CHAPTER 1

Tissues and organs of the immune system

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CHAPTER OVERVIEW

- Lymphoid organs or tissues are specialized anatomic compartments where lymphocytes develop, reside, and function.
- Primary lymphoid tissues are the sites where lymphocytes undergo development, education, and maturation.
- Secondary lymphoid tissues are the main sites where naïve lymphocytes engage foreign antigens to mount a primary immune response.
- Tertiary lymphoid tissues are secondary lymphoid tissue-like structures that are induced at sites of chronic inflammation, and the function of such structures is not fully defined.
- Memory immune responses can occur outside secondary lymphoid tissues. Memory T cells can also be maintained without secondary lymphoid tissues.
- Primary and secondary lymphoid tissues are also necessary for tolerance induction and maintenance.

Introduction

It is the nature of scientists to be perpetually occupied with questions like "where," "how," and "why" things happen the way they do. Immunologists, in particular, are keen on answering the "where" question as it is central to understanding how immune cells are generated, what is required for their maturation, and whether they might mount productive responses against foreign antigens or not. The immune system is a *bona fide* organ system comprising primary and secondary lymphoid tissues (Figure 1.1). Primary lymphoid tissues (the bone marrow and thymus) specialize in generating immune cells from hematopoietic progenitors and transforming immature cells into mature lymphocytes

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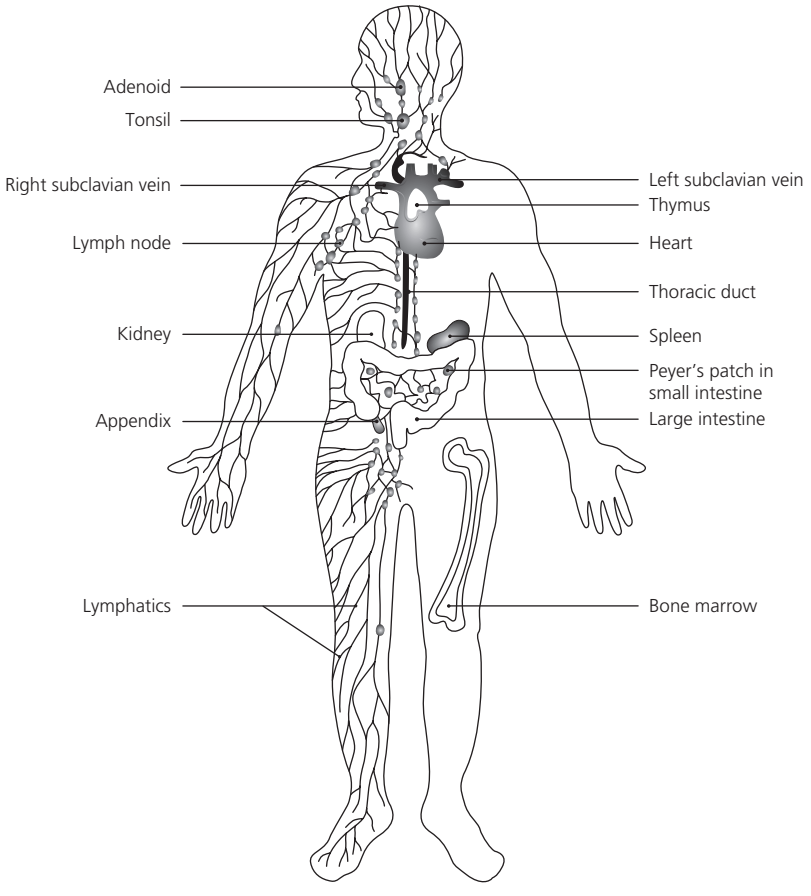


Figure 1.1 Lymphoid tissues of the human body. The primary lymphoid tissues are the bone marrow and thymus. The secondary lymphoid tissues consist of lymph nodes, the spleen, and MALTs. Lymph nodes are arranged in strings along lymphatic vessels where they trap antigens and cells traveling in the lymph. The spleen intercepts antigens and cells circulating in the bloodstream. MALT includes the Peyer's patches, adenoids, tonsils, and appendix. Cells traveling in the lymphatic system re-enter the blood circulation via the thoracic duct. Source: Redrawn from Murphy (2011). Reproduced by permission of Garland Science/Taylor & Francis LLC.

with high specificity to foreign antigens (non-self) but not "self antigens." Secondary lymphoid tissues, namely the spleen, lymph nodes, and mucosa-associated lymphoid tissues (MALTs) on the other hand are organized structures that are strategically located throughout the body to trap foreign antigens and ensure that they are best presented to T and B lymphocytes. The ability of an animal to mount a productive immune response is therefore critically dependent on the presence of the primary and secondary lymphoid tissues as well as the coordinated migration of immune cells into and out of these tissues.

This chapter will provide a comprehensive overview of the anatomy and function of primary and secondary lymphoid tissues and consider their roles in both transplant rejection and tolerance. Tertiary lymphoid tissues, which are secondary lymphoid tissue-like structures that are induced at sites of chronic inflammation, will also be discussed as they are thought to influence allograft outcomes. Controversies and unresolved questions will be highlighted where appropriate to encourage future investigations.

Primary lymphoid tissues

Primary lymphoid tissues are sites where T cells and B cells develop and mature, and mainly include the bone marrow and the thymus in mammals.

Bone marrow

The bone marrow is the site where both red and white blood cells are generated, by a process known as hematopoiesis. The adult human has two types of bone marrow: the red marrow, in which hematopoiesis is actively taking place, and the yellow marrow, consisting mainly of fat cells and lacking hematopoietic activity. At birth, all marrow is red but it is slowly replaced by yellow marrow over time. By adulthood, red marrow is restricted to flat bones (cranium, sternum, vertebrae, pelvis, and scapulae) and the epiphyseal ends of long bones (e.g., the femur and humerus), while the remaining marrow cavities are being occupied by fat cells. The bone marrow also provides a place where subsets of lymphocytes (both T cells and B cells), especially those with memory phenotypes reside.

Structure

Histologically, the red marrow consists of hematopoietic islands; such islands are mixed with fat cells, surrounded by vascular sinusoids, and interspersed throughout a meshwork of trabecular bone (Figure 1.2). The hematopoietic islands are organized into three-dimensional structures that provide optimal microenvironment for hematopoiesis. They contain blood cell precursors at different stages of maturation, stromal reticular cells, endothelial cells, macrophages, osteoblasts, osteoclasts, and the extracellular matrix. Both hematopoietic and nonhematopoietic cells in the islands orchestrate blood cell maturation through cell–cell contacts as well as production of growth factors, cytokines, and chemokines. Mature blood cells enter the circulation by migrating through the discontinuous basement membrane and between the endothelial cells of the vascular sinusoids.

