Immunology and Immunopathology

Basic Concepts

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preface

This book represents the authors' views of the essential elements necessary for the understanding of clinical immunology and immunopathology. We have used many diagrams to explain the complex concepts of immunology and immunopathology. In some instances the illustrations may have been oversimplified, and for this we are entirely responsible. Other immunologists and immunopathologists may disagree with our selection of information and references. The authors take full responsibility for any errors or omissions.

This book is intended for students in both medical school and the biological sciences, including medical technology and veterinary medicine. It should also be helpful to medical professionals and others interested in immunology and immunopathology.

We acknowledge with many thanks our appreciation to the individuals who have contributed to make this text possible. They include Drs. Nichols and Kimura for their contributions in Chapters 7, 15, and 17, respectively; Mr. Allen Perreira for his detailed illustrations in Chapters 1, 2, 5, 10, 11, and 12; Mr. Jeffrey Teraoka for the drawings in Chapters 3, 7, 8, and 13, and Mr. Glenn Kimura for the illustrations in Chapters 14 and 17; Drs. Eugene Yanagihara and Hong-Yi Yang for the H&E histological tissues in Chapters 1 and 12, and Drs. Takuji Hayashi and Joiner Cartwright for the electron microscopic photographs in Chapters 1 and 8; Mrs. Haruko Hazama, Mrs. Cleo-Mae Mrozek, and Miss Mary Lam for their meticulous typing; Mr. Bert Weeks, Mr. Jeffrey Teraoka, and Miss Katherine Shiraki for the photographic work; and to Mrs. Haru Hokama for proofreading the drafts of Chapters 1 through 6 and 8 through 13.

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Y. H.
R. M. N.

Contents

1 : The Immune System: Cellular Basis

Humans normally possess numerous complex defense mechanisms to protect themselves against pathogenic microorganisms and harmful foreign substances, such as bacteria, viruses, parasites, and toxins, to which they are constantly exposed. This capacity to withstand the deleterious agents within the environment is *native* or *innate* immunity. For the most part native immunity is genetically determined within a species with respect to a particular agent or *antigen* (Ag). *Antigen* is a term given to substances (see Chapter 4) that stimulate an immune response when administered to a host: substances that are generally foreign in molecular structure to the host, which recognizes them as *non-self.* This inherited capacity may vary within species and between individuals. Specifically *acquired immunity,* on the other hand, is an individual property dependent on a person's previous experience with an antigen, a particular harmful agent, or a toxin.

Native Immunity

Native immunity can be considered under two broad general areas: (1) *active* and (2) *passive* immunity. The active defense mechanisms of the host involve the tissues and structures of the body and those components of them that are in part or wholly functionally directed for active resistance to harmful external or opportunistic endogenous microorganisms and their toxins. The passive defense mechanism, or nonsusceptibility, involves no specific tissues or structures but is, rather, an environmental situation within the host that fortuitously is unsuited for initiation of a disease process by a particular pathogenic microorganism.

Active Defense

EPITHELIAL SURFACES. The outer covering of the body (the skin), together with the mucous membranes, composed of specialized epithelial cells lining the respiratory and gastrointestinal tracts, serve as the first line of defense against infection. These surfaces act primarily as mechanical barriers to invasive and pathogenic microorganisms. In addition, substances such as fatty acids found on the surface of the skin are bactericidal to certain pathogenic microorganisms. An example of this is the bactericidal effect of lactic acid against some species of the genus *Salmonella.* The specialized epithelial cells of the mucous membranes secrete mucus (protein-carbohydrate complexes), enzymes, and other bactericidal factors that contribute to the host's ability to resist

