelial cells or MHC⁺ mesenchymal cells contribute equally or not.

MESENCHYMAL STROMA

Macrophages, dendritic cells, and perivascular reticular connective tissue comprise the mesenchymal stroma of the thymus. Macro-

phages are distributed throughout the cortex and medulla, with a slight increase in density at the corticomedullary border. Immunohistology and enzymatic reactions reveal heterogeneity among thymic mononuclear cells. Nonspecific esterase-positive cells are more prevalent in the medulla, but rare scattered cells are present in the corticomedullary junction and cortex (Duijvestijn et al., 1982). Few of the esterase-positive mononuclear phagocytes are Ia-antigen positive. The cortical macrophages and some of the medullary macrophages contain phagocytosed nuclear debris and periodic acid-Schiff (PAS) positive granules. Dendritic cells at the corticomedullary junction are acid phosphatase positive and are strongly reactive with antibodies specific for class I and II MHC antigens.

The nonepithelial dendritic cells in the thymus control development of CD4⁺ T cells. Monoclonal antibodies to Ia antigen produced profound immunosuppression and loss of the dendritic cells in the medulla without affecting Ia antigen expression of thymic epithelial cells (Kruisbeek et al., 1985). Anti-Ia treatment selectively reduced development and release of CD4⁺ T cells into the periphery without affecting development of CD8⁺

cells or immature thymic cortical cells. Thus helper T cell development is entirely dependent on functional dendritic cells, and development of immature T cells and suppressor cells occurs through interactions with different cells.

Noradrenergic and peptidergic fibers enter the thymus with nerve bundles and plexuses around blood vessels, penetrate into the cortex from subcapsular plexuses, and branch among lymphocytes in the thymic cortex (Felten et al., 1985). The vasculature and lymphatic tissue of the outer and deep cortex are innervated by these fibers. The medulla is remarkably free of innervation except for fibers associated with blood vessels. Neuropeptide antigens are present in varicosities of thymic neurons. Vasoactive intestinal peptide (VIP)-reactive neuronal varicosities and acetylcholinesterase-positive profiles are present in the deep cortex and corticomedullary regions, respectively.

BLOOD-THYMUS BARRIER

A blood-thymus barrier was proposed because of differences in *ex vivo* antibody formation in thymus fragments after injection of antigen directly into the thymus or after intravenous inoculation (Marshall and White, 1961; Horiuchi and Waksman, 1968). Studies with tracers of blood vessel permeability showed that cortical thymocytes, in contrast to those of the medulla, are protected from circulating macromolecules (Raviola and Karnovsky, 1972). Blood vessels in the thymic cortex were not permeable to the protein tracers, but postcapillary venules in the medulla permitted tracers to leak along the clefts between endothelial cells. The tracers had limited distribution in the thymic parenchyma because macrophages in the perivascular space ingest and retain much of the leaked tracer. Phagocytic cells and basal lamina at the border between the cortex and medulla captured any tracer that may have escaped endocytosis in the vessel wall. Thus hematogenous antigen has access, albeit restricted, to the thymic medulla.

LYMPH-THYMUS BARRIER

Although the thymic cortex has a blood barrier, it is freely permeable to particulates or soluble proteins in lymph fluids originating in

the peritoneal cavity. Antigens deposited in the peritoneal cavity rapidly pass through diaphragmatic and mediastinal lymphatics to the parathymic lymph nodes and lymphatics surrounding the thymus capsule, where they diffuse into the lymphoid parenchyma of the superficial cortex (Eggli et al., 1986; Stet et al., 1987; Pitt and Anderson, 1988) on a concentration gradient produced by the unique cortical blood vasculature. Soluble antigens deposited in the outer cortex may deliver a tolerogenic signal to developing T cells (Cowing et al., 1985).

T CELL DEVELOPMENT IN THYMUS

The thymus provides a receptive microenvironment for bone marrow pre-T cells to enter, clonally expand, select antigen and MHC haplotype specificities, mature, and exit to the periphery after elimination of rogue clones. The thymus releases fewer than 5% of its daily cell production, the remainder apparently dying within the organ. The route of traffic of pre-T cells to specific regions of the thymus and out again has long been debated, especially with regard to the number of precommitted cell lineages that enter and the parenchymal locations where they develop. A single stem cell can recolonize an embryonic thymus and produce nearly all T cell subsets, however (Kingston et al., 1985). The conventional maturation pathway is presented here. Pre-T cells multiply in the cortex, begin to mature, and then migrate toward the medulla where recirculating cells exit via postcapillary venules near the corticomedullary junction, and nonrecirculating cells exit via efferent lymphatics in the capsule and interlobular septae (Weissman, 1967). Proliferation of immature T cells occurs in various discrete cortical microenvironments including inside cytoplasmic envelopes of nurse cells located in the outer cortex (Wekerle et al., 1980). Immature T cells enclosed by nurse cells do not differentiate into cytotoxic T cells, and a minority express markers indicating helper cell commitment (Andrews et al., 1985), but the ultimate fate of T cells educated by nurse cells is unknown. Other T cells divide and mature in association with distinctive epithelial cells and/or mononuclear cells of bone marrow origin. Mature T cells that exit the thymus enter the circulation and many become com-

ponents of the recirculating pool. CD4⁺ cells that recirculate do not return to the thymus, but CD8⁺ cells may. A population of CD4⁺/CD8⁺ T cells are released from the thymus into the circulation, where they lodge in the spleen to complete maturation into specific immunoregulatory or effector cells. The complete sequence of phenotypic and functional changes occurring in developing T cells, and the genetic rearrangements in the T cell receptor for antigen which occur during development will be discussed by Shevach in Chapter 6.

Peripheral Lymphoid Organs

Although it is widely believed that "sensitization" may occur anywhere in the body, the peripheral lymphoid organs provide the most efficient sites for interaction of T and B cells with presented antigen by way of characteristic mechanisms for acquiring antigen and by the presence of vascular interfaces for large-scale circulation and recirculation of immunocompetent cells. Lymph nodes are filters of lymph, the spleen is a filter of blood, and mucosa-associated lymphatic tissues acquire environmental antigens by pinocytosis and transfer through specialized follicle-associated epithelial cells.

Skin and Diffuse Lymphatic Tissues

Skin and epithelial surfaces are the first line of defense against environmental antigens that threaten an organism's integrity. Epithelium with underlying loose connective tissue, blood vessels, and lymphatics may be considered part of the diffuse lymphatic system, especially for contact-sensitizing antigens that fix to epithelial cells. Epithelium functions primarily as a physical barrier, but keratinocytes, mononuclear cells, and nerve endings included in the epidermis secrete a large number of biologically active substances that may effect differentiation and activation of T cells locally or in regional lymph nodes (Kato et al., 1981; Luger et al., 1981; Rubinfeld et al., 1981; Chu et al., 1983).

Lymphocytes and Thy1⁺ dendritic epidermal cells (Koning et al., 1987) are infrequently seen in the dermis or in intraepidermal loca-

tions of normal skin. Because of the vast surface area, the total number of lymphocytes in the skin may be great despite the small numbers in any single location. Although there is considerable speculation about homing of lymphocytes to the skin, few are present in afferent lymph (Rannie and Ford, 1978). Langerhans cells populate the epithelial layer above basal cells and keratinocytes in a uniformly distributed pattern, presumably limited by contacts among extended dendritic processes. Mononuclear phagocytes are usually found in perivenular locations in the superficial dermis. Precursors for Langerhans cells and monocytes enter the skin from the blood to replace those that regularly exit into the afferent lymph of regional lymph nodes (Hoefsmit, 1979; Streilein et al., 1982). The mean turnover time of Langerhans cells in mouse skin is about three weeks (Katz et al., 1979; Toews et al., 1980). Langerhans cells in afferent lymph draining the skin are regarded as the sentinels of skin-associated lymphoid tissue (Kaplan et al., 1987). The so-called passenger leukocyte that initiates allograft rejection is also a Langerhans cell, and removal of the cell (Stingl et al., 1981; Streilein et al., 1982) or ablation of the afferent lymphatics (Barker and Billingham, 1968) prevents sensitization (Billingham and Silvers, 1971; Silvers et al., 1976).

Lymph and Lymphatics

Lymph is a clear body fluid that clots like blood. Lymph forms when dissolved proteins and solutes filter out of venules and capillaries because of local differences in luminal hydrostatic and osmotic pressure. Whenever the epithelial barrier is breached, the neuropeptide "substance P" is released by axons and increases lymph production through hyperemic effects (Helme et al., 1987). Afferent lymphatic vessels originate in the reticular connective tissue beneath the epithelium of the skin, gut, and urogenital tract and within the mesenchymal tissues of all organs (Yoffey and Courtice, 1956). The endothelial cells of lymphatics are anchored to the surrounding reticular fiber meshwork, causing the lymphatics to dilate rather than collapse when tissue hydrostatic pressure exceeds the pressure of fluid in the lymphatic. This opens valvelike

junctions between endothelial cells and allows intravasation of tissue fluids (Leak and Burke, 1968). Lymph flows unidirectionally toward lymph nodes because valves prevent backflow under normal physiologic conditions. Lymph capillaries merge into larger lymphatics which drain into lymph nodes. Efferent lymph from regional lymph nodes may drain into one or more additional nodes before flowing into major efferent lymphatics.

LYMPH NODES

The main function of lymph nodes is to filter and trap antigens and cells containing antigen that flow into them via afferent lymphatics and to provide a site for clonal expansion of lymphoid cells recruited from the millions of cells that enter and leave via various routes (Fig. 2-5). Lymph nodes are connective tissue bags filled with mobile cells organized into functional compartments by a meshwork of reticular cells and fibers, specialized blood vessels, and nerve fibers. A "cortex" and a "medulla" are distinguishable in histologic preparations by the relative density of small lymphoid cells contained in the reticular ma-

trix. The superficial cortex contains a lymphatic sinus, macrophage-rich zone, and B cell follicles. The deep cortex is a traffic zone where migrant or recirculating T and B lymphocytes enter from the blood.