Function

Hematopoietic stem cells (HSCs) are a pluripotent self-renewing cell type in the bone marrow that give rise to progenitor cells. These progenitor cells in turn generate all cells of the megakaryocytic (platelet), erythroid (RBC), myeloid,

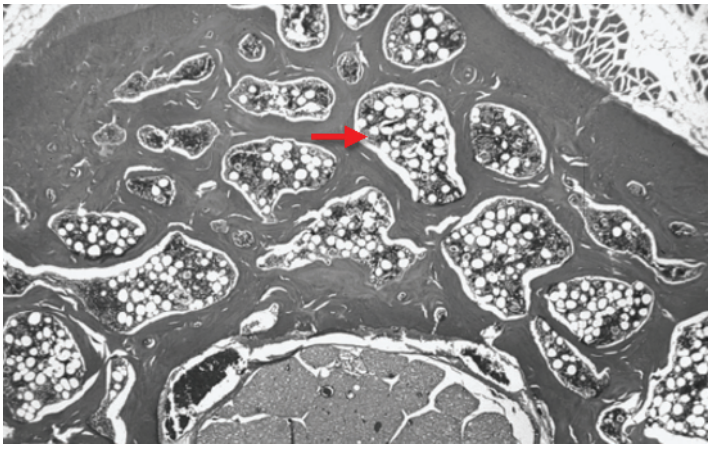


Figure 1.2 Structure of the bone marrow. Example of red bone marrow (vertebra). Arrow points to a hematopoietic tissue island. Note fat cells (white globules) admixed with hematopoietic cells. Trabecular bone fills the space between islands. Source: Reprinted from Travlos (2006). Reproduced by permission of SAGE publications.

and lymphoid lineages (Figure 1.3). Myeloid cells (monocytes, dendritic cells or DCs, neutrophils, basophils, and eosinophils), natural killer (NK) cells, and B lymphocytes develop in the bone marrow, whereas T cell progenitors (prethymocytes) migrate to the thymus where they undergo further maturation (see section "Thymus"). The bone marrow also contains mesenchymal stem cells that give rise to nonhematopoietic tissues such as adipocytes, chondrocytes, osteocytes, and myoblasts. Mesenchymal stem cells have attracted considerable interest among transplant immunologists because of their immunosuppressive properties and prolonged survival features when adoptively transferred in select models.

The bone marrow is the site where most stages of B cell maturation occur in mammals. B cell development in the bone marrow proceeds in a stepwise fashion from pro-B cells to pre-B cells, and lastly to immature B cells. During maturation in the bone marrow, B cells rearrange their immunoglobulin genes and express cell-surface IgM (the B cell receptor for antigen). These steps require close interactions with bone marrow stromal cells, which provide critical adhesion molecules, growth factors, chemokines, and cytokines (e.g., Flt3 ligand, thrombopoietin, CXCL12, and IL-7). Finally, autoreactive immature B cells are "weeded out" in the bone marrow through either clonal deletion or receptor editing before they are allowed into the circulation and complete their maturation in secondary lymphoid tissues.

In addition to serving as a primary lymphoid organ, the bone marrow is also a reservoir for mature myeloid and lymphoid cells. The bone marrow contains large numbers of neutrophils and monocytes that are mobilized into the circulation when needed (e.g., after infection). It is also the homing site

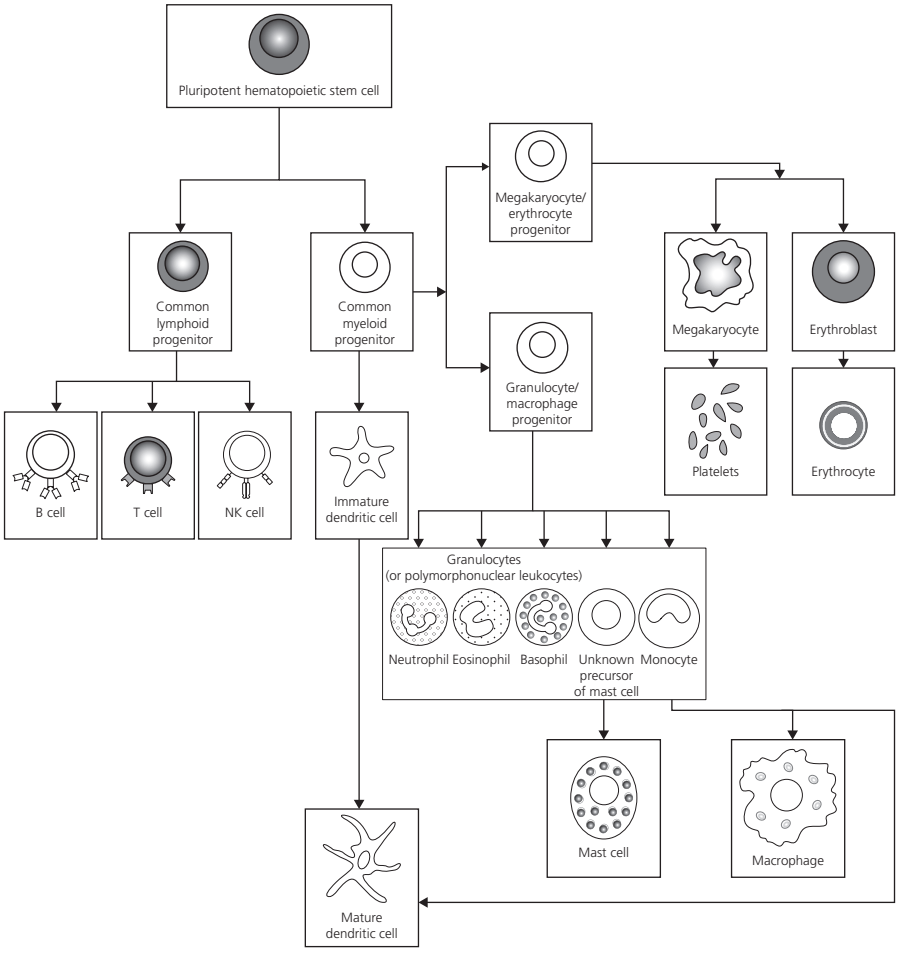


Figure 1.3 Ontogeny of immune cells. Cells of the immune system arise from pluripotent HSCs in the bone marrow. The common lymphoid progenitor gives rise to B cells, T cells, and NK cells. The common myeloid progenitor gives rise to dendritic cells (DCs), monocytes, neutrophils, eosinophils, and basophils.

for mature plasma cells, which are maintained in the bone marrow through the action of IL-6. Plasma cells are the principal source of antibodies in sensitized transplant recipients; therefore, investigators addressing the pathogenesis of antibody (historically referred to as "humoral") rejection are increasingly interested in these bone marrow-resident plasma cells. There is also strong evidence that memory T cells home to or reside in the bone marrow where they can be activated by antigens. Other experiments have suggested that activation of naïve T cells could occur in the bone marrow under certain circumstances, raising the possibility that the bone marrow may additionally serve as a secondary lymphoid tissue (see section "Secondary lymphoid tissues").

Cell trafficking

Cell trafficking is a dynamic process underlying allorecognition and transplantation responses, and remains a potential target in therapeutic strategies. Mature myeloid cells and certain precursor lymphoid cells (pre-thymocytes, immature B cells) migrate out of the bone marrow and enter the circulation. Conversely, mature lymphocytes (e.g., plasma cells) migrate into the bone marrow. The HSCs are also known to exit and re-enter the bone marrow. These trafficking events are primarily regulated by adhesion molecules and guided by chemokines. The integrin VLA-4, for example, maintains the developing B cells in tight contact with stromal cells by binding to VCAM-1. The chemokine CXCL12, which is produced by stromal reticular cells and osteoblasts, is responsible for retaining HSCs as well as myeloid and lymphoid cells in the bone marrow by binding to its receptor CXCR4. Some individuals with "gain of function" mutations in CXCR4 (e.g., WHIM syndrome) have pan-leukopenia because of increased retention of leukocytes in the bone marrow. Conversely, CXCR4 antagonism with the drug plerixafor mobilizes HSCs and myeloid and lymphoid cells from the bone marrow into the circulation. In the mouse, there is abundant evidence that CCR2 and its ligand CCL2 are responsible for monocytes exiting from the bone marrow into circulation.

