Lecture II

Experimental systems in immunology

Cells and organs of the immune system

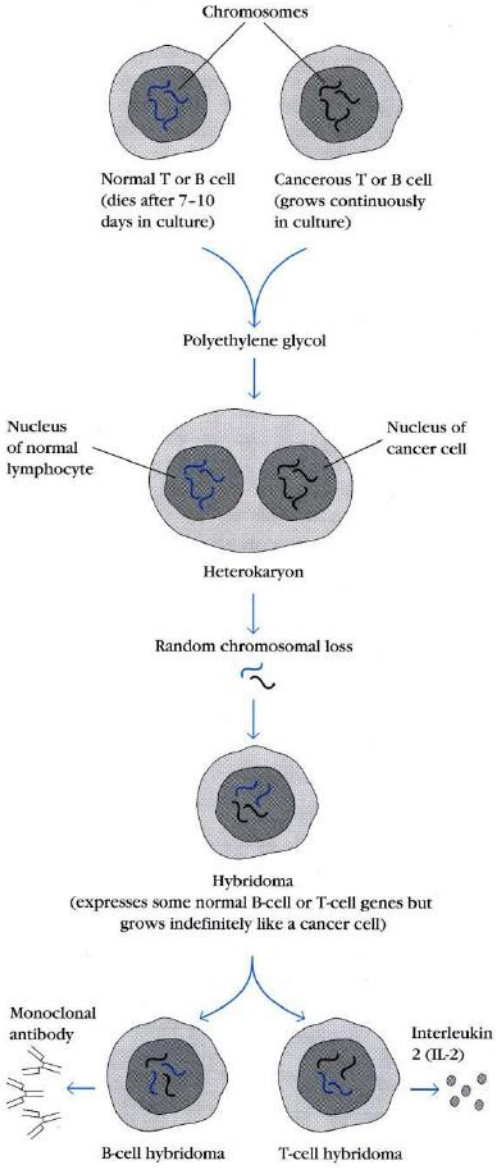


FIGURE 2-2

Production of B-cell and T-cell hybridomas by somatic-cell hybridization. The resulting hybridomas express some of the genes of the original normal B or T cell but also exhibit the immortal-growth properties of the tumor cell. This procedure is used to produce B-cell hybridomas secreting monoclonal antibody and T-cell hybridomas secreting various growth factors.

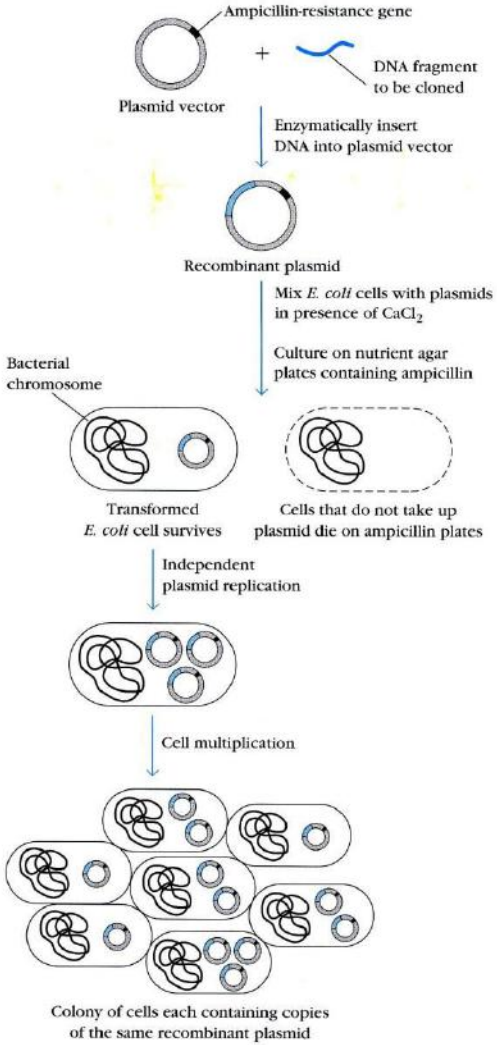


FIGURE 2-3

cDNA cloning using a plasmid vector. A plasmid containing a replication origin and an ampicillin-resistance gene is cut with a restriction endonuclease that produces blunt ends. Following addition of a poly-C tail to the 3′ ends of the cDNA and of a complementary poly-G tail to the 3′ ends of the cut plasmid, the two DNAs are mixed, annealed, and joined by DNA ligase, forming the recombinant plasmid. Uptake of the recombinant plasmid into *E. coli* cells is stimulated by high concentrations of $CaCl_2$. Transformation occurs with a low frequency but the transformed cells can be selected in the presence of ampicillin. [Adapted from Harvey Lodish et al. 1995, *Molecular Cell Biology*, 3rd edition, Scientific American Books.]