Lymphocytes in the deep cortex are directed toward specific B or T cell microenvironments where they may be activated by antigen-presenting cells, or the lymphocytes crawl into lymphatic channels and out of the lymph node. The medullary cords contain sessile B lymphoblasts and plasma cells which accumulate there after immune reactions, and the medullary lymph sinuses are the highways out of the lymph node. The cortex is organized into hemispheric lobules, where the flat surfaces face the afferent lymph supply, and the round central borders merge with stromal chords and sinuses to form the medulla. Cortical lobules in lymph nodes of all mammalian species have a constant vertical dimension. The lateral dimensions of cortical lobules are highly variable, accounting for the rapid two- to fourfold node enlargement that occurs 6-18 hours after antigen inoculation (Anderson and Reynolds, 1979).

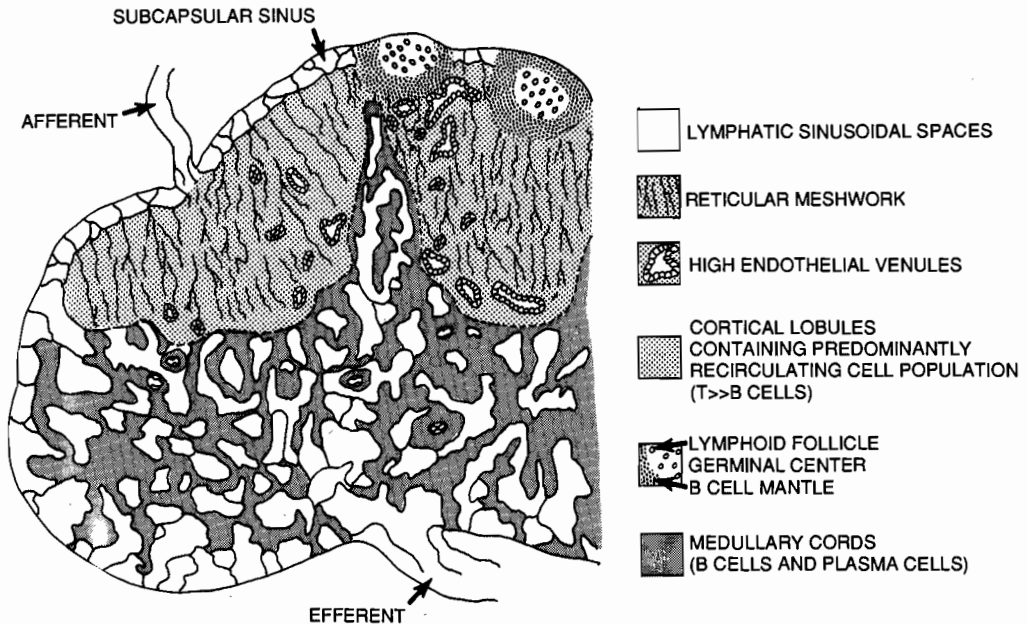


Figure 2-5. Diagram depicting the general topography of rat or mouse lymph nodes where tissue organization is simplified. The lymph node lymphoid compartments, as represented by the patterns in the squares, include cortex, medulla, follicles, lymph sinuses, and high endothelial venules.

ANTIGEN ACQUISITION AND PRESENTATION

Afferent lymph drains into a flat lymphatic antechamber (the subcapsular sinus), which distributes the lymph over the superior surface of cortical lobules packed with lymphocytes, scattered macrophages, and interdigitating dendritic cells. Motile cells and soluble materials percolate directly into the superfi-

cial cortex by passing through "pores" in the floor of the subcapsular sinus. Larger particles and dead cells pass into lymphatic capillaries (intermediary sinuses), which form an extensive perivenular plexus in the deep cortex before emptying into the medullary sinuses (Fig. 2-6). There is considerable phagocytic activity at all portals of antigen access

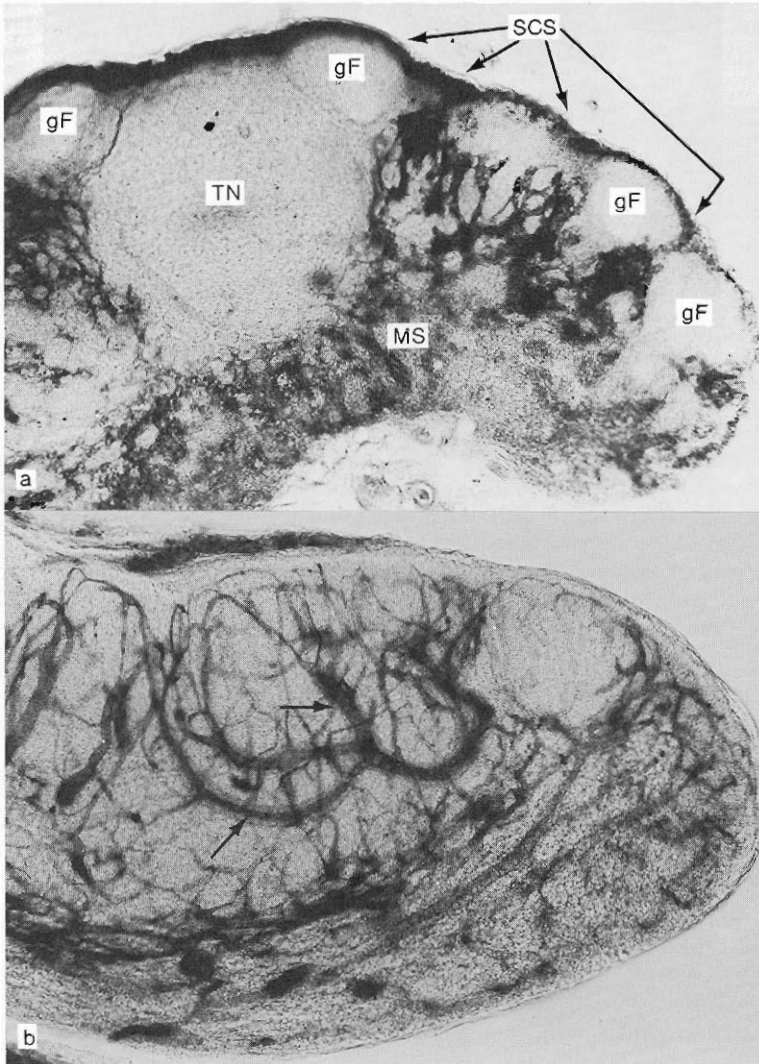


Figure 2-6. (a) The "diffuse" cortex is permeated with fine plexuses of lymphatic capillaries, as illustrated by this cleared slice of lymph node stained with carbon by intralymphatic injection. The structures that readily fill with carbon tracer include subcapsular sinus (SCS), intermediary sinuses (arrows), and medullary sinuses (MS). Note that germinal follicles (gF) and T cell-reactive nodules (TN) are not penetrated by tracer. (b) This cleared slice of lymph node stained by intravascular perfusion with alcian blue dye (Anderson and Anderson, 1975) illustrates that the intermediary sinuses are spatially related to networks of high endothelial venules (arrows) in the deep cortex.

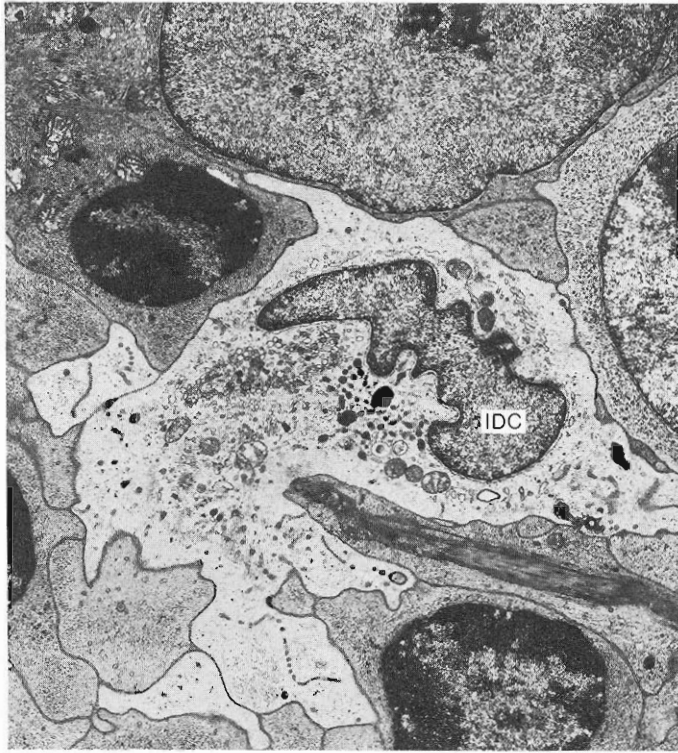


Figure 2-7. Interdigitating dendritic cells (IDC) with characteristic electron-lucent cytoplasm and branching processes are located among the T cells in the deep cortex. The processes (or veils) extend and insinuate among numerous lymphoid cells. It has been estimated that one IDC makes contact with at least one macrophage and 200 or more lymphocytes (Anderson and Warren, 1982). These cells are strongly and constitutively Ia-antigen positive; they are not phagocytic but have cytoplasmic organelles that are ideally suited for glycosylation and phosphorylation—functions that might contribute to their assumed role as antigen-presenting cells.

to lymph nodes. Macrophages are found in the subcapsular sinus near the pores and suspended from reticular fibers that cross the lymphatic channels. Macrophages are also prevalent in the large lymphatic sinuses in the medulla. Antigens that escape phagocytosis within a lymph node face phagocytosis in other lymph nodes through which the lymph must pass before entering major efferent collecting ducts. Antigens that succeed in eluding lymph node entrapment will ultimately be captured by blood monocytes or macrophages in the spleen, liver, or bone marrow.