Role in rejection and tolerance

The role of the bone marrow in organ transplantation has been studied in the context of tolerance induction. Investigators hoping to achieve solid organ allograft acceptance without immunosuppression have used simultaneous bone marrow and solid organ transplantation from the same donor. In this regimen, recipients receive "partial" myeloablative conditioning, followed by infusion of donor HSCs, with the goal being to induce mixed hematopoietic chimerism (both donor and host cells co-exist). Stable mixed chimerism can be attained in small experimental animals. It is more difficult in nonhuman primates (NHPs) and humans, but does result in long-lasting tolerance to allografts as re-emerging donor-specific B cells and T cells in transplant recipients are deleted or become anergic upon encountering donor antigens in the bone marrow and thymus, respectively. One obstacle to the widespread clinical use of the mixed chimerism is the need for toxic myeloablation conditioning prior to HSC infusion, as donor HSC engraftment is dependent on the presence of unoccupied hematopoietic niches or "space" in the recipient's bone marrow. A very small proportion (0.1–1%) of niches are unoccupied under homeostatic conditions, severely limiting the number of exogenous HSCs that could engraft, even if infused in very large numbers. While irradiation and cytotoxic drugs (e.g., cyclophosphamide) have been the mainstay of "freeing up" niches in the recipient, less toxic therapies that target chemokines and chemokine receptors in the bone marrow are currently being investigated. Finally, the bone marrow alone is insufficient for sustaining primary immune responses as seen in mice that lack secondary

lymphoid tissues but have an intact bone marrow are severely compromised in their ability to reject a transplanted organ.

Thymus

The thymus is *the* primary lymphoid organ where mature T cells are generated from bone marrow-derived progenitors (pre-thymocytes). The emergence of the thymus in evolution coincides with the emergence of adaptive immunity in jawed fish. In mammals, it is located in the upper anterior thorax above the heart. It owes its name to its lobular shape, which in the eyes of the Greek physician Galen resembled the *thyme* leaf. The thymus was considered to be a nonimmune organ for a long time until seminal work in mice by Jacques F. A. P. Miller and others in the 1960s demonstrated its central role in T lymphocyte development. It was found that removal of the thymus at birth leads to severe immune defects, including the abrogation of skin allograft rejection. Thymectomizing mice that had already reached puberty, on the other hand, had no significant effects on the immune response, indicating that the mature T cell repertoire had completely formed by then. The critical role of the thymus in T cell development in humans was confirmed in individuals with the congenital absence or severe hypoplasia of the thymus (DiGeorge syndrome). These individuals have very few T cells but normal B cell counts. Unlike in mice, removal of the thymus in infants or children does not lead to any obvious immune abnormalities, as T cell development in humans is largely completed prior to birth. Although the human thymus involutes significantly in size after puberty, thymic function persists in adults, especially in those who become lymphopenic secondary to either infection (e.g., HIV) or lymphodepletion (e.g., induction therapy at the time of transplantation).

Structure

Histologically, thymic lobes are made up of two clearly distinguishable areas: the cortex and the medulla and are separated by a highly vascularized corticomedullary border (Figure 1.4). The stroma in both regions consists of a three-dimensional network of thymic epithelial cells (TECs) surrounded by T cells. TECs are known by the acronyms cTECs and mTECs depending on whether they are located in the cortex or medulla, respectively. The cortex is densely populated with immature T cells in various stages of development, while the medulla harbors less tightly packed mature T cells. In addition to T cells, the thymus also contains DCs, macrophages, and B cells. As will be highlighted in the next section, cTECs, mTECs, DCs, and B cells play critical roles in T cell development, selection, and education. The thymic medulla in humans also contains distinct structures known as Hassall's corpuscles that consist of concentric layers of keratinizing epithelium. These structures are a prominent site of T cell apoptosis and of thymic stromal lymphopoeitin (TSLP) production. TSLP is an IL-7-like cytokine believed to activate thymic DC (see section "Function").

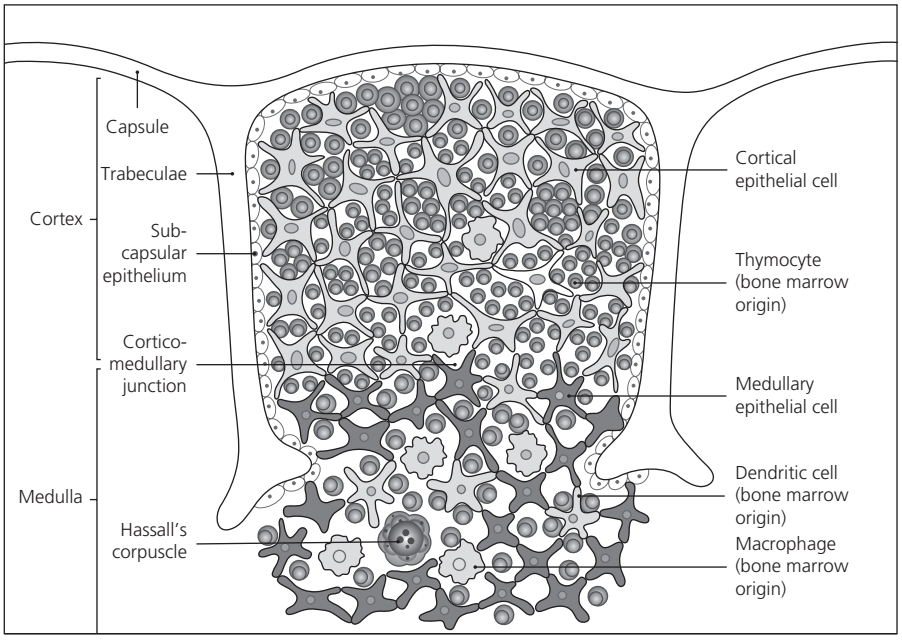


Figure 1.4 Structure of the thymus. The thymus consists of outer (cortical) and central (medullary) regions. Thymocyte maturation and T cell selection occur in both the cortex and medulla, with the outer cortical layer containing mainly proliferating, immature thymocytes and the deeper cortical and medullary areas containing immature T cells undergoing selection. Cortical and medullary epithelial cells, as well as bone-marrow derived DCs and macrophages, participate in the selection process. Source: Redrawn from Murphy (2011). Reproduced by permission of Garland Science/Taylor & Francis LLC.

Function

The thymus is the primary site where T cell maturation and selection occur. The end result of these processes is the generation of a mature T cell repertoire that recognizes myriad foreign peptides in the context of self-MHC (self-restricted) and yet has been successfully purged of autoreactive T cells. Bone marrow-derived T cell progenitors enter the thymus via venules near the corticomedullary junction and begin their maturation in the thymic cortex where they proliferate extensively, acquire classical T cell markers (e.g., the CD4 and CD8 co-receptors), and undergo random rearrangement of T cell receptor (TCR) genes to form mature TCRs. Thymocytes that express functional TCRs then undergo positive and negative selection. Most (>95%) die by "negative selection" because they either fail to sufficiently bind self-MHC and self-peptide complexes, and thus be destined to be poor antigen recognizing cells (leading to immunodeficiency) or bind these complexes too strongly (destined to become potentially autoreactive cells). Only those with proper TCR affinity for self-MHC and peptide complexes are selected to undergo further maturation (positive selection).

T cell selection begins in the cortex but is completed in the medulla, after which mature CD4 and CD8 T cells exit the thymus to enter the blood circulation. Positive selection is mediated by cTECs because they constitutively express both MHC class I and II molecules and can process and present self-peptides. Negative selection is mediated by both cTECs and mTECs for the same reasons mentioned above. Bone marrow-derived DCs are also crucial contributors to both processes. A subset of TECs in the corticomedullary junction is responsible for the production of IL-7, a cytokine critical for T cell survival. Thus, T cells are absent in IL-7 gene knockout mice. Importantly, the expression of a gene called autoimmune regulator (AIRE) allows TECs to present peptides derived from proteins in nonthymic tissues (e.g., insulin). AIRE therefore ensures that autoreactive T cells specific to all possible self-antigens are deleted in the thymus. Humans who harbor AIRE mutations develop autoimmune polyglandular syndrome type I, also known as autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy (APECED).