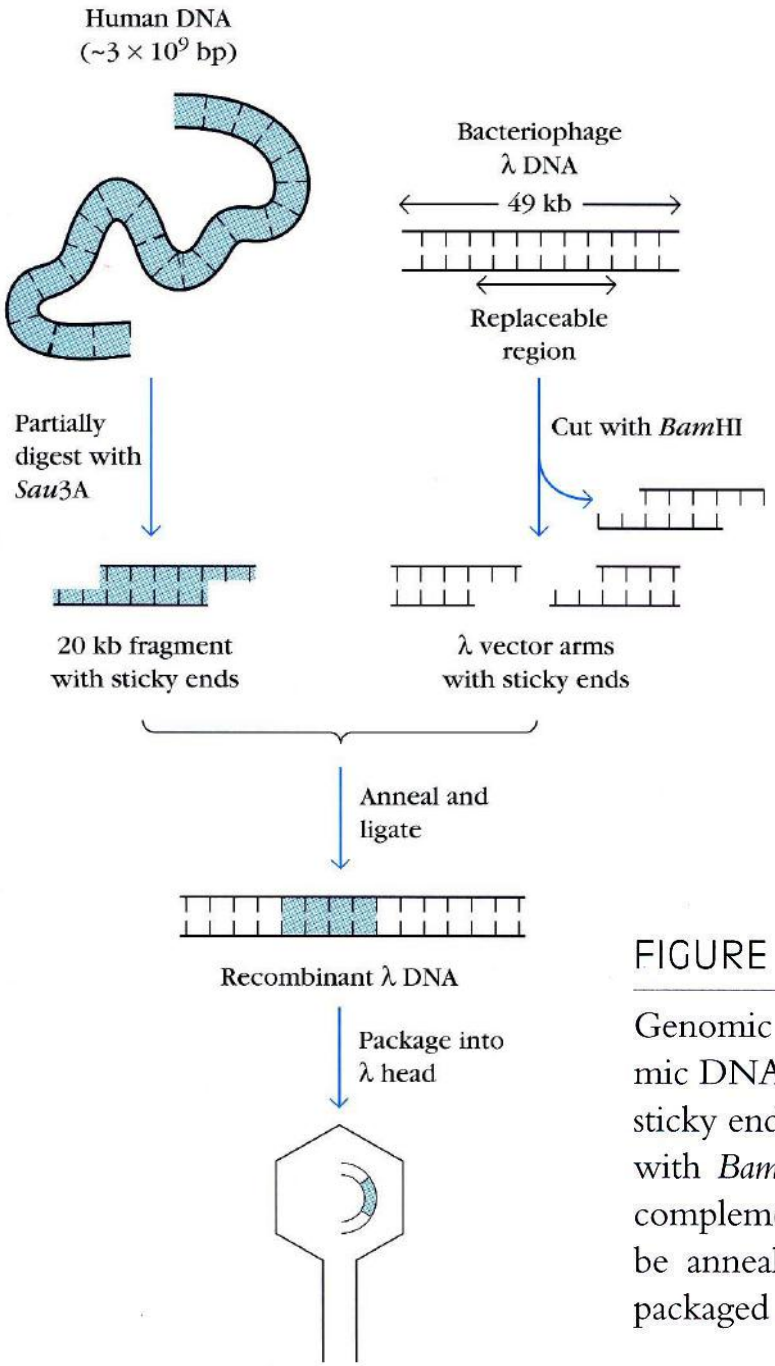


FIGURE 2-4

Genomic DNA cloning using bacteriophage λ as the vector. Genomic DNA is partially digested with *Sau*3A, producing fragments with sticky ends. The central 15-kb region of the λ-phage DNA is cut out with *Bam*HI and discarded. These two restriction enzymes produce complementary sticky ends, so the genomic and DNA fragments can be annealed and ligated. After the resulting recombinant DNA is packaged into a λ-phage head, it can be propagated in *E. coli*.

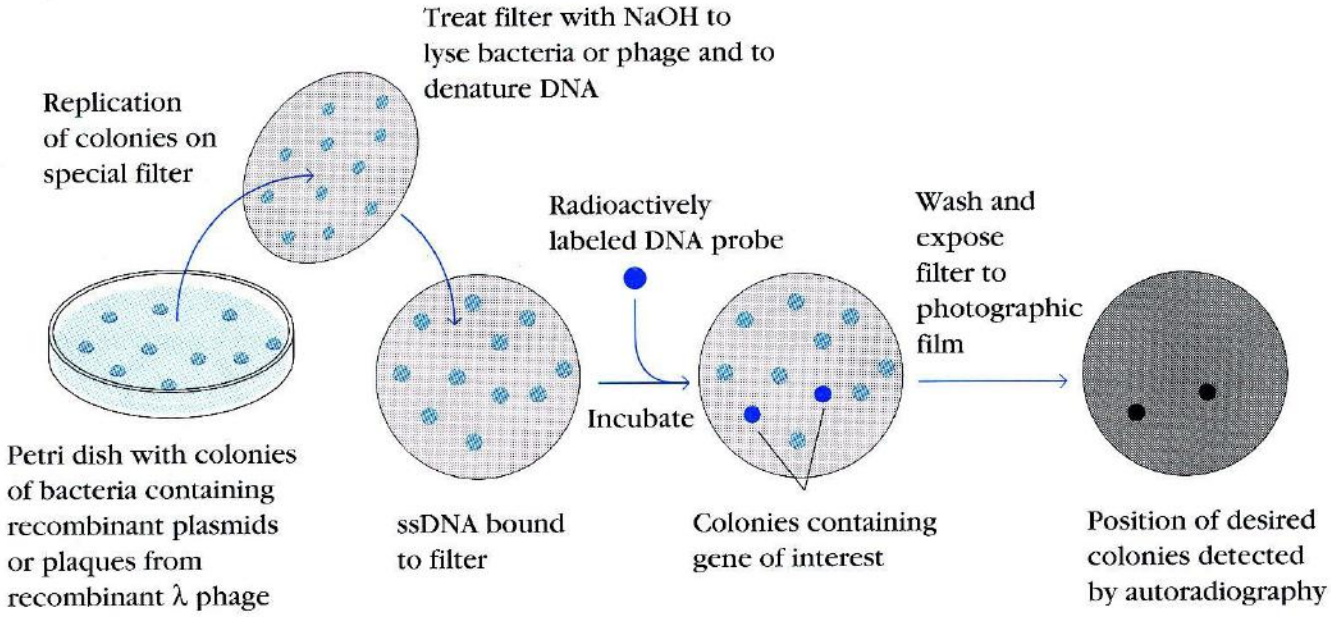


FIGURE 2-5

Selection of specific clones from a cDNA or genomic DNA library by in situ hybridization. A nitrocellulose or nylon filter is placed against the plate to pick up the bacterial colonies or phage plaques containing the cloned genes. After the filter is placed in a NaOH solution and heated, the denatured ssDNA becomes fixed to the filter. A radioactive probe specific for the gene of interest is incubated with the filter. The position of the colonies or plaques containing the desired gene is revealed by autoradiography.

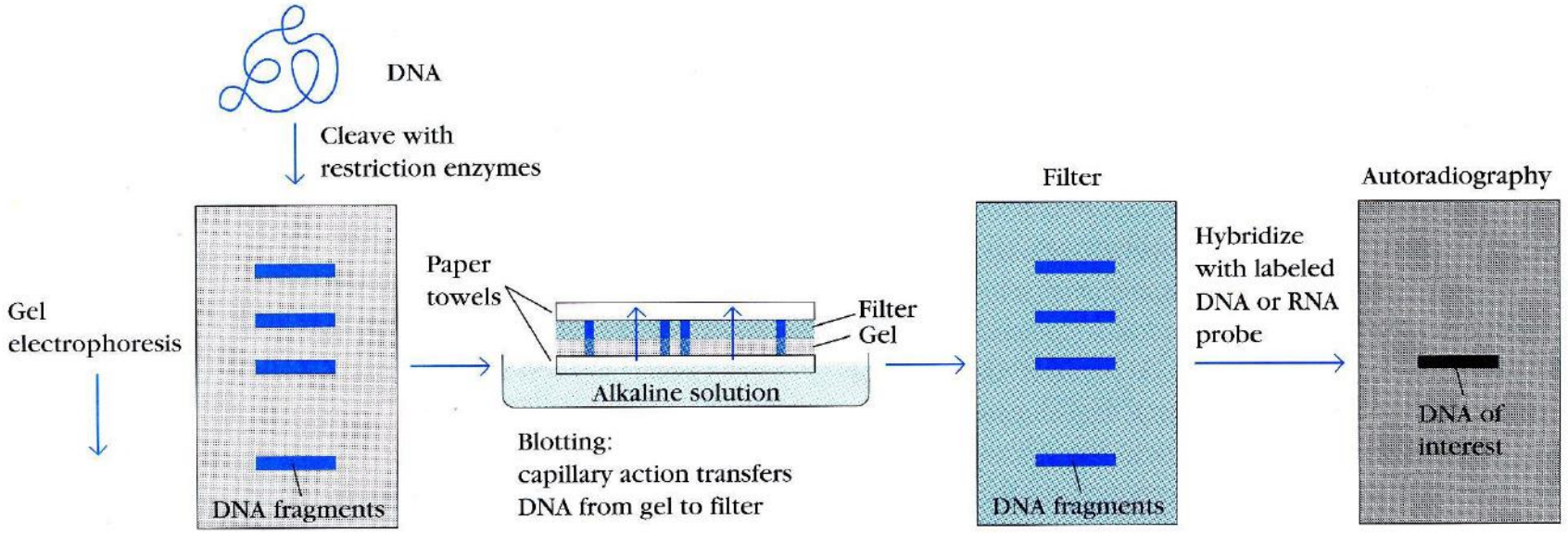


FIGURE 2-6

The Southern blot technique for detecting specific sequences in DNA fragments. The DNA fragments produced by restriction-enzyme cleavage are separated by size by agarose gel electrophoresis. The agarose gel is overlaid with a nitrocellulose or nylon filter and a thick stack of paper towels. The gel is then placed in an alkaline salt solution, which denatures the DNA. As the paper towels soak up the moisture, the solution is drawn through the gel into the filter, transferring each ssDNA band to the filter. This process is called blotting. After heating, the filter is incubated with a radiolabeled probe specific for the sequence of interest; DNA fragments that hybridize with the probe are detected by autoradiography. [Adapted from James Darnell et al., 1990, *Molecular Cell Biology*, 2nd ed., Scientific American Books.]