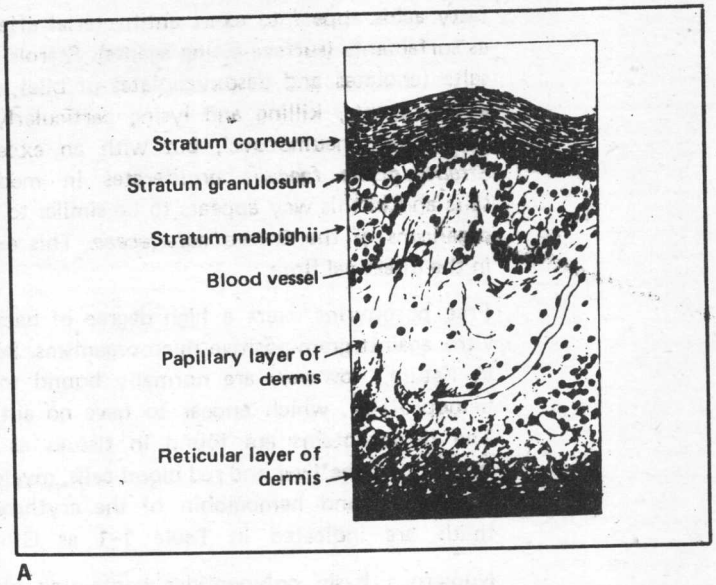
Table 1-1 : Antimicrobial Substances in Tissue and Body Fluids

Molecular Substance	Major Source	Chemical Type	Type of Bacteria Affected
Group I			
Fatty acids	Ubiquitous	Short- and long-chain fatty acids	Gram-negative
Bile salts	Gall bladder, liver, intestines	Steroids	Gram-positive
Heme	Red blood cells, white blood cells	Porphyrins	Gram-positive
Group II			
Polylysine, polyarginine	Thymus, tissues rich in histones	Low molecular weight basic peptides	Mainly gram-positive
Protamine	Sperm	Low molecular weight basic peptide	Gram-positive
Spermine, spermidine	Pancreas, prostate	Basic polyamines	Gram-positive
Group III			
Lysozyme	Ubiquitous	Low molecular weight basic protein (enzyme)	Mainly gram-positive
Phagocytin	Leukocytes	Globulin	Gram-negative
Beta lysin	Serum	Multiple enzymatic system; protein	Gram-positive
Properdin	Serum	Serum proteins (see Chapter 9)	Gram-negative
Opsonin	Serum	Globulins (natural antibodies?)	Gram-negative
Complement	Serum	Complex group of serum proteins consisting of 15 components	Gram-negative

attack by pathogenic microorganisms and harmful agents. Figures 1-1 and 1-8 illustrate those tissues and cells of the skin and mucous membrane that are associated with active defense of the host.

TISSUE AND BODY FLUIDS. When microorganisms enter the host after trauma or injury to the epithelial surfaces, they are immediately confronted by secondary defense substances found in body fluids: some in serum and others in tissue fluids. When isolated from animal tissue and body fluids, these compounds show antimicrobial activity. Characteristics of some of these chemical substances are summarized in Table 1-1. Information as to whether all these materials exert antimicrobial activity in the body itself is as yet incomplete. Nonetheless, since these molecular compounds are found distributed throughout the tissues, their potential function as bactericidal agents cannot be excluded.

The antimicrobial effect, in the laboratory, of short-chain and long-chain fatty acids has long been known. Short-chain fatty acids may act as metabolic inhibitors against microorganisms and in this manner exert their killing effect, whereas long-chain