Mononuclear cells spaced throughout the deep cortex participate in antigen processing and presentation. Not all macrophages in these sites express surface Ia antigen or perform antigen-presentation functions, but all phagocytic cells may participate in process-

ing by ingesting and degrading antigens for use by Ia antigen-expressing cells. Interdigitating dendritic cells (IDC) constitutively express Ia antigen and exhibit physical contacts with macrophages and numerous cortical T cells (Veldman, 1970). This capacity to distribute its cell surface over numerous lymphocytes (Fig. 2-7) may explain the IDC's relative effectiveness in stimulating mixed lymphocyte culture reactions (Steinman and Witmer, 1978). Although IDC do not secrete lymphocyte activating factors, macrophages located near IDC secrete cytokines which enhance interaction and stimulation of T cells by IDC. It is not known whether the interdigitating dendritic cell is directly derived from Langerhans cells from the skin (Stingl et al., 1978), by local differentiation of a migrant precursor cell (Anderson et al., 1981), or

by differentiation of daughter cells produced through local division of resident mononuclear cells. Fibroblastic reticular cells in lymph nodes also bind antigen without phagocytosis. Three to 4 days after inoculation, labeled antigens are found on the surfaces of dendritic cells within B cell areas of the lymph node cortex (Nossal and Ada, 1971). Cytophilic antibody (Fc receptors) and complement (C3b receptors) facilitate binding of antigen by the dendritic cells within germinal centers of B cell follicles. Antigen

remains bound to follicular dendritic cells for a long period of time, and the same antigen will bind to these cells with greater avidity on secondary exposure, presumably due to cytophilic antibody.

GERMINAL FOLLICLES

Germinal follicles (Fig. 2-8) are discrete lymphoid compartments where B cells that are stimulated by T-dependent antigens undergo division, isotype switching, and memory development (Nieuwenhuis and Opstelten,

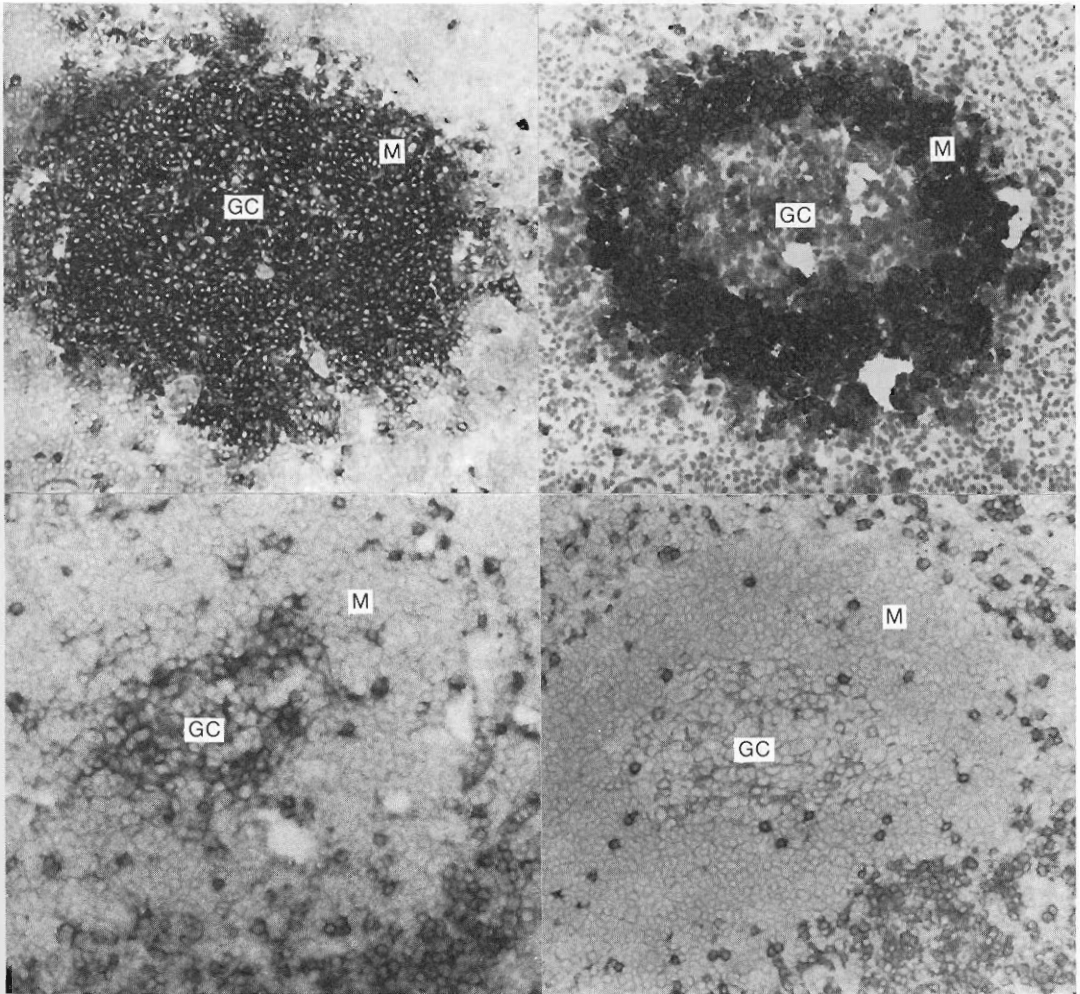


Figure 2-8. Immunohistochemical staining of lymph node frozen sections for IgM, IgD, CD4, and CD8 gives the following patterns for germinal follicles. Lymphocytes in the follicular mantle (M) and germinal center (GC) are predominantly B cells, with IgM⁺/IgD⁺ small cells in the mantle and IgM⁺/IgD⁻/2° isotype⁺ large cells in the germinal center. There are few T cells in follicles. CD4⁺ cells are present in the mantle and the germinal center. In contrast, CD8⁺ cells are limited to the mantle. Each panel is labeled with the phenotype selected by enzyme-linked monoclonal antibodies.

1984). B cell follicles are found in all peripheral lymphatic tissues including foci of chronic inflammation. Although some germinal follicles develop oligoclonally (Kroese et al., 1987), there are far fewer germinal follicles than there are antigen reactivities among B cells. In lymph nodes, B cell follicles partially interrupt the subcapsular sinus in the superficial cortex. A germinal center develops in a follicle as proliferating B cells displace the cortical reticular fibers into a basketlike enclosure that separates central lymphoblasts from the peripheral mantle of small B cells. Lymphoblasts, follicular dendritic cells, and "tingible-body" macrophages reside inside the enclosure. On the outside, the mantle zone is comprised almost entirely of small B lymphocytes with IgM^+/IgD^+ surface phenotype. A sprinkling of $CD4^+$ and $CD8^+$ T cells are also present in the mantle zone. In the germinal center, the large B lymphoblasts and intermediate cells have lost surface IgD ; 80–90% now express IgM , and 30% another isotype such as IgG ; IgM^+/IgD^+ lymphocytes in follicular mantle zones have not yet switched, whereas germinal center B cells have. Therefore, it is likely that rearrangement of the immunoglobulin heavy chain genes occurs in (or en route to) germinal centers with mantle-zone B cells serving as a preswitch pool. Switched B cells initiate low-level transcription of messenger RNA for the "secondary" isotype while still expressing membrane IgM (Schweitzer et al., unpublished). IgM is deleted and the "secondary" isotype is expressed usually after a second exposure to antigen. Inside germinal centers the activated B cell sees antigen on follicular dendritic cells and receives help from $CD4^+$ T cells and/or their factors. The frequency of $CD4^+$ T cells in germinal follicles is small relative to that of B cells, but they outnumber $CD8^+$ T cells 3:1. B cells divide and migrate rapidly out of the follicle as lymphoblasts, which leave the node to lodge in the spleen, inflammatory foci, and other lymphatic tissues. Some large IgM^+ B cells lodge in the marginal zone of the spleen and switch after they reencounter antigen. Labeled B lymphoblasts "home" to germinal centers without accumulating in the small cell mantle after intravenous infusion (Opstelten et al., 1981).

LYMPHOCYTE TRAFFIC-DEPENDENT CORTEX

The deep cortex of lymph nodes, which appears in histologic sections as a diffusely cellular area containing specialized vascular structures called high endothelial venules (HEV), has also been called the T-dependent cortex because of the adverse effect of neonatal thymectomy upon maturation of recirculating lymphocytes (Parrott et al., 1966). In "resting" lymph nodes, the reticular connective tissue surrounding HEV contains numerous small lymphocytes in cords of reticular cell matrix between the HEV and lymphatic vessels of the intermediary sinuses (Anderson and Anderson, 1975). The size of the deep cortex depends upon the rate of lymphocyte immigration via HEV, the transit time of cells traversing the reticulum, and the rate of egress of immigrants via efferent lymphatic channels. Indeed, the paracortex is capable of rapid three- to fivefold enlargement 6–24 hours after a strong stimulus induced by viral infection, injection of immunologic adjuvants (Anderson and Reynolds, 1979), or inoculation of alloantigens (Anderson et al., 1975). Accelerated lymphocyte traffic usually results in logjams of small lymphocytes in intermediary sinuses. Although most of the acute enlargement of the deep cortex during the first 24 hours after inoculation is due to increased lymphocyte traffic into the node, some of it is due to sequestration and activation of antigen-reactive cells, causing them to remain, to proliferate or to release lymphokines. The outcome of this selection by antigen and cytokines is the subsequent outpouring of antigen-reactive cells in the efferent lymph between 72 and 100 hours after inoculation.