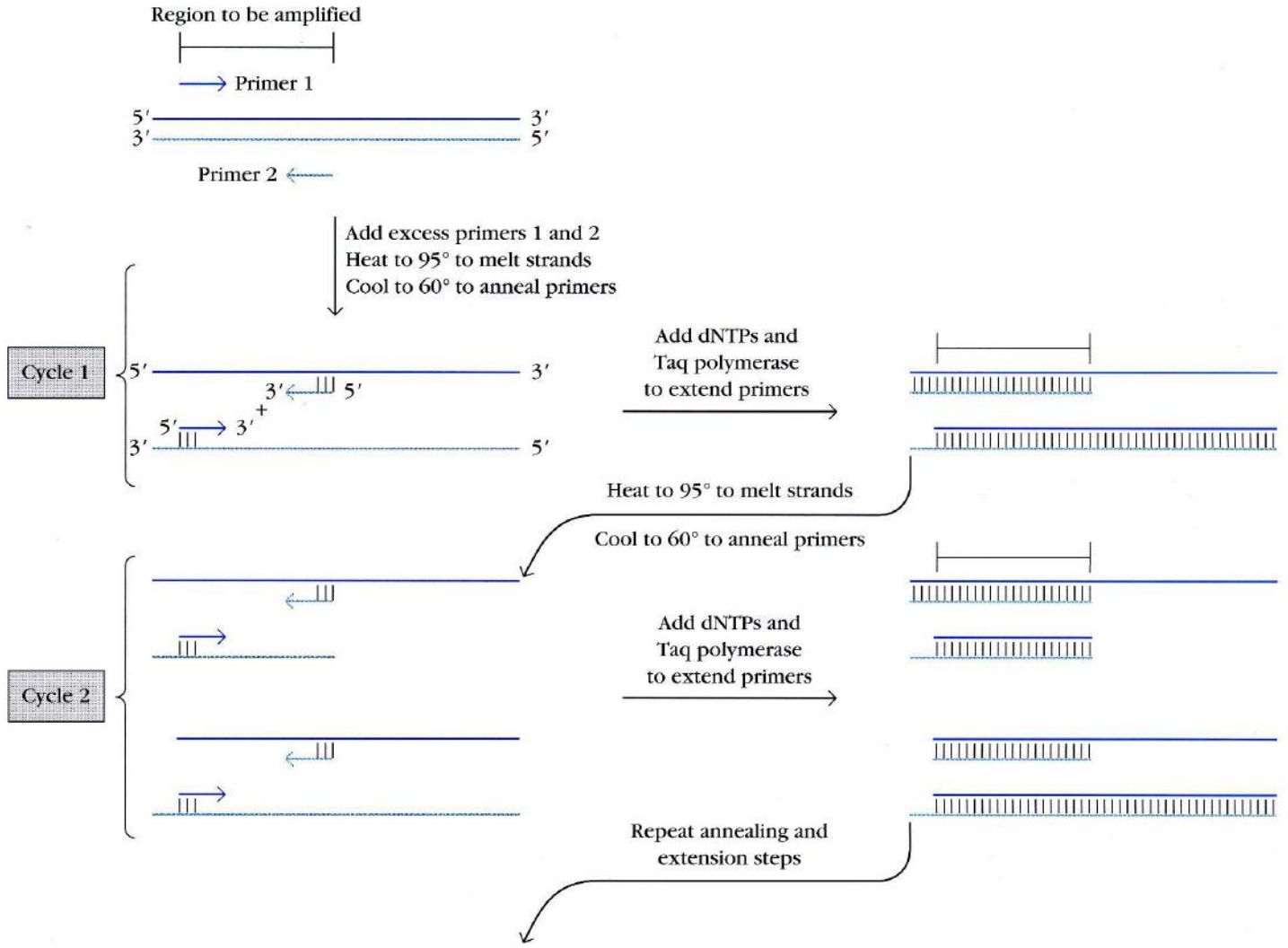


FIGURE 2-7

The polymerase chain reaction (PCR). DNA is denatured into single strands by a brief heat treatment and is then cooled in the presence of an excess of oligonucleotide primers complementary to the DNA sequences flanking the desired DNA segment. Taq polymerase, a heat-resistant DNA polymerase obtained from a thermophilic bacterium, is used to copy the DNA from the 3′ ends of the primers. Because all of the reaction components are heat stable, the heating and cooling cycle can be repeated many times, resulting in alternate DNA melting and synthesis, and rapid amplification of a given sequence. [Adapted from Harvey Lodish et al., 1995, *Molecular Cell Biology*, 3rd ed., Scientific American Books.]