In addition to generating mature T cells (mostly with αβ TCRs), the thymus is also indispensable to the production γδ T cells, NKT cells, and natural regulatory T cells (nTreg). γδ T cells originate from the same progenitors as αβ T cells but exit the thymus at an early "double negative" stage (i.e., they lack both CD4 and CD8 expression) and populate epithelial sites such as the gut and skin. NKT cells are NK-like T cells that express an invariant TCR and are positively selected in the thymus by the nonclassical MHC molecule CD1d. Their main function is recognition of glycolipids produced by certain microbes such as mycobacteria. nTreg are CD4+ T cells that express conventional αβ TCR with an intermediate affinity to self-MHC and self-peptide complexes (thus, they are positively selected). They regulate immune responses by suppressing the function of effector T cells. The discovery of thymic-derived nTreg came from elegant observations in mice and humans. Although thymectomy at birth led to severe immunodeficiency in mice, immunologists noted that thymectomy on the third post-natal day paradoxically caused severe autoimmunity. The autoimmunity could be attributed to the absence of nTreg. It was later discovered that the transcription factor Foxp3 is required for the development of Treg and thus loss of function Foxp3 mutations in mice and humans cause fatal autoimmune disease (scurfy in mice and immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome, or IPEX in humans).

Cell trafficking

Chemokines play a key role in guiding cell trafficking in the thymus. The entry of progenitor cells from the bone marrow into the thymus is dependent on the chemokines CCL21 and CCL25, which bind the receptors CCR7 and CCR9, respectively. The chemokines CCL21 and CXCL12, the ligand for CXCR4, direct the migration of immature thymocytes during their maturation journey through the thymic cortex and medulla. Disruption of CCL21 expression causes defective deletion of autoreactive T cells. The chemokine that is central to the emigration

of mature T cells from the thymus into the blood is CXCL12. CXCL12 repels mature T cells out of the thymus, which contrasts to its function in retaining myeloid and lymphoid cells in the bone marrow (see section "Cell trafficking"). Emigration also requires binding of sphingosine-1-phosphate (S1P), which is present in high concentrations in the blood and lymph, to its receptor $S1PR_1$ on mature T cells. This interaction drives mature T cells to the periphery and alteration of cell trafficking through this pathway in transplantation has been tested in transplantation using the S1P agonist FTY720, which had adverse effects in transplant patients and was not further developed for this indication.

Role in rejection and tolerance

In solid organ transplantation, the thymus is exploited for tolerance induction because it is indispensable to the generation of nTreg and for the negative selection of newly developing T cells (commonly referred to as central tolerance). As mentioned earlier, unless performed at birth, thymectomy does not prevent allograft rejection. However, thymectomy around day 3 after birth eliminates nTreg and prevents graft acceptance in mouse models in which Tregs are needed for tolerance. In the mixed hematopoietic chimerism approach, the process of thymic T cell selection is recapitulated; DCs derived from donor HSC induce the apoptosis of newly developing, donor-reactive T cells in the thymus, leading to central tolerance. The key role of thymus in tolerance has prompted some investigators to co-transplant thymus and kidney or heart (referred to as thymo-kidney, thymo-heart or thymo-islet) grafts from the same donor to MHC-mismatched recipients. The rationale behind this procedure rests on the ability of epithelial cells of the thymic graft to express donor antigens, including donor MHC, and to delete donor-reactive T cells from the recipients, thus leading to donor-specific, central tolerance. Finally, the thymus also plays a role in the maintenance of allograft tolerance in some animal models by either providing a steady source of nTreg or a site for continual negative T cell selection.

Secondary lymphoid tissues

After exiting the thymus and bone marrow, mature naïve T and B cells populate the secondary lymphoid tissues, which include not only compartmentalized or encapsulated tissues such as the lymph nodes, spleen, tonsils, appendix, and Peyer's patches of the small intestine but also other lymphoid structures that are relatively less well delineated. Secondary lymphoid tissues are strategically located throughout the body at sites where antigen and antigen-presenting cells (APCs) are efficiently concentrated. Specifically, lymph nodes drain distinct organs or geographical areas of the body via afferent lymphatic vessels, the spleen traps antigens that enter the blood circulation, and MALTs capture antigens at mucosal surfaces (Figure 1.1). This arrangement maximizes the chance

that antigen-specific T and B cells, which exist in very low frequencies (about 1 in 100,000 T cells express the TCR specific for an individual antigen), encounter their cognate antigens. Importantly, the architecture of secondary lymphoid tissues is such that contacts between APCs, T cells, and B cells occur not only in the most effective way but also in the correct sequence to initiate productive responses. T and B cells that have been successfully activated by antigens then leave secondary lymphoid tissues to enter the blood circulation and eventually the tissues to execute their function. Secondary lymphoid tissues are the quintessential sites for initiating primary immune responses.

We will focus on lymph nodes, spleen, and Peyer's patches and discuss their direct relevance to solid organ transplantation. The roles of secondary lymphoid tissues in rejection and tolerance will be discussed collectively at the end of the section.

Lymph nodes

Lymph nodes are encapsulated, bean-shaped structures distributed along the lymphatic system throughout the body. The lymphatic system drains the extracellular fluid from the tissues and returns it to the blood via the thoracic and right lymphatic ducts in the chest. Antigens and migrating APCs are present in the lymphatic fluidand, therefore, lymph nodes are ideally positioned to capture the antigens arriving via the lymph for presentation to T and B cells in the nodes. Lymphocytes enter the lymph nodes through specialized blood vessels known as high endothelial venules (HEVs) that are localized to specific areas within the lymph nodes (see section "Structure" below). Lymph nodes are classified as either peripheral or mucosal, based on subtle differences in the adhesion molecules they express as well as their function (see sections "Function" and "Cell trafficking and lymph nodes"). Peripheral lymph nodes encompass most lymph nodes in the body, while mucosal lymph nodes include the mesenteric, cervical, sacral lymph nodes as well as bronchial lymph nodes, which participate in mucosal immune responses.

Structure

From a histological perspective, lymph nodes are organized structures surrounded by a capsule and sinus (Figure 1.5). Lymph flows from the afferent lymphatics into the subcapsular sinus and exits the lymph node through the efferent lymphatic vessel via the medullary sinus. A conduit system lined by fibroblastic reticular cells and collagen, fibrillin, and laminin layers allows lymph-carrying antigens and APCs to penetrate deep into the parenchyma of the lymph node. Antigens diffuse across sinus and conduit walls and are taken up by APCs that reside within lymph nodes or are picked up by DCs that extend cellular processes (dendrites) into the lymph. Mature APCs carrying antigens that reach lymph nodes from peripheral tissues via lymphatic vessels migrate across the sinus and conduit walls and then localize in T cell areas under the guidance of chemokines (see section "Cell trafficking and lymph nodes").

A point of entry for T and B cells to lymph nodes is the highly specialized HEV. HEV are post-capillary vessels unique to lymph nodes and Peyer's patches. They are lined by tall, cuboidal endothelial cells, which express adhesion molecules and chemokines necessary for the transmigration of T and B cells (see section "Cell trafficking and lymph nodes"). Importantly, T and B cells are not randomly distributed in the lymph node but are highly organized into two anatomic regions in the cortex. B cells are grouped within follicles in the outer cortex, while T cells are more diffusely distributed in the paracortical (or deep cortical) areas underneath the B cell follicles. The areas with T and B cells also contain stromal cells, macrophages, and DCs. A network of follicular dendritic cells (FDCs) located in the B cell follicles is specialized to capture antigens or antigen–antibody complexes. They play an important role in the formation of germinal centers, which are the central part of lymphoid follicles where memory B cells and antibody-producing plasma cells are generated (see section "Function"). FDCs are distinct from other DCs in that they are not derived from bone marrow precursors; they neither express MHC class II molecules nor do they have phagocytic activities. The innermost region of the lymph node consists of medullary cords that protrude into the medullary sinus (Figure 1.5); such medullary cords are rich in macrophages and plasma cells.