The deep cortex shrinks rapidly after chronic thoracic duct drainage (Goldschneider and McGregor, 1967; Anderson et al., 1976) or neonatal thymectomy (Parrott et al., 1966), both of which deplete recirculating lymphocytes. Although the deep cortex is commonly known as the T-dependent cortex, it is not comprised solely of T cells. The lymphocytes populating the deep cortex are about 75% T cells and 25% B cells; 90% of them are small lymphocytes, and virtually all belong to the recirculating pool. There are slightly more suppressor T cells than helper T cells in resting lymph nodes, but the ratio can vary considerably. This also is true of the

could be artifact of reutilization of 3H -T.

ratio of T cells to B cells. After alloantigenic stimulation, lymph nodes fill up with T cells. In contrast, after inoculation of an antigen that selectively induces an antibody response, the deep cortex contains nearly 60% B cells (Anderson and Reynolds, 1979; Kraal and Twisk, 1984). Therefore, the deep cortex is a dynamic lymphatic compartment which responds to stimulation by cytokines, antigens, and other biologic response modifiers.

LYMPH NODE VASCULATURE

The vasculature of the lymph node cortical lobules is well suited to the demands of a tissue populated by transient cells (Anderson and Anderson, 1975). Arterial vessels enter lymph nodes at the hilum and branch once or twice as they cross cortical lobules to supply a network of capillaries and arteriovenous communications in the superficial cortex, beneath the floor of the supracapsular sinus. Arteriovenous communications and short post-capillary venules course beneath the floor of the subcapsular sinus to connect with high endothelial venules (HEV) lined by large polygonal endothelial cells (Fig. 2-9). Distal segments of HEV open into elastic capacitance vessels draining segments of cortical lobules. These segmental veins merge to form lobular veins near the hilum. Circumferential smooth muscle bundles form contractile structures at sites where segmental and lobular veins join before connecting with the systemic circulation. Contractile vascular structures may serve to prevent dramatic intranodal circulatory changes from influencing the systemic blood flow.

Studies of microvascular changes in stimulated lymph nodes demonstrated a biphasic shift in blood flow after antigen inoculation (Hay and Hobbs, 1977; Herman, 1980). An early transient increase in flow up to 30 times normal was attributed to hyperemia. The increased flow, caused by increased arteriovenous shunting, resulted in increased lymphocyte traffic rates (Ottaway and Parrott, 1979). This was followed by a second, more gradual wave of increased blood flow due to angiogenesis and enlargement of the network of HEV between 3 and 6 days after alloantigenic stimulation (Anderson et al., 1975) or after inoculation with adjuvants (Anderson, 1985). These antigen-induced alterations in the mi-

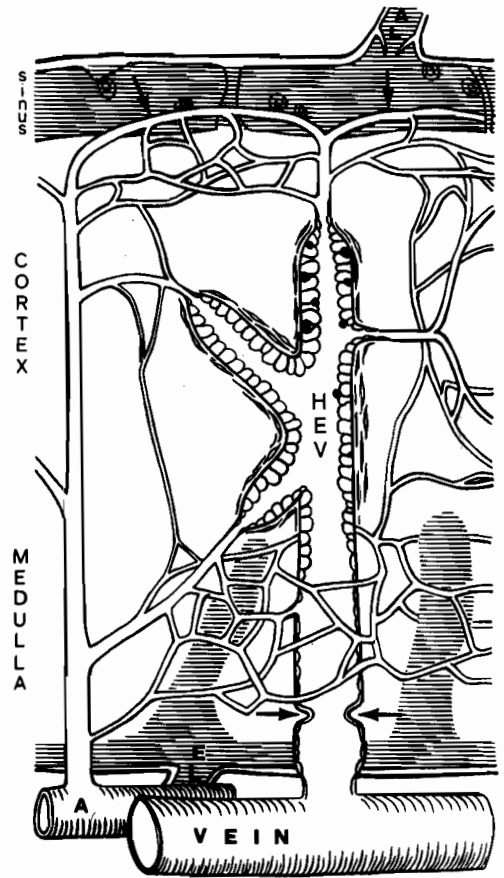


Figure 2-9. The location of high endothelial venules (HEV) between arteriovenous communications (solid arrows) and venous sphincters (open arrow) provides a unique system for regulating blood flow and pressure in lymph node venules. Alterations in blood flow that accompany inflammatory reactions to antigen inoculation result in tissue fluid and venous hydrostatic pressure differences that lead to lymphaticovenous gradients at the HEV. Cytokines, neuropeptides, and other mediators can regulate lymphocyte traffic into lymph nodes by inducing hemodynamic changes that lead to formation or reversal of lymphocyte chemotactic gradients in HEV walls.

crocirculation directly affected transvascular lymphocyte migration into lymph nodes, initially by increasing the rate of immigration, and subsequently by enlarging the surface area of HEV endothelium available for migration.

Myelinated and unmyelinated nerve fibers are found coursing through the stroma of

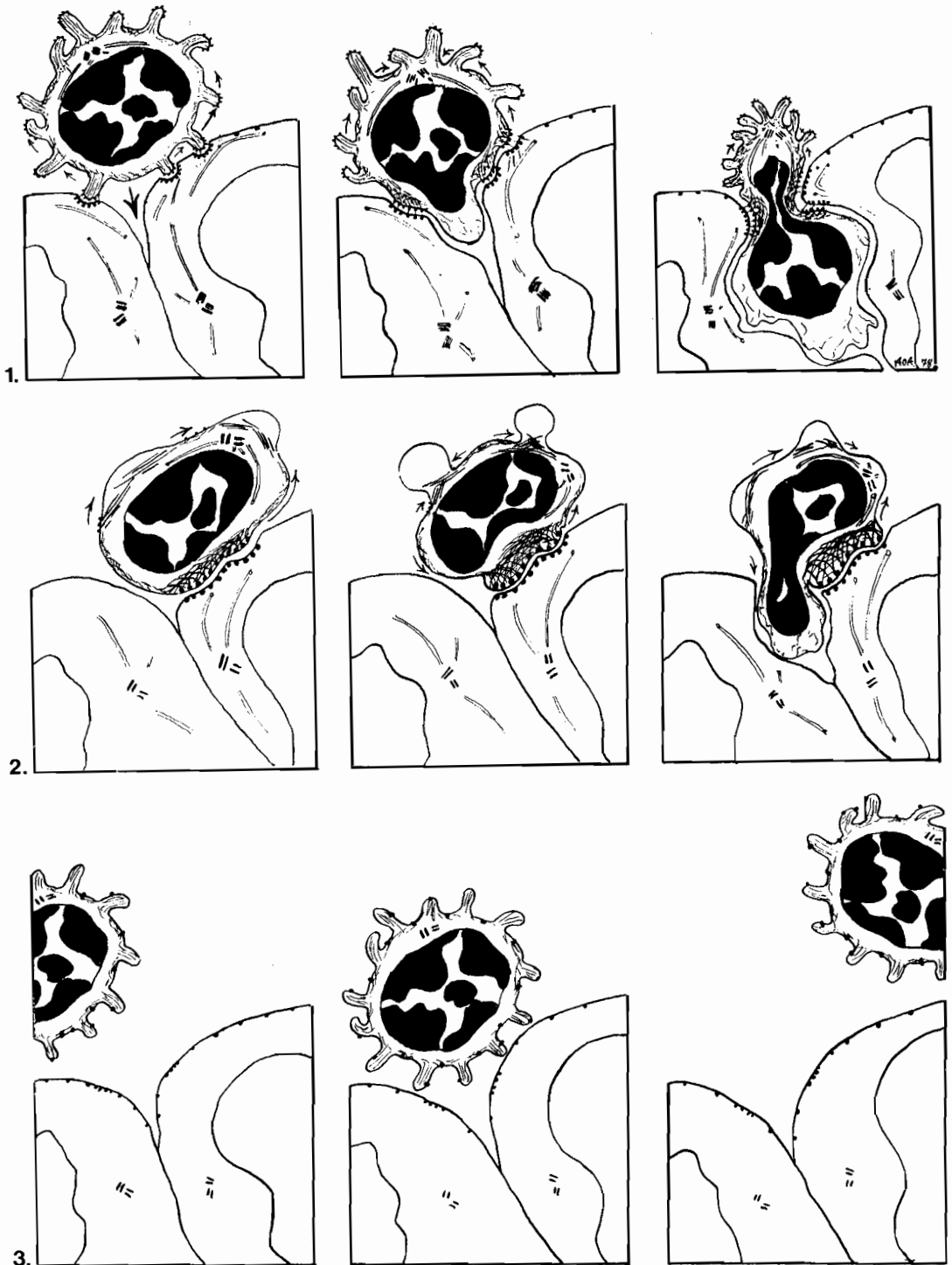


Figure 2-10. Circulating lymphocytes adhere to luminal surfaces of HEV before emigrating into lymph node parenchyma. Translocation across the vascular interface is an active process that requires cytoplasmic polarization and cytoskeletal contraction (Anderson et al., 1979). (Row 1) Normal, receptor-mediated binding is shown, followed by polar redistribution of sites of adhesion toward the centriolar pole of the lymphocyte. If the complementary sites on the endothelial cells remain stationary, the net result is forward propulsion into the interendothelial cleft. (Row 2) Poisoning of the actin-dependent cytoskeleton by treat-

lymph nodes, where they are associated with arteriovenous communications and venous sphincters (Anderson and Anderson, 1975). Recent studies by Felten et al. (1985) clearly indicate that noradrenergic and peptidergic nerve fibers supply diffuse areas populated with lymphoid cells in addition to the vasculature. Bundles of nerve fibers enter lymph nodes at the hilus and follow arteries and veins to the floor of the subcapsular sinus where the nerves form a plexus. Noradrenergic fibers extend into the paracortex near lymphoid aggregates adjacent to high endothelial venules. Furthermore, norepinephrine, which was depletable by 6-hydroxydopamine, was found in lymph nodes. Depletion of norepinephrine with that agent resulted in a diminished immune response in draining lymph nodes after subcutaneous injection of antigen (Felten et al., 1984), suggesting an immunoregulatory role for the products of neural secretion in lymph nodes.