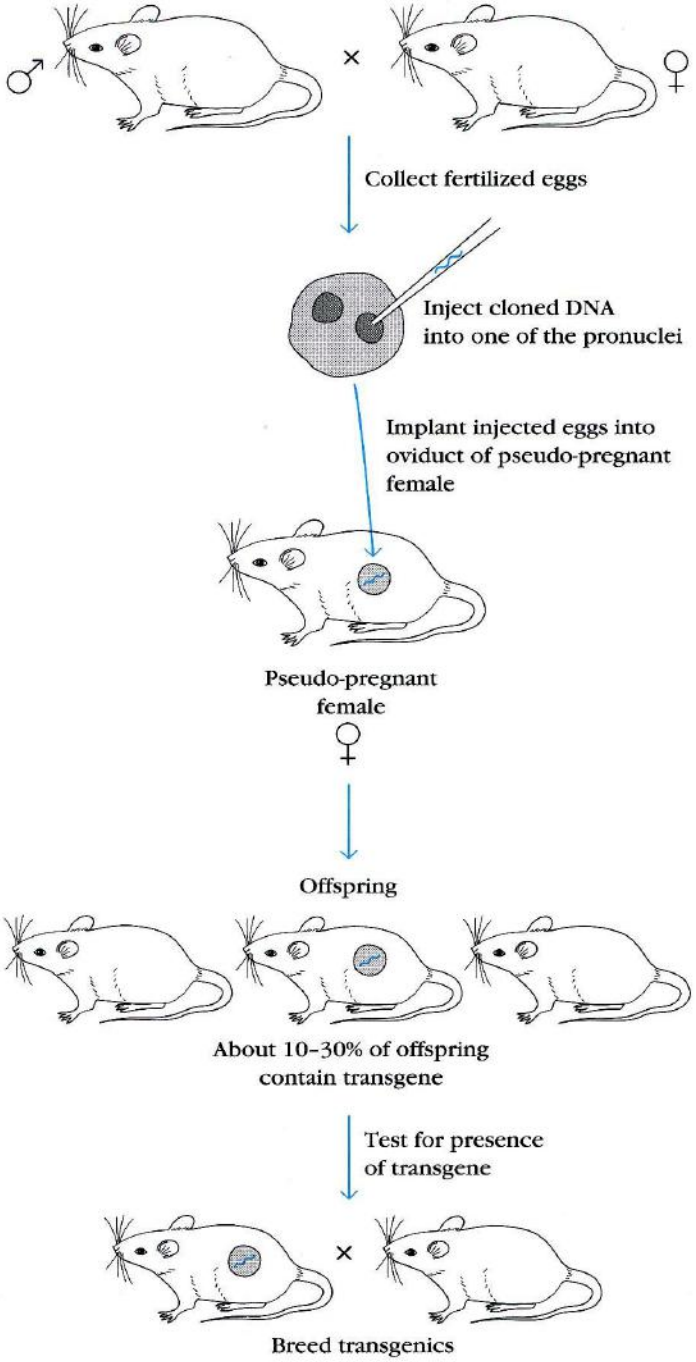


FIGURE 2-11

General procedure for producing transgenic mice. Fertilized eggs are collected from a pregnant female mouse. Cloned DNA (referred to as the transgene) is microinjected into one of the pronuclei of a fertilized egg. The eggs are then implanted into the oviduct of pseudo-pregnant foster mothers (obtained by mating a normal female with a sterile male). The transgene will be incorporated into the chromosomal DNA of about 10%–30% of the offspring and will be expressed in all of their somatic cells. If a tissue-specific promoter is linked to a transgene, then tissue-specific expression of the transgene will result.

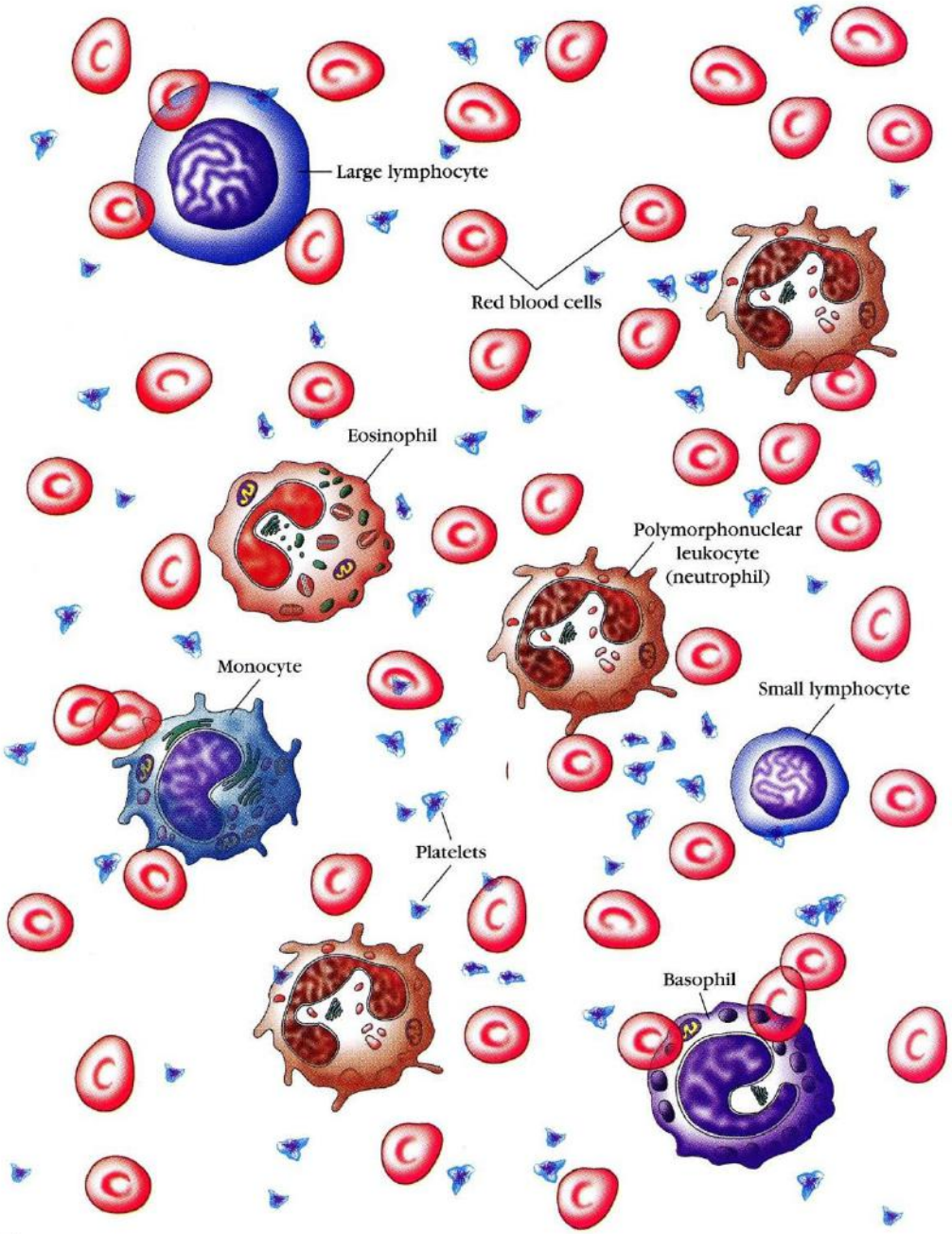


FIGURE 3-1

Morphology and staining characteristics of various types of blood cells. Red blood cells and platelets, which both lack nuclei, are the most numerous. Among the leukocytes responsible for immune responses, the neutrophil is the predominant cell type.