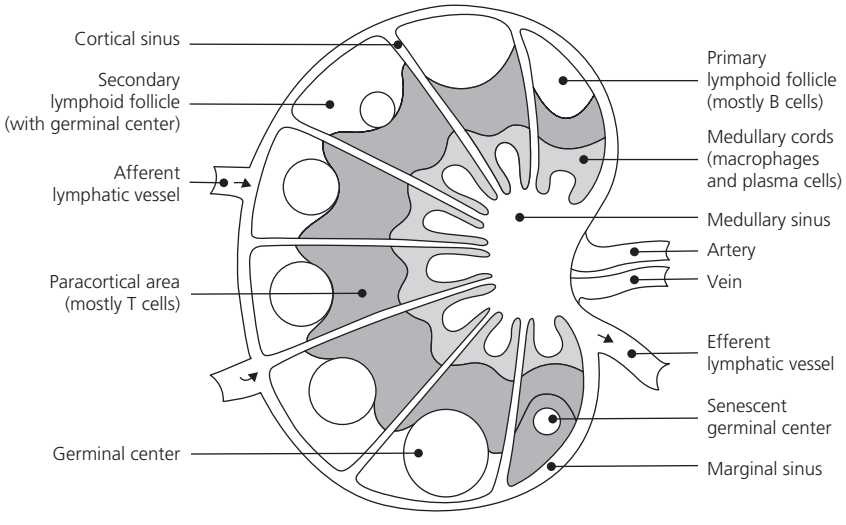


Figure 1.5 Structure of the lymph node. A cross-section of a lymph node reveals the outer cortex where B cell follicles reside and the paracortical (or deeper cortical) areas where the T cell zones are. The medulla (or medullary cords) are rich in plasma cells and macrophages. Lymph-carrying antigens and DCs arrive from peripheral tissues via the afferent lymphatic vessel and empties into the cortical sinus. Activated lymphocytes leave lymph nodes via the efferent lymphatic vessel and eventually enter the bloodstream via the thoracic ducts. Source: Redrawn from Murphy (2011). Reproduced by permission of Garland Science/Taylor & Francis LLC.

Function

Lymph nodes are the principal sites where naïve T and B cells are exposed to antigens to initiate activation and maturation. Mice that lack lymph nodes have abnormalities in their adaptive cellular and antibody immune responses. Lymph nodes are also crucial for the maintenance of naïve and memory T cells, especially those of the CD4+ subpopulation. This is due to the presentation of self-antigens by MHC on lymph node-resident DC, which provides survival signals to T cells. Lymph nodes and the spleen are also the sites where immature B cells that emerge from the bone marrow complete their final maturation during which autoreactive B cells that bind abundant self-antigens are deleted and purged.

The activation of naïve T cells occurs in the cortical zones (T cell zone), whereas B cell priming occurs in the follicles in the outer cortex. Classical imaging studies have established that T and B cell activation in lymph nodes occurs following a sequence of events. Naïve T and B cells enter lymph nodes via HEV and migrate to the T cell zones and B cell follicles, respectively. T cells sample DCs as they journey within the T cell zone. An antigen-specific T cell that encounters its cognate antigen on DCs leads to a protracted contact (or synapse) with the DC, and divides with daughter cells differentiating into effectors. T cells that do not encounter cognate antigen rapidly exit the lymph node via cortical sinuses, eventually returning to the blood via the thoracic duct. Naïve T cells may re-enter other lymph nodes via the lymph. Activated T cells on the other hand are retained for a few days in the lymph node to complete their proliferation and differentiation before exiting. Retention is in part mediated by the membrane lectin CD69 induced on activated T cells. Many follicular T helper cells (referred to as Tfh), however, migrate toward the B cell follicles. B cells activated in the follicles localize to the border between the follicle and the adjacent T cell zone where they are much more likely to interact with helper T cells. This interaction leads to B cell proliferation and differentiation into plasma blasts. Some plasma blasts migrate back to the center of the follicle where they continue to divide and differentiate into memory B cells or antibody-producing plasma cells, forming what is known as germinal centers where immunoglobulin gene rearrangements and affinity maturation occur. Affinity maturation refers to the selection of B cells that produce antibodies with high affinity to antigen. Mature plasma cells arising in germinal centers either migrate to the medullary cords or leave the lymph node altogether. Like T cells, B cells that do not encounter their cognate antigens rapidly exit lymph nodes via the efferent lymphatic vessel and then into the blood.

Cell trafficking and lymph nodes

DC, T cell, and B cell trafficking into, within, and out of lymph nodes is orchestrated by chemokines. DCs express the chemokine receptor CCR7 and migrate from nonlymphoid tissues to lymph nodes in response to the CCR7 ligand CCL19 and CCL21. CCL21 bound to extracellular fibrils in the walls of lymphatic vessels

and in the paracortical areas (T cell zones) of the lymph node guides DC migration by a process known as haptokinesis. Naïve T cells and a subset of memory T cells (central memory T cells) also express CCR7; therefore, they co-localize with DCs in the paracortical areas. CCR7 gene knockout mice lack T cell zones and have impaired primary T cell responses. Unlike T cells, naïve B cells express the chemokine receptor CXCR5 and home to follicles in the cortex in response to the chemokine CXCL13. Upon activation by antigens, B cells express CCR7 and migrate toward the periphery of the follicle in response to CCL21 (produced by mature DC) where B cells receive help from activated CD4+ T cells that express CXCR5. Follicular helper T cells (Tfh) also express CXCR5 and reside in B cell follicles in the lymph nodes, spleen, and other secondary lymphoid tissues. Tfh cells participate in B cell activation and germinal center formation. More recently, a subset of regulatory T (Treg) cells known as follicular Treg has been described. These cells inhibit B cell activation and humoral immunity.

The process by which naïve T cells enter lymph nodes via HEV has been well characterized. Initial adhesion of T cells on HEV is mediated by the binding of L-selectin (CD62L) on T cells to the sugar moieity (sulfated sialyl-Lewis^x) of mucin-like molecules (CD34 and GlyCAM-1) on endothelial cells. CD34 and GlyCAM-1 are specific to HEV in peripheral lymph nodes and are referred to as peripheral node addressins (PNAd), while MAdCAM-1 is expressed on endothelial cells in mucosal lymph nodes and Peyer's patches. MAdCAM-1 is the ligand for the integrin $\alpha_4\beta_7$ expressed on gut homing T cells. Firm adhesion and subsequent transendothelial migration of T cells is dependent on the binding of high-affinity LFA-1 on T cells to ICAM-1 on endothelial cells. LFA-1 is a β2 integrin that changes its confirmation from low to high affinity in response to G protein-coupled chemokine receptor signaling. Pertussis toxin, a potent inhibitor of Ga_i, therefore, abolishes T cell entry into lymph nodes. The exit of effector T cells from the lymph node is dependent on sphingosine-1-phospate (S1P), which is also required for the egress of mature T cells from the thymus. The immunosuppressive drug FTY720 is an S1P receptor agonist that causes the retention of activated T cells in secondary lymphoid tissues, presumably by rapid downregulating S1P receptors. Although effective in preventing organ transplant rejection at high doses (but with significant side effects), it is currently approved for the treatment of multiple sclerosis where lower doses appear to be effective and with less side effects.

Spleen

The spleen is a highly vascularized organ that serves several functions. First, it clears aging and abnormal blood cells and platelets in the blood as well as opsonized bacteria and immune complexes. Second, it serves as a site for extramedullary hematopoiesis in conditions where the bone marrow is compromised. Third, it is an organized secondary lymphoid tissue that plays an important role in initiating adaptive immune responses. Unlike lymph nodes, the

spleen is not connected to lymphatic vessels but only to the bloodstream. In the absence of the spleen, humans are at higher risk for sepsis caused by encapsulated bacteria such as *Streptococcus pneumoniae*, which are normally opsonized and cleared by the spleen. Splenectomy in experimental animals also leads to certain immunologic defects and has been used clinically in some forms of aggressive solid organ transplant rejection.