LYMPHOCYTE MIGRATION INTO LYMPH NODES

Gowans and Knight (1964) demonstrated that large-scale emigration of recirculating lymphocytes from blood to lymph occurred across the walls of high endothelial venules. Accumulation of lymphocytes in HEV lumina was due to selective adhesion of circulating lymphocytes to endothelial surfaces via receptor-ligand-like interactions (Anderson and Anderson, 1976; de Bono, 1976; Stamper and Woodruff, 1976). Polymorphonuclear leukocytes never adhered to HEV in vivo unless the lymph node was involved in acute inflammation (Marchesi, 1964). Adhesion with HEV luminal surfaces occurred via lymphocyte microvilli which interdigitated with pits in the endothelial cell membrane (Fig. 2-10), a cellular interaction that resisted hydrodynamic shear forces and required an intact cytoskeleton (Loor, 1977; Anderson et al., 1979). The existence of a glycoprotein "receptor"

for recognition in lymphocyte homing was hypothesized a long time ago (Gesner and Ginsburg, 1964; Marchesi and Gowans, 1964). Inhibition studies with monoclonal antibodies recently identified two lymphocyte membrane glycoproteins which may be responsible for organ-selective adherence of lymphocytes to HEV in mucosal or peripheral lymphatic tissues (Rasmussen et al., 1985; Chin et al., 1986). Substantially less information is known about the endothelial moiety recognized by putative homing receptors. Specific oligosaccharides inhibited in vitro binding of lymphocytes to HEV, thereby demonstrating that the lymphocyte receptor recognizes a carbohydrate moiety on the HEV (Stoolman and Rosen, 1983; Stoolman et al., 1984; Rosen et al., 1985). Thus, the lymphocyte "receptor" behaves like a lectin that binds to a sulfated carbohydrate on the HEV endothelium.

Luminal lymphocytes polarize and squeeze between endothelial cells to enter the perivascular reticular cell sheath. The unique permeability of the HEV from lymphatic tissue toward the blood permits cytokines or other factors in adjacent lymphatic tissue to diffuse along spaces between HEV endothelial cells to yield chemotactic gradients (Anderson and Anderson, 1976). When antigen inoculation causes altered blood flow, cytokine release, and increased tissue fluid pressures in regional lymph nodes, the resultant interendothelial gradient in HEV accelerates lymphocyte migration into the lymphatic tissue (Parrott and Wilkinson, 1981; Anderson et al., 1982; Anderson, 1985). After entering the deep cortical interstitium, lymphocytes crawl along cell processes and through a lattice-work of reticular fibers until they reach reactive foci in T or B cell areas. They also cross lymphatic endothelium to enter intermediary sinuses that deliver them into the efferent lymph. Contact guidance along fibro-

ment with long-acting cytochalasin A results in accumulation of paralyzed lymphocytes on luminal surfaces of HEV. The cells can still recognize and bind to HEV, but they cannot translocate. (Row 3) Treating the recipient with colchicine, which disrupts microtubule function and phosphatidylinositol pathways in cell membranes, results in failure of untreated lymphocytes to recognize and bind to HEV, suggesting that release of complementary receptor elements in endothelial membranes from their stabilization by microtubule-dependent cytoskeleton or by alterations in membrane phospholipid composition is responsible for the failure of lymphocytes to home.

↙ blastic reticular cells was proposed by Chang et al. (1979) for intranodal redistribution of migrants because lymphocytes bind to these cells and migrate rapidly on their surfaces *in vitro*. Aggregation or repulsion of lymphocyte sets was also proposed to explain compartmentalization of lymphoid cells within lymphatic tissues (Curtis and De Sousa, 1975). The precise mechanisms that govern accumulation and distribution of lymphocyte subsets within specific lymphoid compartments are not known.

Spleen

STRUCTURE

The spleen filters blood and is the largest single lymphoid organ in the body. It has a dense fibrous capsule with muscular trabeculae extending inward to subdivide the spleen into lobules and provide anchors for the reticular meshwork supporting the cellular parenchyma (Fig. 2-11). The spleen is grossly divisible into white pulp, formed of cylindrical collections of lymphocytes around the arteries, and red pulp, which contains erythrocyte-rich blood in cords of reticulum. The splenic cords are a continuous spongelike reticular tissue which crisscrosses between fenestrated walls of splenic venous sinuses. The cords contain erythrocytes, lymphocytes, macrophages, granulocytes, and plasma cells. The splenic venous sinuses are lined by elongated endothelial cells that resemble barrel staves. The venous sinus wall is ringed by hoops of reticular fibers. All blood cells liberated into the splenic cords must squeeze through slits between these endothelial cells to enter the sinuses and return to the blood circulation. Splenic sinus lining cells express antigens associated with monocytes, macrophages (OKM5), endothelial cells (factor VIII, transferrin receptor), and T lymphocytes (OKT8 and OKT4) (Buckley et al., 1985). The spleen monitors for abnormal cells in blood by mechanical sieving in venous sinuses and by exploration of surface antigens by mononuclear phagocytes (Chen and Weiss, 1973).

BLOOD AND LYMPH VASCULATURE

The spleen is supplied by the splenic artery, which enters through the hilus and branches

to pass through trabeculae into medium-sized arteries surrounded by a sheath of lymphatic tissue (periarteriolar lymphatic sheath, PALS). These vessels terminate in small arterioles which pass through PALS and empty into the reticulum of the red pulp cords or pulp sinuses. Within the PALS, narrow perpendicular branches of the central arteriole supply a ring of sinuses within the marginal zone of the PALS. These sinuses fill readily with radio-opaque perfusates (Herman, 1980) and materials used to make vascular corrosion casts for scanning electron microscopy; otherwise they would not be noticed. Marginal sinuses are critically important to lymphocyte recirculation in the spleen. Their supply vessels arise perpendicularly, thus possibly contributing to concentration of lymphoid cells through skimming the cells from the peripheral stream of the central arteriole. Blood from red pulp cords and marginal sinuses empties into the venous sinusoidal system which connects by gradual transition to pulp veins, trabecular veins, and splenic veins. The spleen has no afferent lymphatic vessels, but efferent lymphatics arise within the white pulp near central arterioles. The efferent lymphatics cross perpendicularly between PALS areas to form an interconnecting network in cleared preparations of spleens whose lymphatics are labeled with carbon (Anderson et al., 1982). The structure of the lymphatic network provides for cellular communication among the anatomically separated white pulp areas. Lymphatic trunks pass through trabeculae, merge with one another, and exit at the hilus. Major efferent lymphatics of the spleen wind down the hilar blood vessels to join the thoracic duct near the pancreas. Parts of the intrasplenic lymphatic network have anatomic relationships resembling "bridging zones" of Mitchell (1973) because they appear to link white pulp with red pulp in histologic sections.

Noradrenergic fibers enter the hilus of the spleen with the vasculature, follow the trabeculae and branching vasculature, and ramify mainly within the white pulp along the central arterioles and periarteriolar lymphatic sheath. Fibers branch from a dense periarterial plexus to permeate and terminate among diffuse small lymphoid cells and accessory

INTEGRINS AND CAMS? ECM

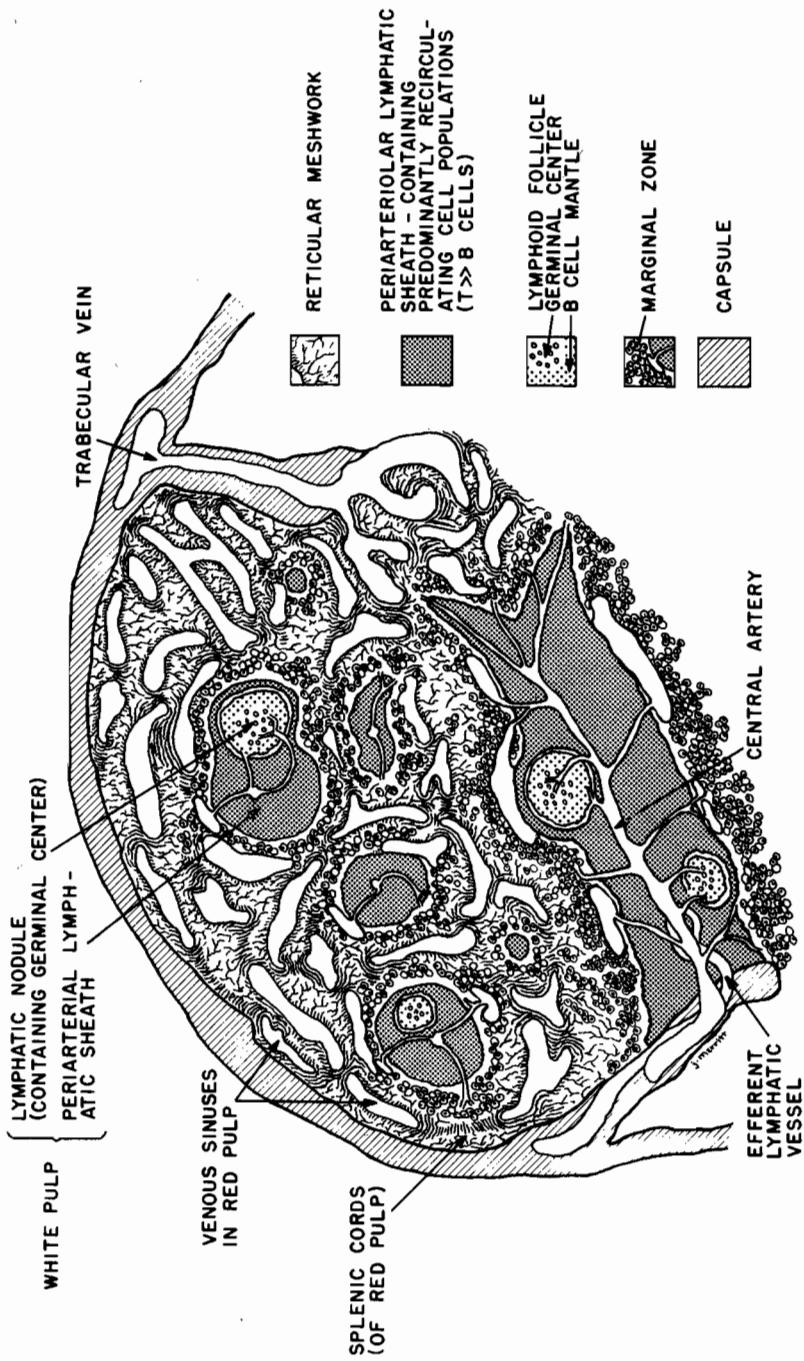


Figure 2-11. Diagram of the red and white pulp of the spleen, using graphic patterns to depict red pulp cords, marginal sinuses, marginal zones, periarteriolar lymphatic sheaths, vascular supply, and efferent lymphatics. (Modified after Weiss, 1972.)