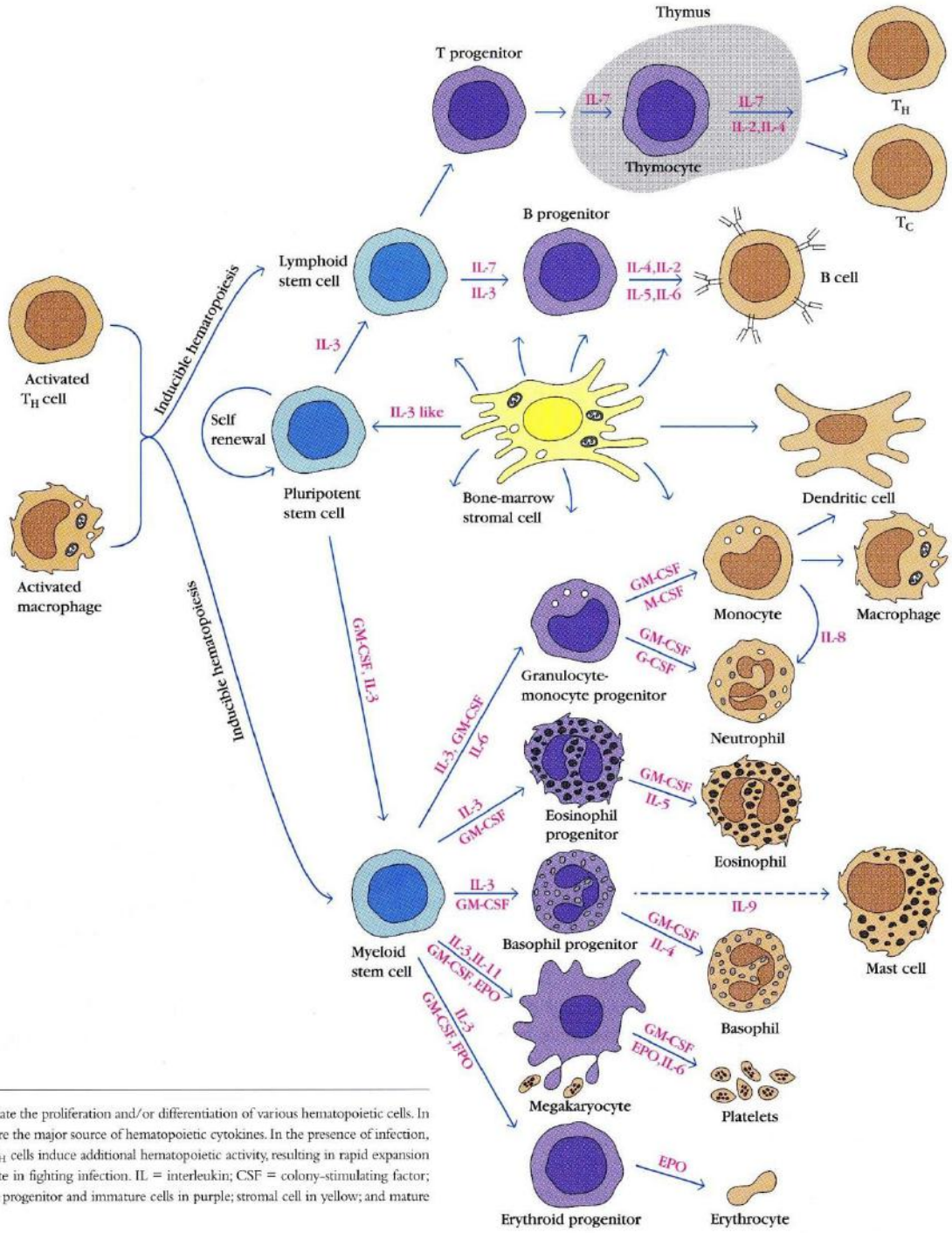


FIGURE 3-2

Regulation of hematopoiesis by cytokines that stimulate the proliferation and/or differentiation of various hematopoietic cells. In the absence of infection, bone-marrow stromal cells are the major source of hematopoietic cytokines. In the presence of infection, cytokines produced by activated macrophages and T_H cells induce additional hematopoietic activity, resulting in rapid expansion of the population of white blood cells that participate in fighting infection. IL = interleukin; CSF = colony-stimulating factor; EPO = erythropoietin. Stem cells are shown in blue; progenitor and immature cells in purple; stromal cell in yellow; and mature differentiated cells in tan.

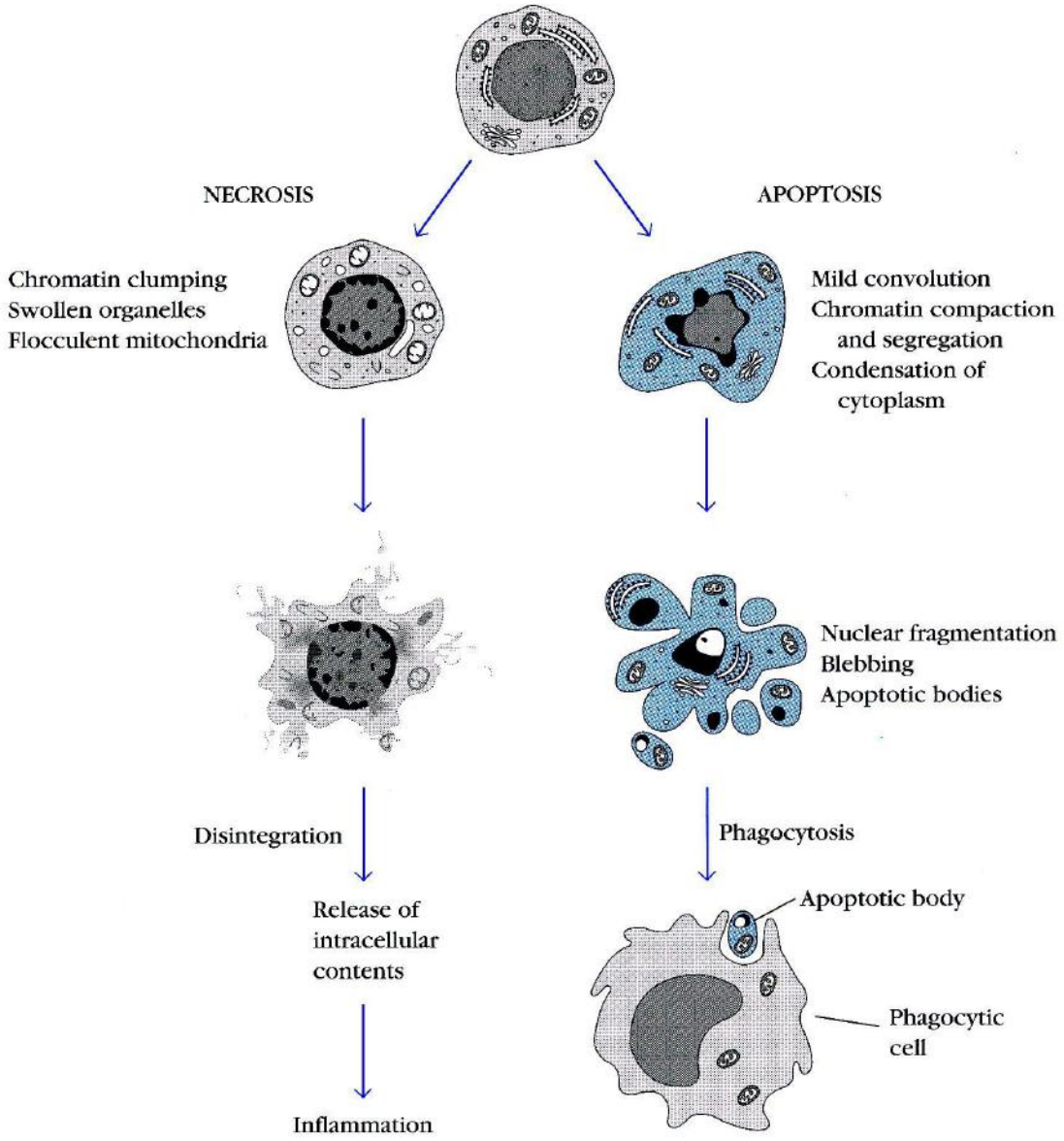
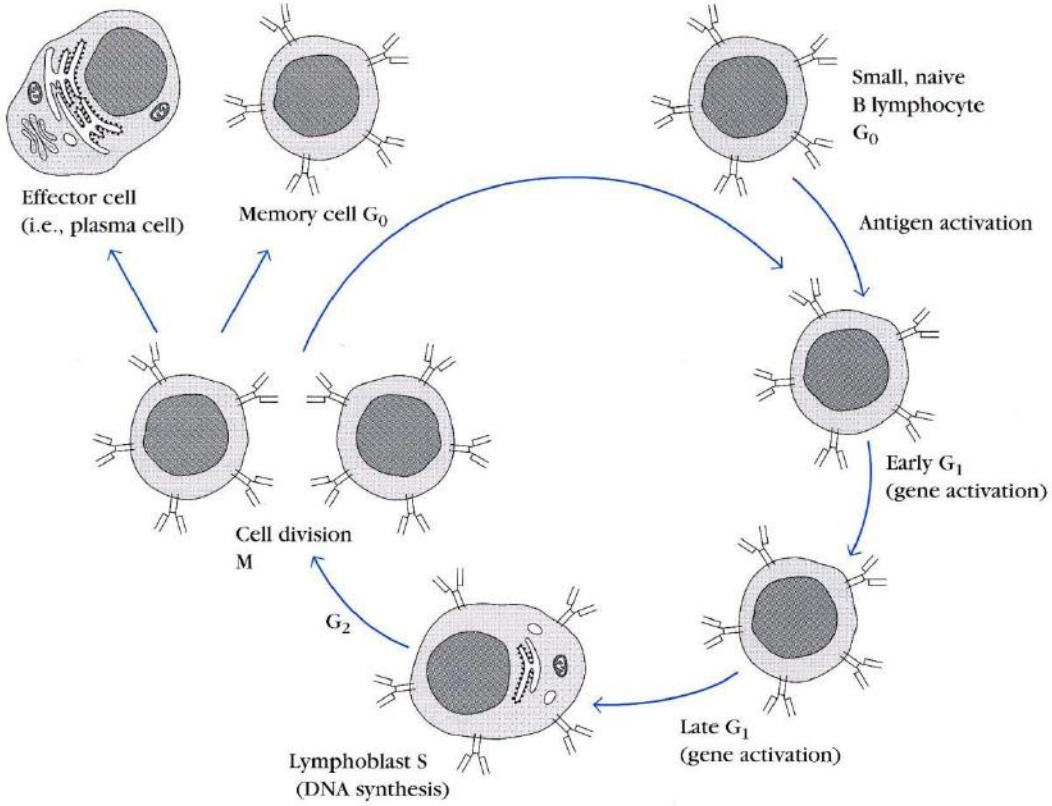


