Beginning with mouse lymph node as the prototype, we examine some fundamental features of all secondary LYMPHOID TISSUES, particularly the segregation of T-DEPENDENT REGIONS containing T-cells (DIFFUSE CORTEX) from T-INDEPENDENT REGIONS containing B-cells (PRIMARY FOLLICLES). The process of MIGRATION (the exit of mature B-cells from the bone marrow, the movement of T-cell precursors from bone-marrow to thymus, and the emigration of mature T-cells from the thymus) is distinguished from that of RECIRCULATION (the process by which both B- and T-cells continually enter lymphoid tissues from the blood and return to the blood via the lymph). The formation of GERMINAL CENTERS in pre-existing primary follicles is seen to be an antigen-dependent and T-cell-dependent process.

Other peripheral lymphoid tissues (e.g. spleen, Peyer's patches), and lymphoid tissues of other species including humans, although differing in details, all show the same basic features of structural organization and cell movement.

Immune responses are not carried out in any single organ, but in a wide variety of structures collectively known as LYMPHOID TISSUE. Lymphoid tissue can be generally categorized as CENTRAL (or "PRIMARY"), versus PERIPHERAL (or "SECONDARY"). Central lymphoid tissues are those which act as a source of immunocompetent cells; these cells then migrate to the peripheral lymphoid tissues which are the sites of immune responses.

<table>
<thead>
<tr>
<th>Central</th>
<th>Peripheral</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymus</td>
<td>Lymph Node</td>
</tr>
<tr>
<td>Bone Marrow</td>
<td>Spleen</td>
</tr>
<tr>
<td></td>
<td>Gut-Associated lymphoid tissue (GALT)</td>
</tr>
<tr>
<td></td>
<td>Tonsils</td>
</tr>
<tr>
<td></td>
<td>Appendix</td>
</tr>
<tr>
<td></td>
<td>Peyer's Patches</td>
</tr>
</tbody>
</table>

The lymphoid tissues we will be discussing are broadly included in the term **Reticuloendothelial System**, or RES. The RES also includes other regions rich in phagocytic cells, notably those in the vasculature of the liver and lungs.

**MOUSE LYMPH NODE**

We will examine the structure of a typical mouse lymph node (diagrammed on next page) to illustrate several key points: Lymphoid tissue is not just a "bag" of lymphoid cells, but consists of a highly ordered structure; it is also a dynamic structure, maintained by a continuous movement of cells into and out of the tissue (via MIGRATION and RECIRCULATION); characteristic anatomical changes can be observed during the generation of an immune response.
The structure of a mouse lymph node can be divided into two areas, CORTEX and MEDULLA.

**Primary follicles:**
collections of densely packed small lymphocytes

**Secondary Follicles:**
follicles containing a germinal center

Region between follicles, less densely packed small lymphocytes; some plasma cells and macrophages

Large numbers of plasma cells and "cords" of macrophages; also lymphocytes, especially medium and large

**Figure 16-1**

**ARCHITECTURE OF A MOUSE LYMPH NODE**

**Figure 16-2**
As fluids from various tissues are collected in the lymphatics, they enter the afferent vessels of a lymph node, percolate through the cortex and through the medulla, and are collected in the single efferent lymphatic through which they leave the node; this fluid eventually ends up in one of the major lymphatics (e.g., thoracic duct) and is dumped into the venous circulation.

_The Primary Follicles of the cortex are made up primarily of B-lymphocytes_ (with occasional T-cells) and are characteristic of a resting or unstimulated node. Upon _antigenic stimulation_, normally following entry of antigen via the afferent lymphatic fluid, a region of intense proliferation develops within the follicle, known as a Germinal Center; the germinal center displaces the remaining densely packed B-cells into a peripheral "mantle", and the follicle is now known as a Secondary Follicle. In addition to containing proliferating cells, the _germinal center_ also is a site of _cell death_, and one diagnostic feature is the presence of macrophages which contain phagocytosed cell debris ("Tingible Body Macrophages").

_The Diffuse Cortex contains mainly T-lymphocytes._ In neonatally thymectomized mice (also in _nu/nu_ athymic mice and congenitally athymic humans) this area is virtually empty of lymphocytes, and for this reason became known as a _THYMUS-DEPENDENT AREA_ (TDA) of lymphoid tissue. In young mice (and continuously, although to a lesser extent in adult animals) mature T-cells which develop within the thymus leave that organ, enter the circulation and colonize the T-dependent areas of lymph nodes and other peripheral lymphoid tissues; this process is one manifestation of _lymphocyte MIGRATION_.