Structure

Histologically, the spleen is made up of two areas: the red pulp and white pulp (Figure 1.6). The red pulp consists of large blood-filled venous sinusoids where red blood cells and platelets are removed. The white pulp, on the other hand, carries out the immunologic functions of the spleen. It consists of discrete, organized collections of lymphocytes surrounding arterioles and interspersed among the venous sinusoids of the red pulp. The arterioles of the white pulp branch off from trabecular arteries and are distinct from those that form the red pulp. A cross-section of a white pulp area reveals a central arteriole, a sheath of lymphocytes around the arteriole (also known as the peri-arteriolar lymphoid sheath or PALS), lymphocyte follicles interspersed at intervals along the PALS, and a peri-follicular zone or marginal sinus surrounding the follicles. PALS contain mainly T cells (like the T cell zones of lymph nodes), while the follicles consist primarily of B cells and FDCs. The marginal zone of the follicles is abundant in macrophages, DCs, and a noncirculating population of marginal zone B cells unique to the spleen. The peri-follicular zone (marginal sinus) consists of small blood vessels that fan out from the central arteriole and empty into open blood-filled spaces surrounding the marginal zone of the follicles. The peri-follicular zone is the site of entry of antigens into the white pulp. The central arteriole and the blood spaces of the peri-follicular zone eventually drain into trabecular veins.

Function

Microbes, antigens, and antigen–antibody complexes present in the blood are picked up by macrophages and immature DCs in the marginal zone of the splenic white pulp. Activated DCs migrate to the T cell zones where they prime T cells with internalized and processed antigen. Activated T cells then either leave the spleen via the blood or migrate to the border of the follicles to provide help to B cells. Activated B cells in turn form germinal centers and differentiate into antibody-producing plasma cells and memory B cells. The spleen is also an important site where immature B cells arriving from the bone marrow undergo their final maturation and selection steps.

A distinguishing feature of the spleen is the presence of unique marginal zone macrophage and B cell populations not present in other secondary lymphoid tissues. The marginal zone has an outer ring of marginal zone macrophages and an inner ring of metallophilic macrophages. These macrophages capture a wide variety of pathogens from the blood through specialized surface receptors. Marginal

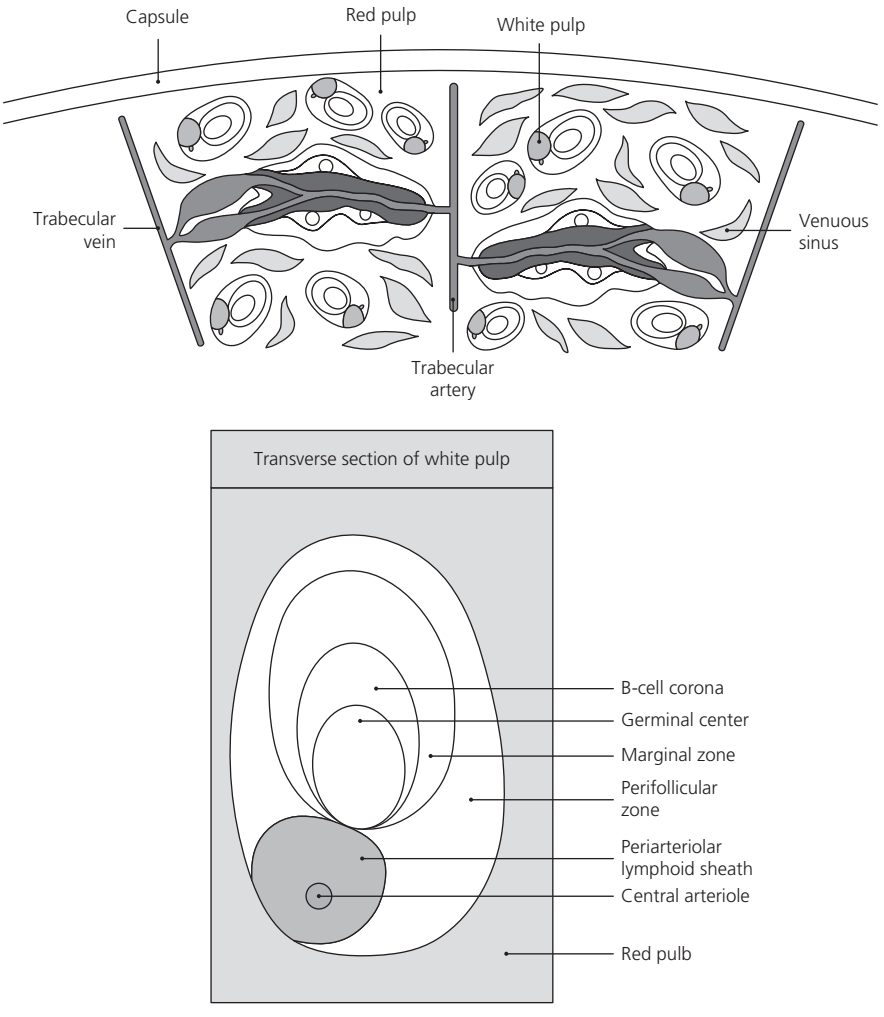


Figure 1.6 Structure of the spleen. The spleen consists of red pulp and white pulp. The red pulp is the site where aging RBCs and platelets are removed. The white pulp is the area in the spleen where lymphocytes are activated. T cells reside in the periarteriolar lymphoid sheaths (PALS) that immediately surround central arterioles while B cells reside in the adjacent follicles (inset). The marginal zone is rich in DCs and macrophages, some unique to the spleen. The spleen has no connections to the lymphatic system. Source: Redrawn from Murphy (2011). Reproduced by permission of Garland Science/Taylor & Francis LLC.

zone B cells are distinct from follicular B cells in that they express high levels of IgM (instead of IgD) and the toll-like receptor (TLR)-9, and have a limited B cell receptor repertoire. They resemble B-1 cells in the peritoneal cavity and likely provide T cell-independent responses to bacterial antigens. The marginal zone, therefore, is often regarded as a defense system strategically positioned to capture pathogens that enter the bloodstream and present them to the adaptive immune cells.

Cell trafficking and the spleen

Unlike lymph nodes, the spleen does not contain HEV and does not have significant PNAd expression. Naïve T cells and B cells enter the splenic white pulp via the marginal sinus and localize to the PALS and follicles in response to the chemokines CCL19/CCL21 and CXCL13, respectively. In contrast to their entry into lymph nodes, the transmigration of lymphocytes into the white pulp is not dependent on G protein-coupled chemokine receptors and the requirement for integrins is not clear. Although ICAM-1, MAdCAM-1, and VCAM-1 are expressed on the endothelial cells lining the marginal sinus, none is absolutely essential for T and B cell entry. The mechanisms and routes of T and B cell egress from the spleen are also not well understood.