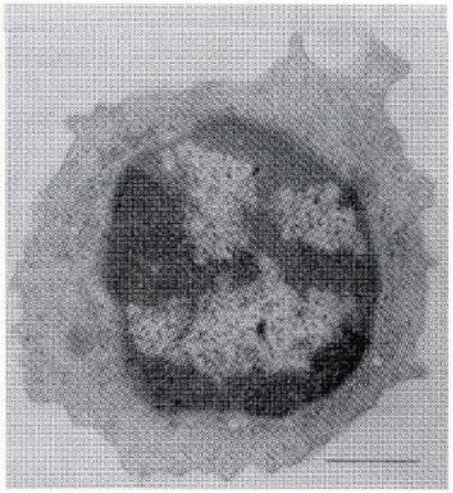
FIGURE 3-4

Comparison of morphologic changes that occur in apoptosis and necrosis. Apoptosis, which is associated with the programmed cell death of hematopoietic cells, does not induce a localized inflammatory response. In contrast, necrosis, the process leading to death of injured cells, results in release of the intracellular contents, which induce a localized inflammatory response.

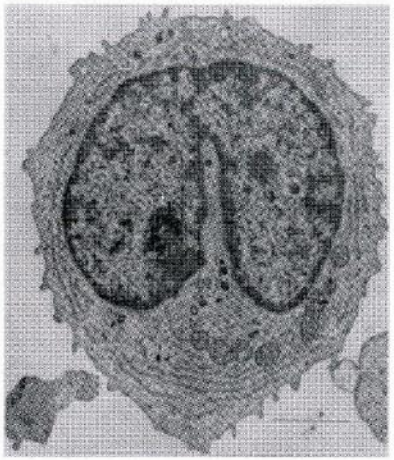
(a)



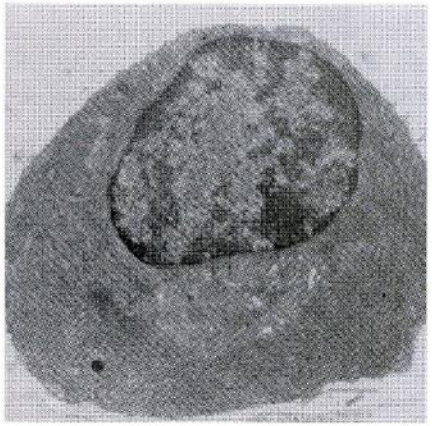
(b)



Small lymphocyte (T or B)
6 µm diameter



Blast cell (T or B)
15 µm diameter

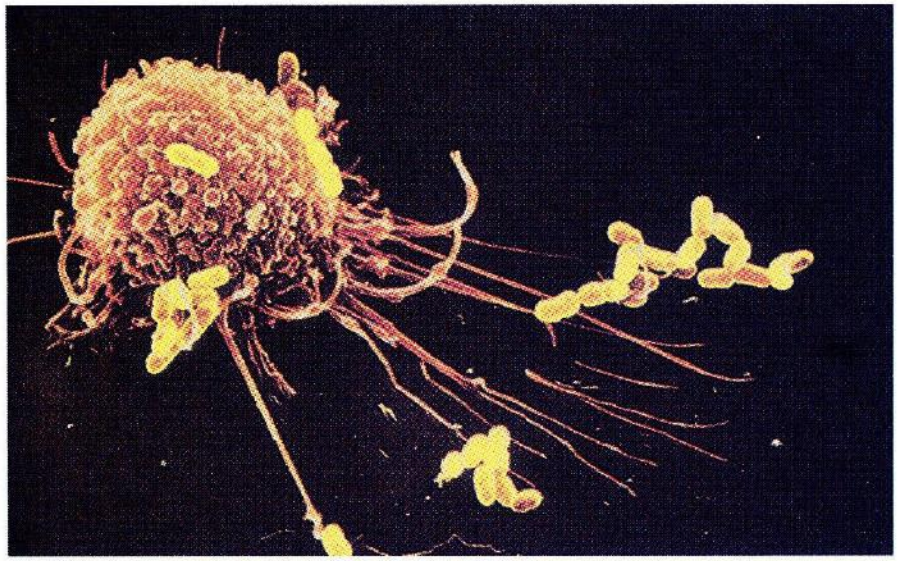


Plasma cell (B)
15 µm diameter

FIGURE 3-10

Fate of antigen-activated small lymphocytes. (a) A small resting (unprimed or naive) lymphocyte resides in the G_0 phase of the cell cycle. At this stage, B and T lymphocytes cannot be distinguished morphologically. Following antigen activation, a B or T cell enters the cell cycle and enlarges into a lymphoblast, which undergoes several rounds of cell division and eventually generates effector cells and memory cells. Shown here are B-cell lineage cells. (b) Electron micrographs of a small lymphocyte (*left*) showing condensed chromatin indicative of a resting cell, an enlarged lymphoblast (*center*) showing decondensed chromatin, and a plasma cell (*right*) showing abundant endoplasmic reticulum arranged in concentric circles. The three cells are shown at different magnifications. [Part (b) courtesy of Dr. Joseph R. Goodman, Dept. of Pediatrics, University of California at San Francisco.]