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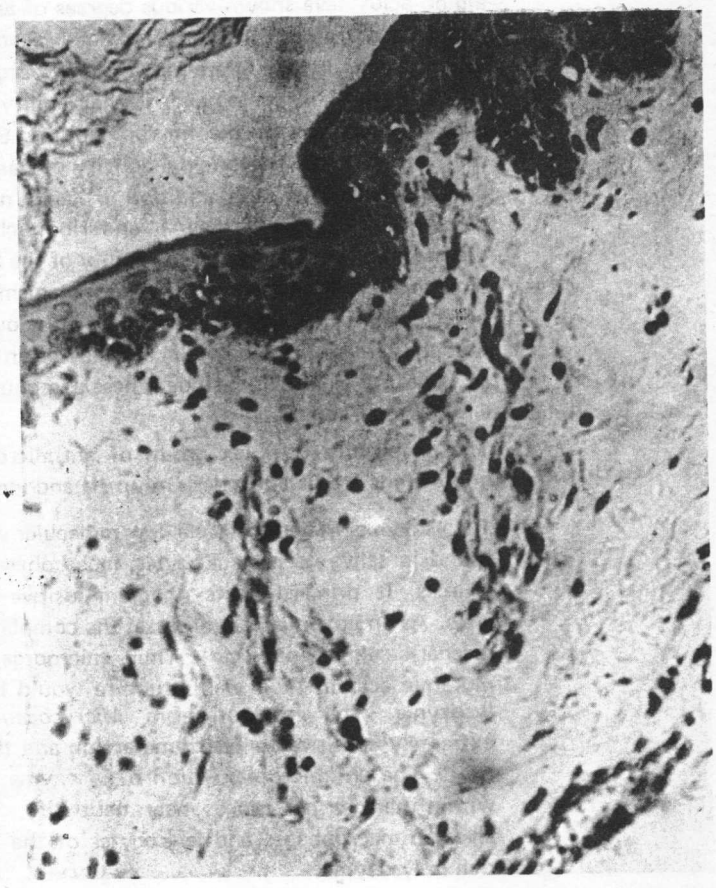

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Fig. 1-1 : A. The human skin, showing the epidermal and dermal layers and the major tissues. B. Histological section (X125) of normal human skin tissue.

fatty acids appear to exert antibacterial effects by their action as surfactants (surface-acting agents). Sterols (lipids), such as bile salts (cholates and desoxycholates of bile), also act as surface-acting agents, killing and lysing particularly the gram-positive capsulated pneumococci, but with an exception: the related *Streptococcus faecalis* proliferates in media containing bile salts and in this way appears to be similar to some gram-negative organisms of the *Enterobacteriaceae.* This explains its presence in the intestinal flora.

Free porphyrins exert a high degree of bactericidal activity in vitro against gram-positive microorganisms. Porphyrins occurring in nature, however, are normally bound to proteins to form hemoproteins, which appear to have no antimicrobial activity. The hemoproteins are found in tissues as enzymes, such as catalases of the liver and red blood cells, myeloperoxidases of the leukocytes, and hemoglobin of the erythrocytes. These body fluids are indicated in Table 1-1 as Group I compounds.

Numerous basic polypeptides containing high levels of basic amino acids have shown various degrees of antimicrobial activity in vitro and are found in a variety of highly specialized tissues. They affect primarily the gram-positive microorganisms. Their bactericidal power seems to reside in their ability to complex or bind with the surfaces of the microorganisms. Studies have shown that, in vitro, the bactericidal activity of basic polypeptides can be neutralized by the addition of acid mucopolysaccharide, deoxyribonucleic acid (DNA), and ribonucleic acid (RNA) to the medium, thus nullifying the ability of the basic compounds to bind to the cell surface of the microorganisms. The fact that the basic polypeptides react with acidic compounds would suggest that the target on the bacterial cell may be an acidic residue, such as a terminal sialic acid residue. These compounds are listed under Group II in Table 1-1.

The chemically complex group of antimicrobial substances of body fluids are listed under Group III and include the following:

1. Lysozyme, a thermostabile, low molecular weight basic protein found in saliva, tears, leukocytes, macrophages, and many other sources. It primarily acts on gram-positive microorganisms in vitro. As an enzyme, it hydrolyzes the complex acetylaminopolysaccharide (muramic acid). Thus, microorganisms that possess muramic acid in their wall structure would be readily lysed by lysozyme. The microorganism *Micrococcus lysodeikticus* is extremely sensitive to lysozyme action and thus is readily lysed by it. The antimicrobial action of lysozyme is chiefly effective within narrow pH ranges near neutrality. The cytoplasm of alveolar macrophages and leukocytes of the blood is especially rich in lysozyme.

2. Phagocytin, a cytoplasmic protein yielded by extraction of leukocytes (white cells) of blood with acid solution. In the test tube, phagocytin appears to have a wide range of antimicrobial action on both gram-negative and gram-positive microorganisms. Its bactericidal effect is greater on gram-negative than on gram-positive microorganisms, however.