Peyer's patches

Peyer's patches are organized lymphoid aggregates present in the intestinal mucosa. They are present in the ileum but are absent in the jejunum and duodenum. Like lymph nodes and the spleen, they consist of B cell follicles and T cell zones (Figure 1.7). The area just underneath the intestinal epithelium is rich in DCs and is known as the subepithelial dome. DCs are attracted there by the chemokines CCL20 and CCL25 (TECK) produced by epithelial cells. The receptors for these chemokines are CCR6 and CCR9, respectively, and are expressed on gut-homing DCs. Peyer's patches do not have afferent lymphatics, so antigen uptake is dependent on specialized epithelial cells called microfold or M cells intercalated between intestinal epithelial cells. M cells lack microvilli and mucus; they are transcytotic, and therefore specialize in transporting antigens (they express receptors for certain pathogens such as Salmonella and HIV) from the gut lumen to Peyer's patches, but are not involved in antigen processing and presentation to T cells. The latter function is carried out by DCs, which acquire antigen directly from the gut lumen via interepithelial dendrites. Naive lymphocytes enter Peyer's patches via HEV that express MAdCAM-1, which binds $\alpha_4\beta_7$ integrin on lymphocytes. Primary immune responses take place in Peyer's patches in a regulated process analogous to what occurs in lymph nodes and the spleen. Newly minted effector cells exit Peyer's patches through efferent lymphatic vessels that drain into the mesenteric lymph nodes. Effector T and B cells eventually reach the circulation via the thoracic duct and re-enter the small intestinal wall via blood vessels. The homing of an effector or memory T cell back to the site where its naïve predecessor was initially primed is an example of "imprinting." In case of the gut, imprinting occurs at the time of naïve T cell activation by DCs in Peyer's patches. Gut DCs induce the expression of $\alpha_4\beta_7$ and CCR9 on effector T cells, mainly through the production of rentinoic acid derived from dietary vitamin A. $\alpha_4\beta_7$ and CCR9 are responsible for the "gut-tropism" of these cells. The $\alpha_4\beta_7$ receptor MAdCAM-1 is expressed on intestinal endothelal cells, while CCR9 ligand CCL25 is secreted by intestinal epithelial cells. It is important to note that not all DC–T cell interactions in the gut induce immune

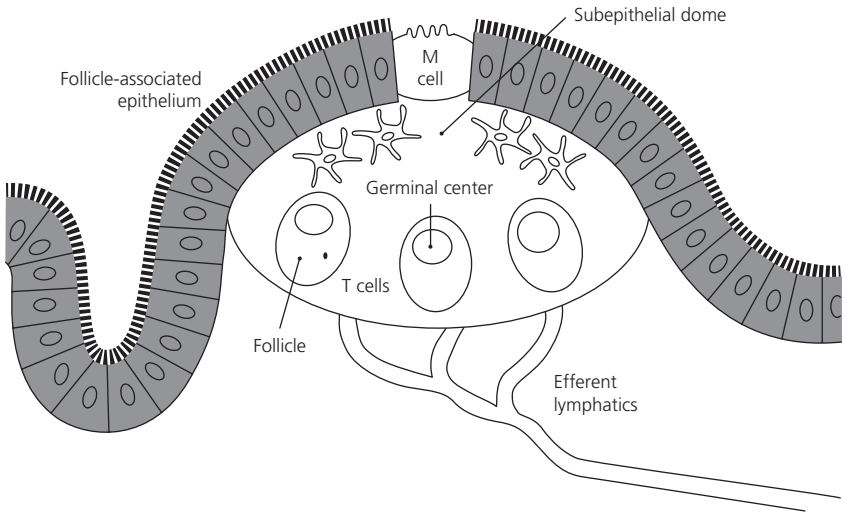


Figure 1.7 Structure of Peyer's patches. Peyer's patches are located in the ileum under the intestinal epithelium. A distinctive feature of Peyer's patches is M cells. They are specialized epithelial cells that transcytose antigens from the gut lumen to the Peyer's patch. The basic architecture of Peyer's patches is similar to that of other secondary lymphoid tissue and is characterized by B cell follicles and more loosely organized T cell zones. Source: Redrawn from Murphy (2011). Reproduced by permission of Garland Science/Taylor & Francis LLC.

responses. In fact, a significant proportion of such interactions are necessary for maintaining tolerance to harmless antigens in the gut, such as those derived from food.

In addition to Peyer's patches, the gut contains a large number of less well-defined DC and lymphocyte aggregates, referred to as "cryptopatches," that are distributed throughout the lamina propria. Cryptopatches are quite plastic; they organize into lymph node-like structures during infection or autoimmunity and participate in the mucosal immune response, but disintegrate back to lose lymphoid aggregates upon resolution of the response. The lamina propria also contains a large number of scattered T and plasma cells. The T cells include effector, memory, and Treg cells, while the lamina propria plasma cells are responsible for IgA production. Another lymphocyte population in the gut residing in the epithelial layer called intra-epithelial lymphocytes (IELs) are virtually all antigen-experienced CD8+ T cells and γδ T cells. They play an important role in host defense against pathogens.

Role of secondary lymphoid tissues in rejection and tolerance

Adaptive immune responses to foreign antigens are mainly initiated within the organized structures of secondary lymphoid tissues. In transplantation immunology, however, controversy has lingered regarding the site where primary

alloimmune responses are initiated. While some contend that the alloimmune response follows the same rules as adaptive immunity to nontransplant antigens, others argue that naïve T cells can be activated within the graft itself, as allografts present a unique form of antigenic challenge: they come with their own DCs and more often, their own vasculature, which can also present donor antigens. It was shown that rejection of skin allografts is dependent on the graft having access to host lymphatics whereas the rejection of vascularized allografts is not. The latter observation led to the "peripheral sensitization" concept that donor endothelial cells lining vascularized grafts are capable of directly activating allospecific naïve T cells. This peripheral sensitization hypothesis has been revisited more recently using genetically modified mice that lack secondary lymphoid tissues; for example, *aly/aly* mice that harbor a mutation in the NF-kB-inducing kinase (NIK) or LTβR gene knockout mice that lack the lymphotoxin (LT) receptor central to the ontogeny of lymph nodes and Peyer's patches (see section "Genesis"). The data so far indicate that rejection of vascularized organ allografts by naïve mice is impaired if all secondary lymphoid tissues are absent, with the exception of small intestinal allografts, which provide their own Peyer's patches, and lung allografts in the mouse, which contain bronchus-associated lymphoid tissues (BALTs). Another key exception is the host that harbors alloreactive memory T cells. Unlike naïve T cells, memory T cells home directly to nonlymphoid tissues (e.g., the allograft) and initiate immune responses within the graft. This is particularly important in the field of organ transplantation because the alloreactive T cell repertoire is composed of both naïve and memory T cells. Alloreactive memory T cells are ubiquitous, even in humans who have never been exposed to alloantigens, due to heterogonous immunity (cross-reactivity). Heterogonous immunity refers to the presence of memory T cells specific to microbial antigens that are also alloreactive.

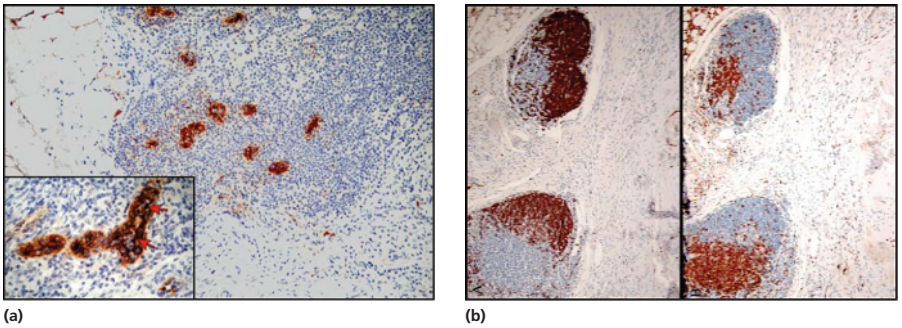
Secondary lymphoid tissues are also important for tolerance induction and maintenance. Transplantation tolerance is an *active* process dependent on the deletion or regulation of mature alloreactive T cells. Both of these phenomena occur predominantly in secondary lymphoid tissues. This is best illustrated by experiments that show that allograft acceptance in mice that lack secondary lymphoid tissues is due to immunologic ignorance and not tolerance. T cells from these mice are not deleted or rendered tolerant because they rapidly precipitate allograft rejection upon transfer to recipients that have intact secondary lymphoid tissues. There is also evidence that Treg maintain tolerance by suppressing immune responses within lymph nodes that drain the transplantation site. In the case of tolerance to gut antigens (e.g., food antigens), Tregs generated in the mesenteric lymph nodes migrate to the *lamina propria* of the intestine where they maintain mucosal tolerance by producing IL-10 and other immune modulatory cytokines.