(a)



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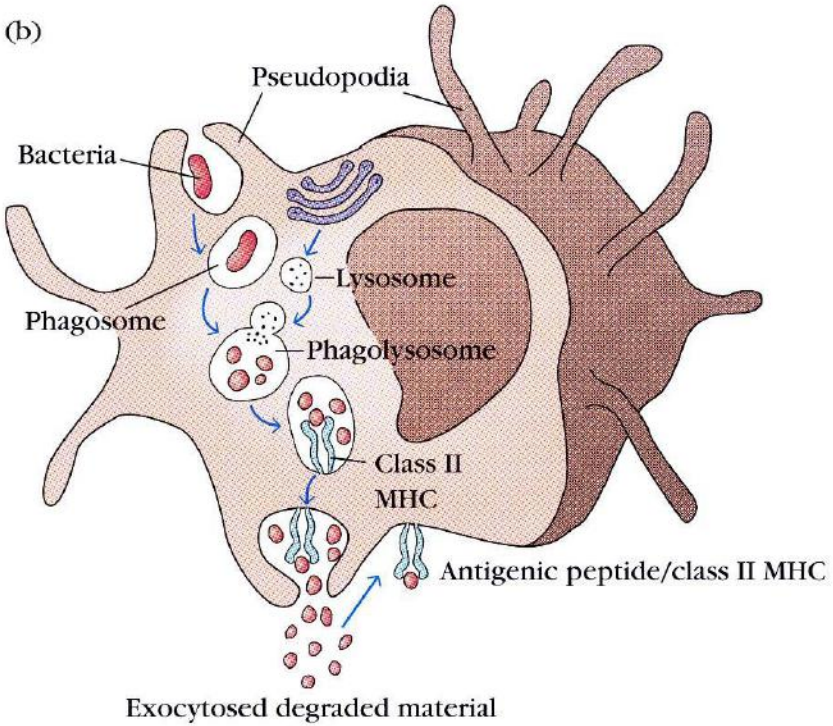
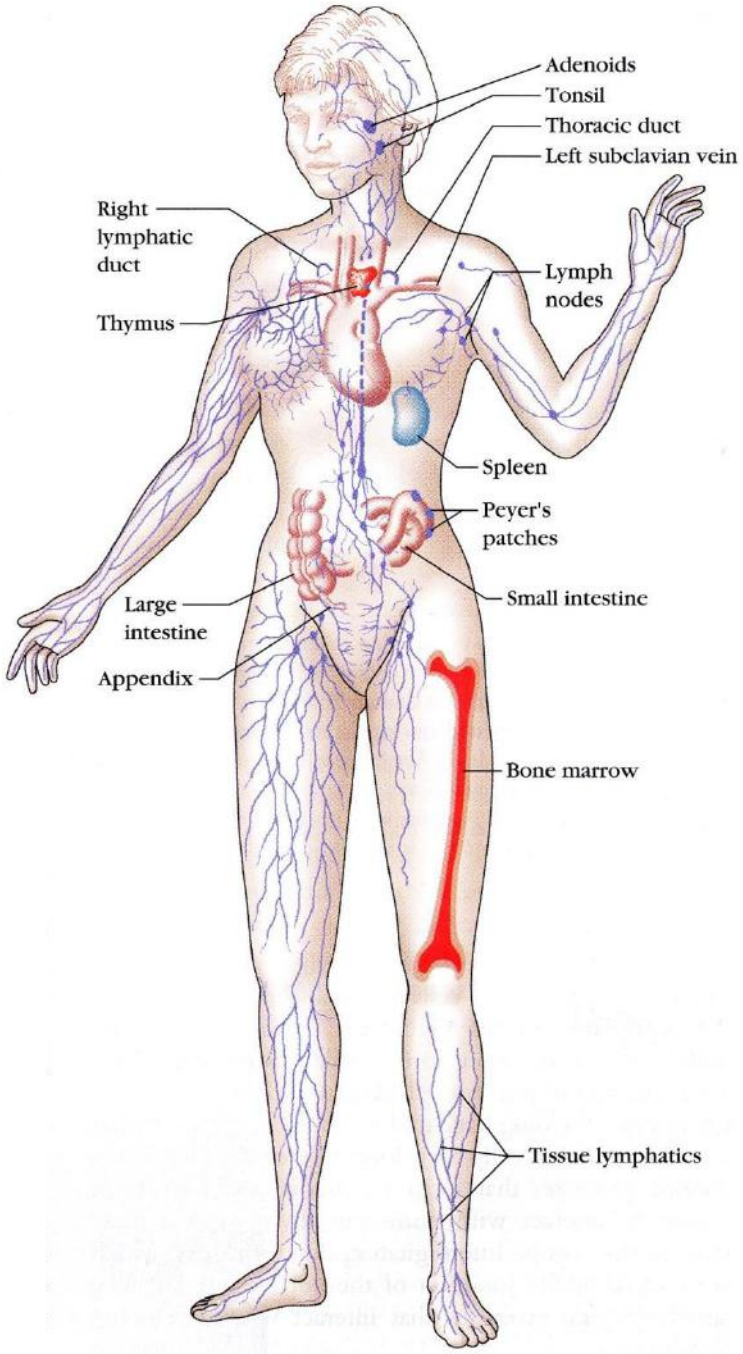


FIGURE 3-12

Macrophages can ingest and degrade particulate antigens, including bacteria. (a) Scanning electron micrograph of a macrophage. Note the long pseudopodia extending toward and making contact with bacterial cells, an early step in phagocytosis. (b) Phagocytosis and processing of exogenous antigen by macrophages. Most of the products resulting from digestion of ingested material are exocytosed, but some peptide products interact with class II MHC molecules, forming complexes that move to the cell surface where they are presented to T_H cells. See text for details. [Photograph by Lennart Nilsson; courtesy of Boehringer Ingelheim International GmbH.]

FIGURE 3-16

The human lymphoid system. The primary organs (bone marrow and thymus) are shown in red; secondary organs and tissues, in blue. These structurally and functionally diverse lymphoid organs and tissues are interconnected by the blood vessels (not shown) and lymphatic vessels (purple) through which lymphocytes circulate. Only one bone is shown, but all major bones contain marrow and thus are part of the lymphoid system. [Adapted from Harvey Lodish et al., 1995, *Molecular Cell Biology,* 3rd ed., Scientific American Books.]