cells in the PALS (Felton et al., 1985). Immunocytochemical data indicate that nerve fibers with neuropeptide Y, Met-enkephalin, cholecystokinin-8, and neurotensin immunoreactivity are present along the central artery, with sparse fibers entering lymphoid tissue. Cholecystokinin-8-bearing fibers are more prevalent in the PALS, however.

FUNCTIONS

The spleen receives a high proportion of the cardiac output which contributes to its effectiveness as a filter of blood and a site for lymphocyte recirculation and lodging. Filtration of effete cells, debris, and microorganisms occurs primarily in the reticulum of the red pulp chords and in the sievelike endothelium of venous sinuses (Chen and Weiss, 1973). Reticular cells provide anchoring sites for mononuclear phagocytes that perform reticuloendothelial functions in the red pulp and marginal zone. The white pulp of the spleen contains a peripheral lymphatic tissue microenvironment for antigen trapping, cellular collaboration, lymphocyte proliferation, and antibody production in addition to providing young bone marrow emigrant B cells a place to complete maturation. The spleen is the primary site for initiation of immune responses to antigens and pathogens that have invaded the blood stream in addition to being a partner in every other immune response in the body. Antigen-laden mononuclear cells and lymphoblasts, released into efferent lymph from other lymphatic tissues 48–100 hours after antigen priming, lodge in the spleen and set up satellite zones of T and B cell proliferation (Anderson and Warren, 1982). During active immune responses, lymphoblastic B cells committed to plasma cell differentiation lodge in the red pulp cords and sinuses, where they mature and commence secretion of antibody.

LYMPHOCYTE MIGRATION IN SPLEEN

Lymphocyte traffic in the spleen moves across a blood-tissue interface into sites of antigen presentation in the marginal zone and periarteriolar lymphatic sheath. Antibodies, enzymes, and drugs that are known to block lymphocyte homing to lymph nodes do not interfere with entry of the PALS by recircu-

lating lymphocytes. Many of the lymphocytes entering the spleen in the arterial blood bypass the white pulp and flow into the reticulum of the red pulp cords and sinuses to exit via the splenic vein. These cells are returned to the blood within 2–3 hours after entering the spleen. However, some small arterioles terminate in sinuses adjacent to the white pulp; others empty directly into the marginal zone. Lymphocytes in the marginal sinuses enter the marginal zone, leaving other leukocytes in the lumen to be carried away in the blood. Lymphocytes arriving in the marginal zone move across a vascular interface that is populated by antigen-binding macrophages and B cells to enter the PALS. Once in the PALS, lymphocytes may remain within the spleen for up to 12 hours. The PALS is analogous to the lymph node paracortex because it contains recirculating T and B cells in addition to interdigitating dendritic cells. Segregation of T and B cells into discrete zones within the white pulp is not rigorous. Follicles located in the border between the PALS and the marginal zone contain predominantly B cells, and more recirculating T than B cells are found in the PALS. Follicles in the spleen are identical to those described in the section on the lymph node. After variable periods of residence in the PALS, some recirculating cells exit through the efferent lymphatics, whereas others move into the red pulp sinuses. The magnitude of lymphocyte recirculation via efferent lymph versus venous return has not been determined, but traffic studies with viable lymphocytes whose motility has been arrested indicate that over 80% of the lymphocytes that leave the spleen in the venous blood never migrate into white pulp (Anderson et al., 1979). If immunocompetent cells encounter an appropriate antigenic stimulus, they proliferate in the same manner as seen in peripheral nodes; B cells form large germinal centers, and antibody-secreting cells can be found within the splenic cords after migration through the blood or direct movement out from the white pulp.

The marginal zone of the spleen contains a heterogeneous assortment of mononuclear cells with specialized functions. A principal function of the marginal zone is antigen trapping. Intravenously injected tracer antigens

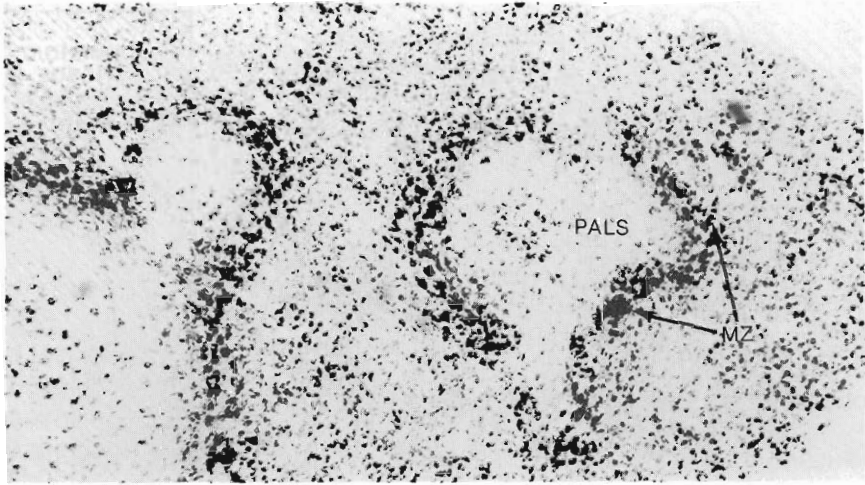


Figure 2-12. Gelatinized carbon is used here to illustrate that the function of the marginal zone (arrows) of the spleen is to capture and display hematogenous antigens to circulating lymphocytes that migrate into white pulp areas at the marginal zone.

reproducibly accumulate in marginal zones regardless of the nature of the antigen (Fig. 2-12). A subset of macrophages in the marginal zone appears to be important in presenting type 2 thymus-independent antigens to B cells. These cells are capable of selective uptake and retention of intravenously injected fluorescein isothiocyanate (FITC)-ficoll. They are negative for surface Ia antigen and expression of the macrophage-related F4/80 antigen (Dijkstra et al., 1985). The antibody-forming cells generated by interaction of B cells with these cells do not necessarily localize in the immediate proximity, however. The marginal zone contains specialized metallophilic macrophages that specifically label with a new monoclonal antibody (MOMA-1). These cells form a ring around the periarteriolar sheath and follicular areas on the inner side of the marginal zone. The same cells show high nonspecific esterase activity and can be distinguished from the marginal zone macrophages by MOMA-1 staining and the lack of selective FITC-ficoll uptake (Kraal and Janse, 1986).

The marginal zone contains a large population of B cells that are membrane IgM^+ and IgD^- , express $Il-2$ receptors, have alkaline phosphatase on their surfaces, and appear to be relatively sessile components of the mar-

ginal zone (MacLennan et al., 1985); that is, they do not show kinetic movement into PALS unless stimulated by antigen to enter B cell follicles. LPS infusion rapidly depletes them from the marginal zone followed by expansion of the IgM^+/IgD^- cells in germinal centers (Groeneveld et al., 1985). Depletion and regeneration of these cells following anti- IgM or $-IgD$ treatment permitted MacLennan to determine that the marginal zone IgM^+/IgD^- B cells were postfollicular IgM^+/IgD^+ cells because the follicle cells regenerated before any marginal zone B cells appeared during recovery from B cell depletion. This population of B cells appears to be enriched in those that selectively respond to type-2 thymus-independent antigens, whereas the follicular B cells respond to thymus-dependent antigens.