Tertiary lymphoid tissues

Tertiary lymphoid tissues are organized, ectopic lymphoid aggregates, often developed in nonlymphoid tissues in response to chronic inflammation, or in the case of transplantation, chronic rejection. They are referred to as tertiary lymphoid tissues or tertiary lymphoid organs (TLOs) because they structurally resemble lymph nodes. The process by which they arise is known as lymphoid neogenesis. In humans, TLO have been described in the thyroid gland (Hashimoto's thyroiditis), central nervous system (multiple sclerosis), thymus (myasthenia gravis), joints (rheumatoid arthritis), salivary glands (Sjogren's syndrome), gastric mucosa (*Helicobacter pylori* infection), liver (primary sclerosing cholangitis and chronic hepatitis C), skin (*Borrelia burgdorferi* infection), and in transplanted kidneys, hearts, and lungs. TLO propagate immune responses locally by functioning as "lymph nodes." There is evidence, however, that TLO also participate in immune regulation.

Structure

TLO contain elements of both chronic inflammation and secondary lymphoid tissues, specifically those of peripheral lymph nodes (Figure 1.8). Structural elements reminiscent of lymph node architecture include (a) HEV, (b) discrete T and B cell areas including follicles and germinal centers, (c) DC and FDC networks, and (d) lymphatic channels. As in lymph nodes, HEV in TLO express L-selectin ligands (PNAd) or MAdCAM-1. Chemokines that attract T cells, B cells, and DCs (CCL19, CCL21, and CXCL13) are produced by stromal cells in TLO.



(a) (b)

Figure 1.8 Tertiary lymphoid tissues in the transplanted heart. Examples of lymph node-like aggregates within the parenchyma of murine cardiac allografts undergoing combined acute and chronic rejection. (a) Lymphoid aggregate rich in high endothelial venules (HEVs) stained positive for peripheral node addressins (PNAds). The insert depicts PNAd+ HEV with stronger staining evident on the luminal side of the endothelium (arrows). (b) Lymphoid aggregates demonstrating distinct compartmentalization of B cells (left panel, CD220+ cells) and T cells (right panel, CD3+ cells). Source: Baddoura et al. (2005). Reproduced by permission of John Wiley & Sons Ltd.

TLO also contain cellular elements of chronic inflammation and are not always organized, including in some instances of scattered HEV surrounded by lymphocyte aggregates. TLO are plastic; they form, dissolve, and reform as inflammation fluctuates. Therefore, TLO are considered as an end-product of inflammation.

Genesis

Generation of TLO is a well-defined process that recapitulates the ontogeny of secondary lymphoid tissues (lymphoid organogenesis). Key to both lymphoid neogenesis and lymphoid organogenesis is the tumor necrosis factor (TNF) family of inflammatory cytokines, namely the lymphotoxins (LT). Lymphotoxins include the secreted cytokine $LT\alpha_3$ and the membrane-bound cytokine $LT\alpha_2\beta_1$. $LT\alpha_3$, which signals through TNFR1 p55, and $LT\alpha_2\beta_1$, which signals through LTβR, is essential for the development of secondary and tertiary lymphoid tissues. LTα or LTβR gene knockout mice lack lymph nodes and Peyer's patches and have a disorganized splenic white pulp. Inactivation of the NIK enzyme downstream of LTβR, either due to a naturally occurring mutation (*aly*) or to a genetically engineered NIK gene knockout, leads to the same secondary lymphoid tissue defects. Mice that lack the LTβ gene on the other hand are devoid of peripheral lymph nodes but retain mucosal (mesenteric, sacral, and cervial) lymph nodes, indicating that $LT\alpha_3$ is sufficient for the genesis of the latter, while $LT\alpha_2\beta_1$ is required for the former. The fusion protein LTβR-Ig is a potent, immunosuppressive agent, which disrupts lymph node and splenic white pulp architecture by interrupting the binding of $LT\alpha_2\beta_1$ to its receptor. This implies that lymphotoxins are important not only for lymphoid organogenesis but also for the maintenance of secondary lymphoid tissues in the adult animal.

During chronic inflammation, lymphoid neogenesis is initiated by bone marrow-derived lymphoid-tissue inducer (LT_i) cells that express $LT\alpha_2\beta_1$ as well as the chemokine receptor CXCR5. Engagement of LTβR on tissue stromal cells by $LT\alpha_2\beta_1$ on LT_i cell leads to the production of secondary lymphoid tissue chemokines (CXCL13 and CCL21, among others). These attract more LT_i cells as well as T, B, and DCs, eventually leading to the formation of tertiary lymphoid structures. The transcription factor RORγt responsible for the development of LT_i is also critical for the development of the Th17 T cell subset. Recent data demonstrated that Th17-mediated autoimmune disorders (e.g., autoimmune allergic encephalitis in mice) are characterized by TLO formation in the affected tissues, suggesting that Th17 cells also initiate lymphoid neogenesis.

Function

Studies in humans and mouse models of autoimmunity have shown that TLO are responsible for T cell and B cell activation and antibody production at the sites where they form; for example, in joints affected by rheumatoid arthritis, the central nervous system in multiple sclerosis, or the pancreas of nonobese diabetic (NOD) mice. These studies provided direct evidence that B cells undergo

antigen-driven proliferation and somatic hypermutation within TLO, leading to the generation of plasma cells that produce high-affinity autoantibodies. The autoantibodies are specific to antigens abundant in the tissue where the TLO are located and often display the phenomenon of epitope spreading. Although direct evidence of T cell activation within TLO is lacking, elegant studies in mice have shown that TLO are sufficient for sustaining T cell-mediated autoimmune diabetes and for mounting antitumor and antiviral T cell responses. Therefore, TLO are beneficial to the host in the setting of tumors or infection but are deleterious in autoimmunity.

Role in rejection and tolerance

Either fully formed TLO or PNAd+ HEV, lymphocyte clusters, and lymphatic channels reminiscent of lymph node architecture have been documented in chronically rejected human and mouse heart and kidney allografts (Figure 1.8). PNAd+ blood vessels, in the absence of lymphocyte clusters, have also been reported in allografts undergoing acute rejection. In lung transplantation, lymphoid neogenesis occurs under the epithelial lining of the airways. These structures are referred to as inducible BALTs (iBALTs) and have been also described after infection with the influenza virus. What function do TLO then serve in transplantation? Most studies point to a pathogenic role for TLO. However, TLO are also observed in allografts accepted long term in mice and humans, suggesting a role in tolerance or immune regulation. Thus, the exact role of TLO in transplantation requires further testing.

Summary

The lymphoid organs and tissues are complex structures that support generation, function, homeostasis, and regulation of lymphocytes. The primary lymphoid organs are sites where T cells and B cells develop and mature. A mature repertoire of lymphocytes consists of cells that are selected and educated to respond to foreign antigens, including alloantigens. Secondary lymphoid organs provide places for mature lymphocytes to reside and also as ideal sites for their activation and functional differentiation. Although T cells and B cells are developed in separate primary sites, they interact extensively at secondary sites to mount productive responses to antigens. Importantly, proper regulation of the immune system and additionally the immune responses to antigens require both primary and secondary lymphoid tissues. The exact role of tertiary lymphoid tissues in the overall immune responses remains incompletely defined. Clearly, immune responses that lead to rejection or even tolerance requires the interaction of multiplicity of cells and precisely choreographed interactions within exquisitely engineered anatomic structures. Strategies to exploit these interactions within lymphoid structures continue to hold promise for the induction of transplant tolerance.

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