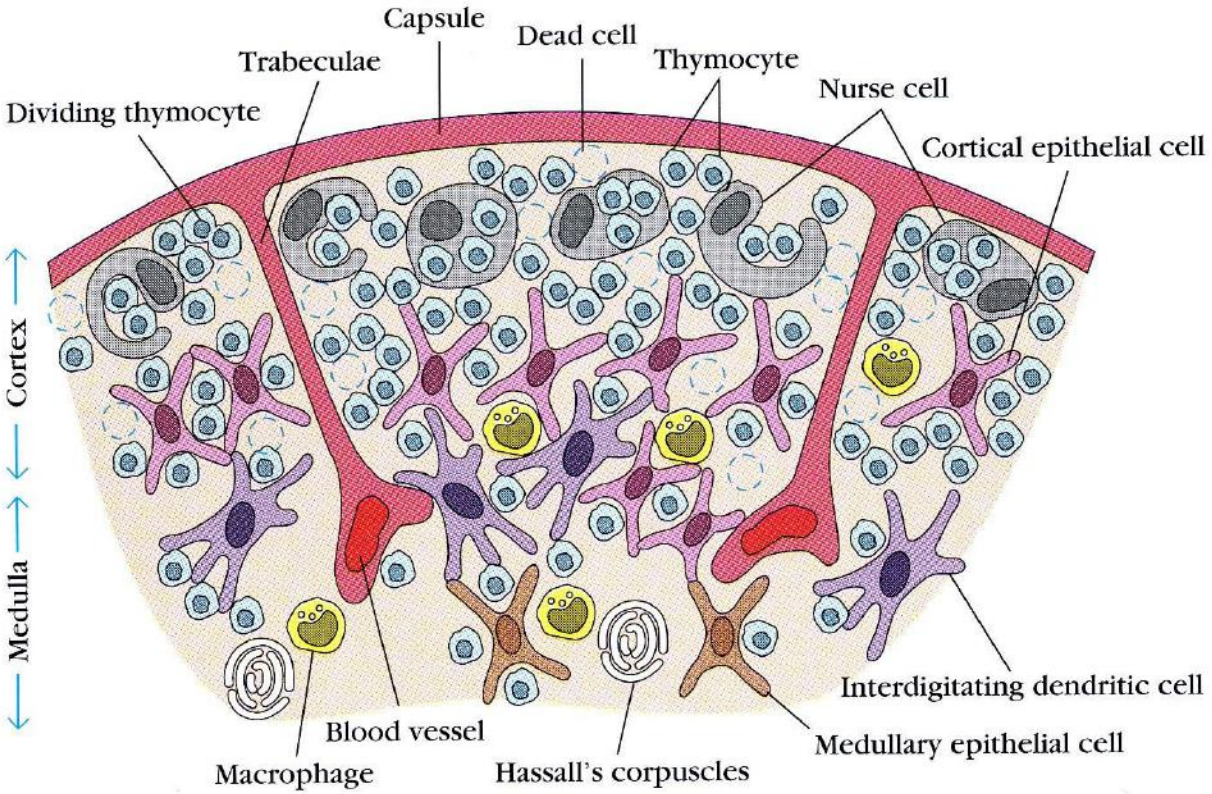


FIGURE 3-17

Diagrammatic cross-section of a portion of the thymus, showing several lobules separated by connective tissue strands (trabeculae). The densely populated outer cortex is thought to contain many immature thymocytes (blue), which undergo rapid proliferation coupled with an enormous rate of cell death. Also present in the outer cortex are thymic nurse cells (gray), which are specialized epithelial cells with long membrane processes that surround up to 50 thymocytes. The medulla is sparsely populated and is thought to contain more mature thymocytes. During their stay within the thymus, thymocytes interact with various stromal cells including cortical epithelial cells (light red), medullary epithelial cells (tan), interdigitating dendritic cells (purple), and macrophages (yellow). These cells produce thymic hormones and express high levels of class I and class II MHC molecules. Hassall's corpuscles found in the medulla contain concentric layers of degenerating epithelial cells. [Adapted from W. van Ewijk, 1991, *Annu. Rev. Immunol.* **9**:591.]

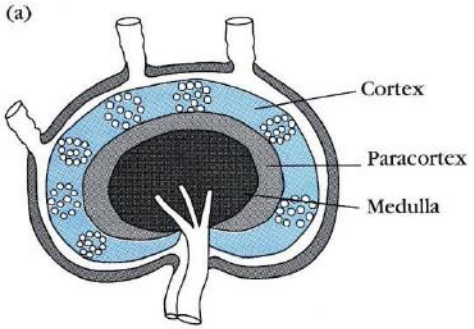
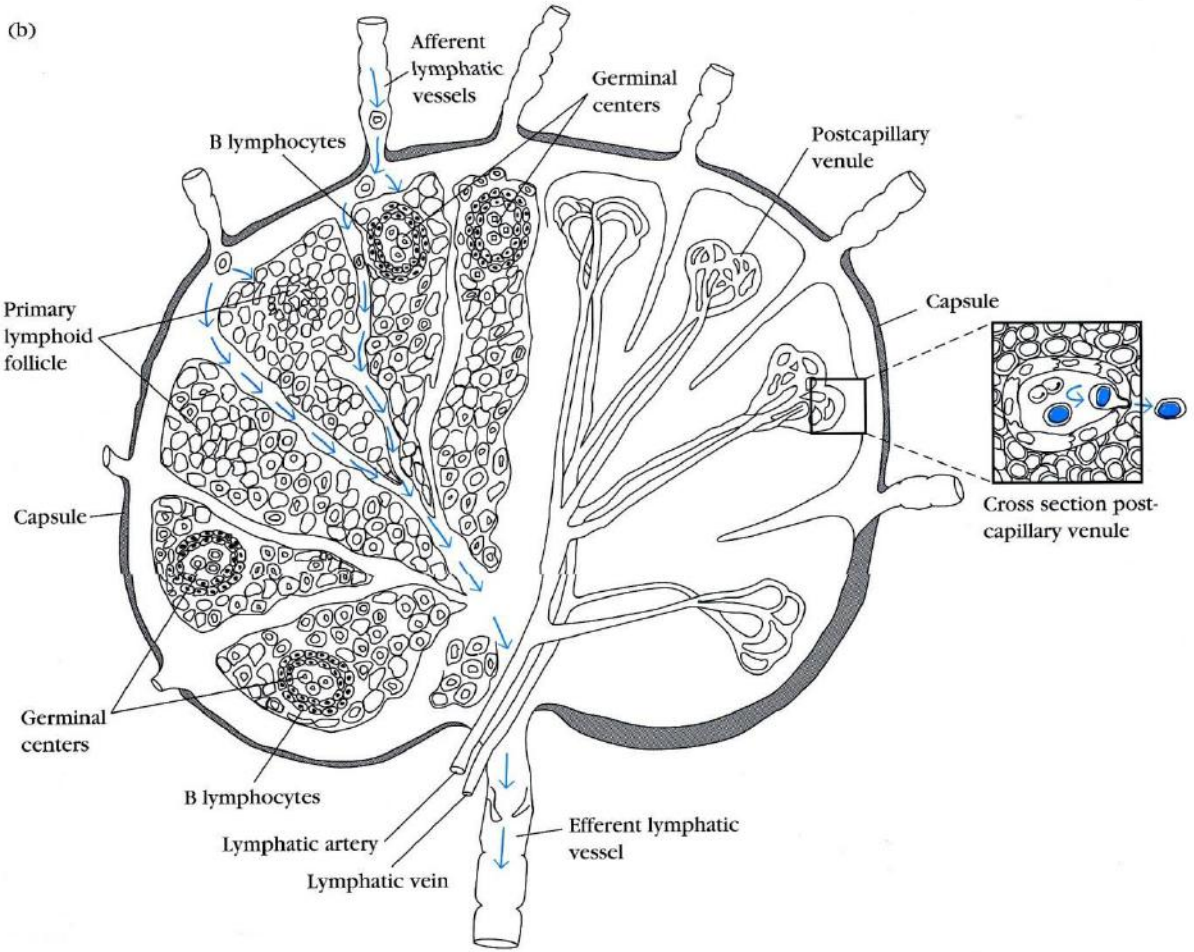


FIGURE 3-21

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



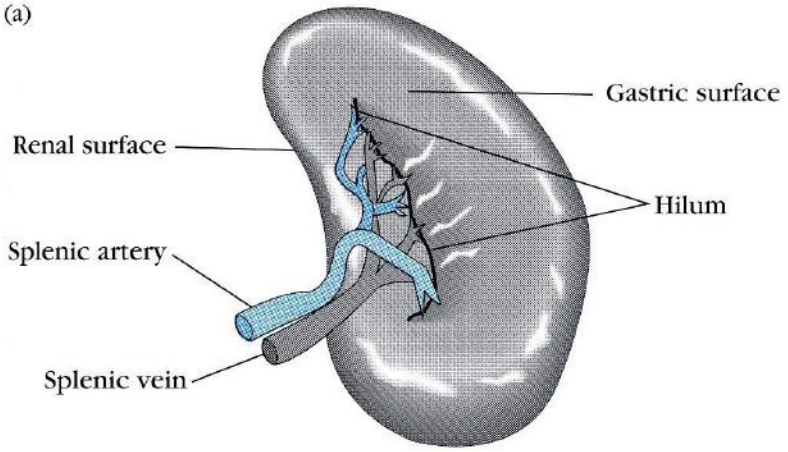


FIGURE 3-22

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