Mucosal Lymphatic Tissues

DEFINITIONS

The mucosal lymphatic tissues are nonencapsulated submucosal lymphoid nodules and diffuse lymphocytic infiltrates in the submucosa of intestinal and respiratory tracts. These organs work collectively with regional lymph nodes and spleen to produce B and T effector cells which lodge in lamina propria and in in-

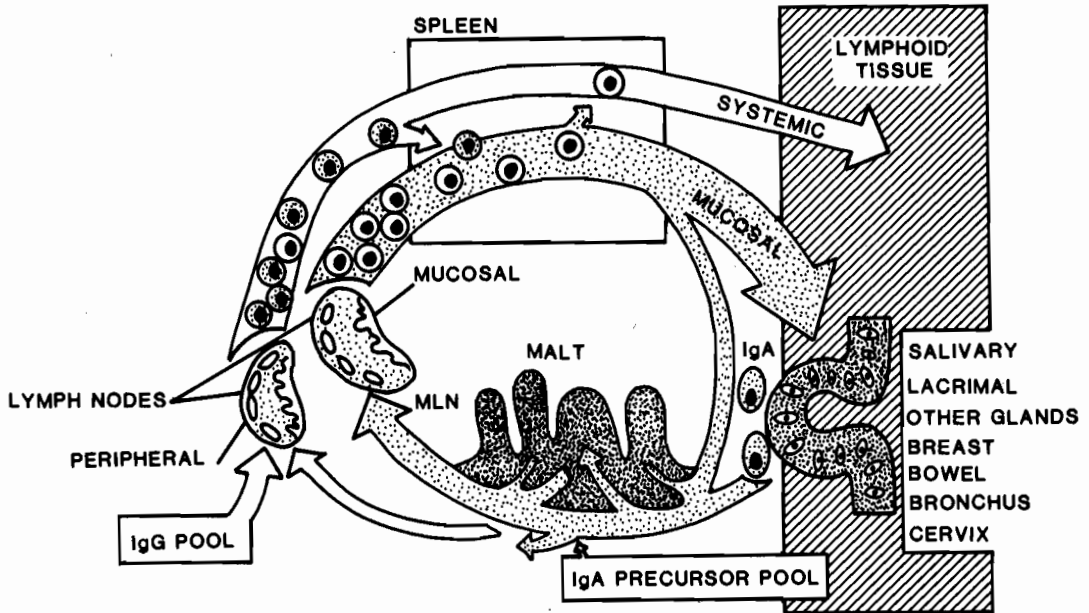


Figure 2-13. Diagram depicting the interconnections among the organs and tissues of the "common mucosal immune system." Lymphocyte recirculation and lodging patterns are especially complex in the mucosal immune system because IgA-committed B cells travel in the blood twice before lodging in the lamina propria of some mucosal site, and mucosally committed T cells exhibit segregated traffic patterns which cross over slightly in the spleen. (Modified after McDermott and Bienenstock, 1979.)

traepithelial locations wherever there is mucosa (Fig. 2-13). Tonsils, adenoids, bronchus-associated lymphatic tissues, Peyer's patches, appendix, and isolated follicles in intestinal mucosae vary with regard to type of surface epithelium (stratified squamous, ciliated columnar, or absorptive columnar) and relative proportions of T and B cells (tonsils have 60% T cells as compared to 25-40% T cells in respiratory or intestinal patches), but the similarity of these tissues to Peyer's patches is greater than the differences, especially because all have "M" cells in their follicle-associated epithelium.

The prototypical mucosal lymphatic tissue is the Peyer's patch (Fig. 2-14), which has a unique dome epithelium that is specialized to sample environmental antigens. Peyer's patches contain lymphoid compartments that are analogous to the deep cortex and follicles of lymph nodes, but there are no afferent lymphatics and no medullary cords for local accumulation of plasma cells (Parrott, 1976).

Each Peyer's patch contains multiple individual B cell follicles separated by diffuse lymphoid tissues in interfollicular areas.

FUNCTIONS

The mucosal immune system has two paradoxically opposite purposes. Mucosal lymphatic tissues amplify development of committed B cells for secretory IgA responses to environmental antigens (Craig and Cebra, 1971; Cebra et al., 1980) and program certain environmental antigens for systemic tolerance induction. The tolerance that results from mucosal immunization does not affect production of B cells committed to IgA secretion. "Mucosal" tolerance is manifested by antigen-specific suppression of delayed cutaneous hypersensitivity and reduced IgG expression (Chase, 1946; Waksman and Ozer, 1976; Mattingly and Waksman, 1978; Ngan and Kind, 1978; MacDonald, 1982; Anderson et al., 1985). The mucosal immune system therefore exerts a yin-yang effect on local

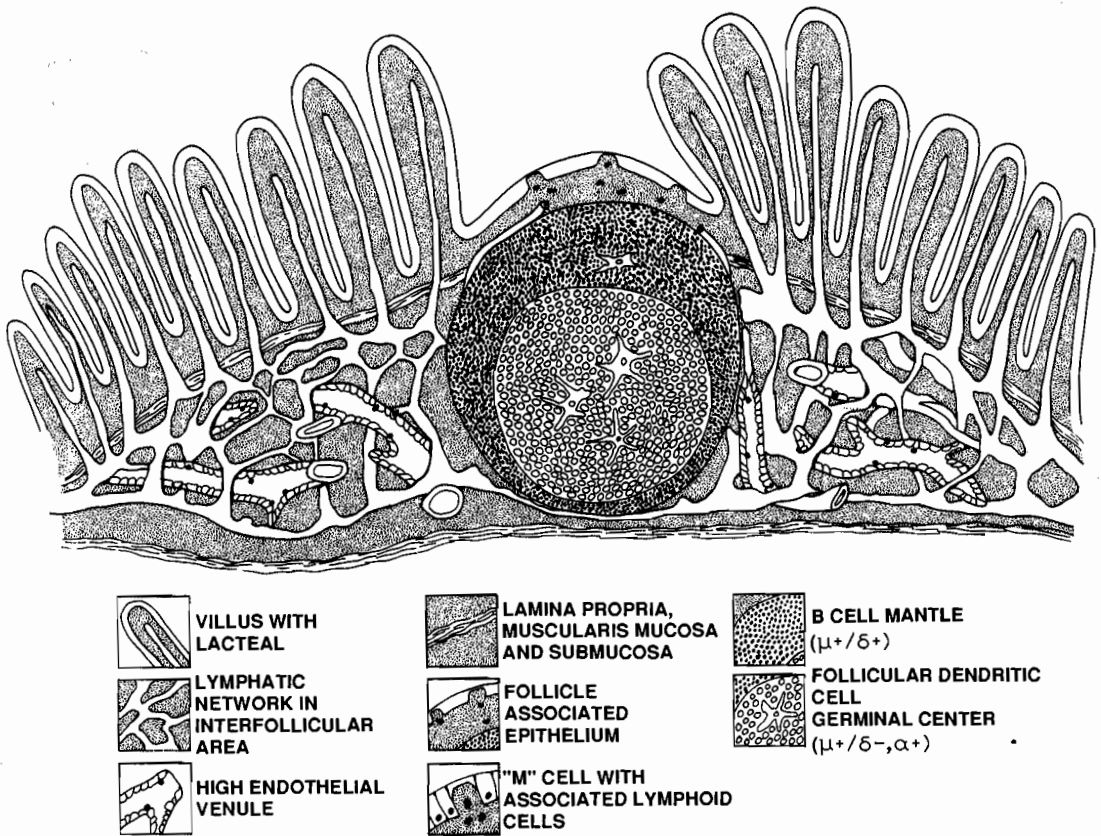


Figure 2-14. Diagram of the murine Peyer's patch, showing the anatomic relationships of the network of lymphatics in the interfollicular areas with the high endothelial venules. Follicle-associated structures, such as "M" cells, dendritic cells, the small cell mantle, and germinal center, are graphically symbolized.

versus systemic immunity and vice versa which is initiated in the unique microenvironments of mucosal versus peripheral lymphatic tissues (Hamilton et al., 1979; Anderson et al., 1988).

M CELLS

The dome epithelium covering each follicle is composed of cuboidal absorptive epithelial cells interrupted by delicate membranous cells which have luminal microfolds instead of microvillus borders (Fig. 2-15). The "M" cells endocytose and transport various materials without lysosomal degradation (Bockman and Cooper, 1973; Owen and Jones, 1974; Owen, 1977; Wolf et al., 1981). Antigen is deposited onto small lymphocytes, small mononuclear phagocytes, and dendritic cells

immediately beneath M cells above the B cell mantle of Peyer's patch germinal follicles (Ermak and Owen, 1986). Minute quantities of intact antigen and products of digestion are transported to the lamina propria and lacteals by ordinary absorptive epithelial cells anywhere in the small bowel. It is important to point out that these products enter interfollicular areas and do not have access to the dome area (Jeurissen et al., 1987). Between the dome epithelium and the follicles there is a thin region of reticulum, containing a delicate plexus of blood vessels and plasma cells.

MUCOSAL FOLLICLES

The follicles, located beneath the dome epithelium, have a mantle of small B cells surrounding germinal centers that is thicker fac-

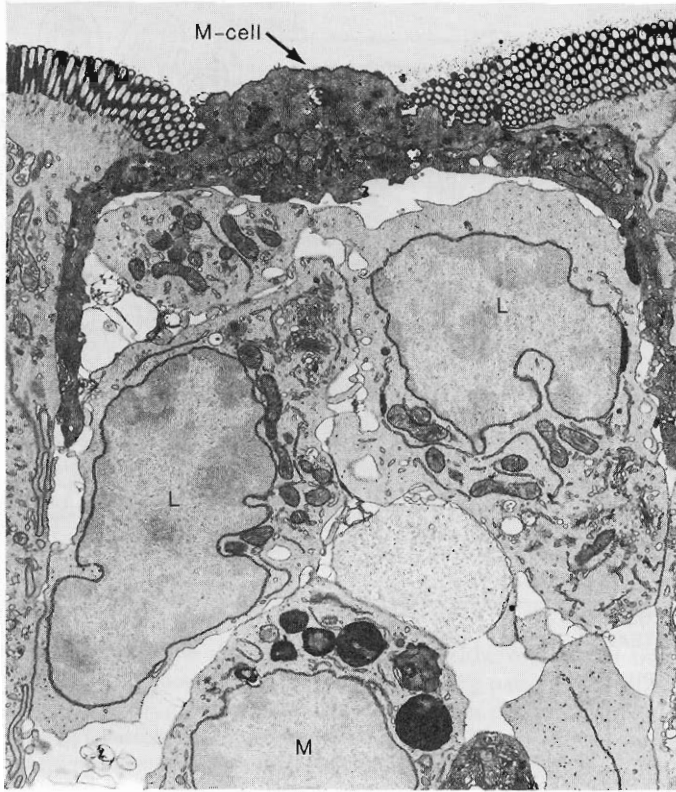


Figure 2-15. Electron micrograph of an M cell sandwiched between two absorptive cells in the follicle-associated epithelium of the Peyer's patch. Note the two lymphocytes (L) and small mononuclear phagocyte (M) enclosed beneath the M cell. (Photograph by courtesy of R. L. Owen.)