A second consequence of neonatal thymectomy is the lack of development of germinal centers upon antigenic stimulation (and, of course, a lack of immune responsiveness to T-dependent antigens). _Thus, although germinal centers develop within B-cell areas (primary follicles), they are T-dependent in their development._

**LYMPHOCYTE RECIRCULATION**

_Lymphocytes leave the lymph node continuously through the efferent lymphatic, enter the blood circulation, and re-enter the lymph nodes through specialized vessels known as High Endothelial Venules_ (HEVs); this process is known as _RECIRCULATION_. If the thoracic duct (a major lymphatic vessel) of a mouse is cannulated and the lymph (and the cells within it) is removed for a period of a week, the diffuse cortex becomes emptied, just as if the animal had been neonatally thymectomized. This is because T-cells recirculate more rapidly than B-cells and are therefore more readily depleted; a longer period of cannulation will eventually remove the B-cells as well.

_The entry of lymphocytes into lymph nodes is a highly specific process, involving recognition by lymphocytes of receptor molecules on the endothelial cells of the HEVs_; erythrocytes, granulocytes and other cells are not capable of carrying out this process. As their name implies, the HEVs are characterized by high cuboidal endothelial cells instead of the squamous cells commonly lining other vessels, and they are present in some other peripheral lymphoid tissues (e.g., Peyer's patches) as well. Recirculation of lymphocytes, in fact, is a universal characteristic of peripheral lymphoid tissues. (NOTE: _Recirculation of lymphocytes also occurs through the spleen, although this organ does not contain HEVs_. In this case lymphocytes enter the spleen by a morphologically distinct route in the "marginal sinus" which surrounds the follicles.)
A diagrammatic representation of lymphocyte migration and recirculation through lymph nodes is shown below. Keep in mind that these processes occur in all peripheral lymphoid tissues (spleen, Peyer's patches, etc.), and that all of them contain follicles as well as thymus-dependent areas.

Figure 16-3

One additional migration pathway which should be mentioned is the one by which bone marrow-derived pre-T cells enter the thymus (this is also shown in the diagram). These are the cells which following entry into the thymus differentiate into mature T-cells, undergoing positive and negative selection to ensure that only suitable T-cell receptors are expressed, and eventually migrate back out into the periphery. This process occurs at a high rate during the early development of the immune system, but persists at a lower rate throughout the life of the animal despite the overall involution of the thymus. (See Chapter 18, TOLERANCE for a more detailed discussion.)

TIME COURSE OF EVENTS IN A "TYPICAL" IMMUNE RESPONSE

Day 0 Antigen introduced in a local site (e.g., skin of the foot).
Day 1 Antigen found within the draining lymph node (e.g., popliteal), having been carried in by activated dendritic cells in the tissues, as well as taken up by resident dendritic cells in the diffuse cortex (also by phagocytic cells of the medulla). These cortical dendritic cells are highly efficient antigen-presenting cells, and it is here that the initial interaction involving antigen-specific T\(_H\) and B-cells is thought to occur.
Day 1-2 Lymph node enlarges, due to increased entry of cells from circulation and decreased exit via lymphatics (i.e. inflammatory response).
Day 2 Wave of T-cell mitosis followed by B-cell mitosis.
Day 4-5 Early germinal center formation, site of class-switching and somatic mutation.
Day 5-6  Early antibody-forming cells exit lymph node via efferent lymphatic, colonize distant lymphoid tissues (spleen, other lymph nodes); large numbers of antibody-producing plasma cells begin to appear in medulla.

The following points should be noted:

- The mitoses seen in the local node represent clonal expansion of antigen-specific precommitted T-cells and B-cells, as well as non-specific proliferation resulting from the many lymphokines present.

- The rapid recirculation of lymphocytes (particularly of T-cells) allows the recruitment of antigen-specific cells to the local site. The enlargement of the node represents the result of such recruitment, in addition to a great deal of non-specific accumulation of cells and fluid.

- Although the initial response is generated within the lymph nodes which drain the local site of antigen deposition, the antibody-forming cells which are produced rapidly spread to other sites, and memory cells rapidly enter the recirculating pool as well as colonize other peripheral lymphoid tissues; the net result is effective systemic immune responsiveness and memory.

- Germinal Centers are a major site for the generation of memory B-cells, isotype switching and somatic generation of diversity for immunoglobulins. While “follicles” are defined as regions of B-cell localization, it is important to remember that germinal centers are both T-dependent and antigen-dependent in their formation.

- As has already been discussed, there exists a class of antigens ("T-independent antigens") which do not depend on T-cell function to generate an antibody response. The response to such antigens does not lead to germinal center formation, and the histological events in such responses will not be discussed here.

CHAPTE 16, STUDY QUESTIONS:

1. Follow a lymphocyte from the bloodstream through its RECIRCULATION pathway(s) back to the blood; identify where it goes and what specific cell interactions must take place during this process. How do T and B-cells differ in this regard?

2. What cellular and molecular events take place in the GERMINAL CENTER? Do all immune responses result in GERMINAL CENTER FORMATION?