either or not both

ing the dome. The germinal centers contain large and intermediate-sized B lymphoblasts, follicular dendritic cells, macrophages, and rare T cells. Regardless of how many follicles a patch contains, each is only one follicle thick, providing an intimate association with the overlying epithelium. Precursor B cells with surface IgM and IgD enter Peyer's patches via HEV and migrate into the mantle zone above the germinal center, where they may come in contact with dendritic cells and T helper cells. IgD is lost when the B cells enter the germinal center to begin proliferating. A small number of cells will express surface IgM with IgA or IgA alone, but most will remain IgM only. Recent data indicate that switching and commitment to IgA expression occurs in nearly 90% of IgM-only germinal center cells, although surface phe-

notypes and antibody produced in short-term culture do not reflect this commitment. In situ hybridization with complementary DNA probes for the rearranged RNA message of IgA does not label any cells in the small cell mantle, lightly labels all of the cells expressing surface IgM only, and heavily labels a minority population that synthesize and secrete dimeric IgA (Cebra et al., unpublished). Immature "mucosal" B cells exit Peyer's patches down mesenteric lymphatics to regional lymph nodes, where they contact additional recirculating helper or immunoregulatory cells. Most B cells pass out of the mesenteric node via efferent lymph to the blood. These cells temporarily lodge in the spleen, where they proliferate and mature over a 5- to 7-day period before returning to the lamina propria of the intestine and other mucosal sites. Once

*McLENNAN - Centrostoma
Centrostoma*

the B cells reach the mucosa, they divide once more and mature into IgA-secreting plasma cells (Husband and Gowans, 1978).

LYMPHOCYTE TRAFFIC AREAS

The interfollicular areas are diffusely infiltrated with small and medium-sized lymphocytes, usually of T cell origin, but numerous IgM⁺/IgD⁺ B cells are also present. Recirculating lymphocytes emigrate from the blood into Peyer's patches via a network of high endothelial venules (HEV) surrounding follicles (Anderson et al., 1976; Yamaguchi and Schoefl, 1983a,b). Lymphocyte recirculation across HEV in patches is very rapid (Anderson et al., 1982; Bjerknes et al., 1986). Accumulation of labeled lymphocytes peaks 90 minutes after infusion, which is the shortest transit time of any organized lymphatic tissue. Lymphocyte traffic to the patch is augmented by recirculation across venules which are not recognizable as HEV. Some lymphoid cells enter the lamina propria via thin-walled venules adjacent to intestinal crypts far away from the Peyer's patch (Jurissen et al., 1987) and flow along submucosal lymphatics until they reach lymphatic plexuses in interfollicular areas (Steer, 1980; Anderson et al., 1982).

T lymphocyte traffic patterns related to mucosal and peripheral lymphatic tissues are segregated, with some crossing over occurring in mesenteric lymph nodes and the spleen. Griscelli et al. (1969) demonstrated the existence of two populations of T lymphoblasts that selectively migrated to the gut lamina propria when the cells came from mesenteric lymph node, or to the spleen when the cells came from a peripheral lymph node. These distinctive traffic circuits were also found for murine T and B lymphoblasts (Rose et al., 1976) but not for small T cells, which migrated randomly to mucosal or peripheral lymphatic tissues regardless of their origin (de Freitas et al., 1977). In sheep, small T cells exhibited a pronounced asymmetry of recirculation (Cahill et al., 1977; Reynolds et al., 1982). Labeled small T cells from intestinal lymph were twice as likely to be recovered in intestinal lymph than in nodal lymph, and vice versa. Furthermore, intestinal recirculat-

ing T cells migrated through the small intestine, whereas nodal T cells did not. Similar lung-associated, gut-associated, and peripheral lymph node-associated traffic patterns were demonstrated in sheep by Spencer and Hall (1984).

Small B cells and B lymphoblasts show a preference for migration into Peyer's patches and gut lamina propria, respectively. There is some preference for "secondary" B cells to return to the mucosa where it first encountered antigen (Pierce and Gowans, 1975; Husband and Gowans, 1978). However, B cells committed to IgA expression lodge in laminae propriae of other mucosae anywhere in the body (Rudzick and Bienenstock, 1974; McDermott and Bienenstock, 1979; Husband and Dunkley, 1985). This ability of Peyer's patch B lymphoblasts to populate mucosal sites in the conjunctiva, upper respiratory tract, bronchi, mammary glands, and gastrointestinal tract is regarded as the "common mucosal immune system," where gut-associated lymphatic tissues supply the bulk of the B cells. The immunoregulatory T cell component of this system is segregated into traffic patterns that permit cross-regulation between peripheral and mucosal immune systems within the spleen and the mesenteric lymph nodes.

The innervation of Peyer's patches is extensive, and the diversity of adrenergic and peptidergic nerve endings in patches is greater than for any other peripheral lymphatic tissue (Ottaway and Greenberg, 1984; Felten et al., 1985; Ottaway, 1985). Noradrenergic fibers enter at the serosal surface, course longitudinally with the muscularis, form interfollicular plexuses that ramify through the diffuse T-dependent areas near HEV. Fibers extend into the mushroom villi between the domes to supply enterochromaffin cells and plasma cells. Blood flow appears to be the limiting factor that determines lymphocyte extravasation in mucosal sites (Ottaway et al., 1983), and vasoactive intestinal peptide, present in peptidergic fibers in Peyer's patches, can modulate affinity of lymphocyte homing receptors for HEV (Ottaway and Greenberg, 1984); therefore, there probably is a role for the extensive innervation of Peyer's patches

in regulating traffic and reactivity of mucosal T and B cells.

IMMUNOGLOBULIN ISOTYPE COMMITMENT

Cebra et al. (1977) proposed that the special feature of mucosal lymphatic tissues that promotes the generation of IgA-only clonal precursors is an environment that allows antigen-driven division without maturation to plasma cells. The feature of the Peyer's patch environment that might promote this situation could include inaccessibility of T helper cells, excessive presence of T suppressor cells, or humoral factors (Faulk et al., 1970; Waksman and Ozer, 1976; Kamin et al., 1974). The tempo of T cell recirculation through mucosal lymphatic tissues is consistent with the segregation of antigen-triggering and subsequent maturation of IgA precursors, compelled to migrate to various lymphatic tissues to complete differentiation. With a mean transit time through the Peyer's patches of under 2 hours, T cells do not remain long enough to participate in local maturation of IgM-expressing IgA-precursor cells. T cells, carrier-primed by the same antigens as the B cells in the Peyer's patches, catch up with IgA precursors in lymph sinuses of mesenteric nodes or in the spleen.

INTRAEPIHELIAL LYMPHOCYTES

Intraepithelial lymphocytes (IEL) are a large heterogeneous population of immune cells in the intestinal epithelium. Two basic characteristics distinguish IEL from peripheral lymphoid cells. First, 80–90% of these cells are CD8⁺/CD4⁻, of which only half are Thy1⁺. In contrast, the predominant phenotype of lamina propria T cells is CD4⁺/CD8⁻. Second, isolated IEL exhibit *in vitro* effector functions that identify them as natural-killer (NK)-like (Parrott et al., 1983) or as cytotoxic cells that are spontaneous or "natural" but without identifying characteristics of NK cells. Further studies by Klein (1986) indicated that 40–70% of IEL bound a monoclonal that recognizes immature thymocytes and B cells, but these cells also expressed the cytotoxic activation antigen despite being Thy1⁻. At least some of the cytotoxic cells in the intraepithelial compartment may therefore be thy-

mus independent. In mucosae, cytotoxicity directed toward virally infected or otherwise parasitized epithelial cells would be prophylactic or protective because the lysed cell would be rapidly eliminated from the body in the mucosal stream. If IgA is also present, it would prevent reinfection of mucosal epithelium.

The mucosal immune system is the most dispersed, the most diverse, and the most complicated lymphocytic system in the body. Like the thymus, it plays a role in generating antigen-reactive lymphoid cells which will become specific effectors upon further maturation; and, on the other hand, the mucosal lymphatic tissues are responsible for inducing tolerance to antigens that are commonly experienced in the enteric canal. There is a great deal remaining to be learned about this important and enigmatic lymphoid system.

Conclusions and Prospects

Successful initiation of specific immune responses requires exquisite coordination among diverse subsets of lymphoid cells and accessory cells. The dispersed and centralized components of the lymphatic system provide microenvironments where new precursor lymphocytes may acquire immunocompetence, and where interactions among T cells, B cells, and antigen-presenting cells, facilitated by neurohormones and cytokines, result in effective induction and amplification of immunity. The types of challenges the immune system must meet are heterogeneous; thus there must be various mechanisms for immune surveillance, antigen acquisition and presentation, and selection of effector populations. The peripheral lymphatic tissues provide environments for optimal sensitization. The skin, regional lymph nodes, spleen, and mucosal lymphatic tissues efficiently handle initiation of immune responses to contact agents, invasive pathogens, hematogenous antigens, and environmental antigens in materials we inhale, eat, or generate within the enteric canal, respectively. Systemic coordination among these peripheral lymphatic tissues, which results in the immune system's

performance as an integrated whole, requires a functioning nervous system, lymph and blood circulatory systems, leukocyte poiesis, and lymphocyte recirculation and lodging. Receptor expression, receptor-ligand inter-

actions, cell signaling, locomotion, adhesion, activation, division, and numerous biologic functions familiar to developmental biology are the subcellular systems that enable the immune system to work in complex organisms.

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