3. Beta-lysin, which probably functions as an enzyme.

4. Properdin, a serum protein that acts via the alternative complement pathway. The complement system (see Chapter 9) comprises a complex group of serum proteins, some of which are heat labile. Bactericidal activity has been exhibited against gram-negative bacteria in the presence of the complement proteins and magnesium ions (Mg^{++}). Properdin, complement, and Mg^{++} ions constitute the components in the alternative pathway of C activation (to be discussed in Chapter 9). The system has also been implicated in the inactivation of a number of viruses.

5. Opsonins, proteins that exist in normal fresh serum of animals and that enhance the phagocytosis by leukocytes of bacteria and inert particles. Opsonins are natural antibodies. Opsonin has no specificity and in this respect differs from a specific antibody (Ab), but it does appear to have predilection for gram-negative microorganisms and inert particles such as carbon. Like properdin, opsonin activates complement. A nonimmunoglobulin, α-globulin, has been shown to have opsonic properties also. Opsonin is measured or titrated by determining the number of microorganisms ingested per phagocyte (phagocytic index) in normal fresh serum and then comparing this with the number of microorganisms ingested per phagocyte in fresh serum of a patient. This gives the opsonic index:

$$\text{Opsonic index} = \frac{\text{Patient's phagocytic index}}{\text{Normal phagocytic index}}$$

6. Complement (C) made up of a group of several distinct functionally interrelated protein entities. Complement is found in the blood serum of animals and occurs in a variety of species. It generally decreases or increases in concentration during some disease processes. Differences in complement activity among various species are attributable to variations in proportion of the constituents of the C components. Details of the complement system are presented in Chapter 9.

CELLULAR ELEMENTS IN INNATE IMMUNITY. The phagocytes, or eating cells, of the blood and reticuloendothelial system are one of the most important active defense mechanisms in native resistance to pathogenic microbes. They act by ingesting bacteria or other foreign particles. Metchnikoff recognized this signifi-

cance of leukocytes as an outgrowth of his earlier observation of intracellular digestion by cells of the water flea.

In the animal body there are two main varieties of phagocytic cells, microphages and macrophages. The microphages include the polymorphonuclear leukocytes, which make up more than half the white cells of the blood. Of these the neutrophils exhibit the greatest phagocytic activity and constitute the major cells in the acute inflammatory response to infection; the number and proportion rises rapidly in a case of acute appendicitis, for example. Eosinophils and basophils exhibit less phagocytic activity.

The cells of the reticuloendothelial system are composed of macrophages, either sessile or wandering. *Sessile,* or *fixed,* macrophages are found lining the capillary endothelium and sinuses of the liver (Kupffer's cells), spleen, bone marrow, lymph nodes, and other organs. They phagocytize foreign bodies from the blood as they flow past. The wandering macrophages, also known as *histiocytes,* include the blood monocytes that migrate through the endothelium and tissues and assist in the repair of damaged tissue by ingesting dead tissue materials and inert particles and further aid in the disposal of inactive erythrocytes and leukocytes that have passed through into the injured areas of the tissues. The characteristics and biochemical mode of action of these cells are discussed in Chapter 8.

Passive Defense

A variety of conditions that may be closely synonymous with innate resistance create an indifference or passivity on the part of animal tissue such that the tissue fails to offer a nurturing environment for the ubiquitous pathogens. Some of the more significant conditions include body temperature, tissue metabolites, oxygen tension, and nonsusceptibility to toxins.

It is known that animals of different species have different body temperatures. It is to be expected that animals with different body temperatures will exhibit different inhibition of microbes. An instance of such a phenomenon was shown by Pasteur, who demonstrated that chickens, which have high body temperatures, are normally resistant to *Bacillus anthracis.* They became susceptible when their body temperature was lowered by immersion in cold water. On the other hand, the *Bacillus* is highly infectious for mammals in general. Viruses and bacteria require certain specific basic chemical compounds or metabolites in order to grow. Thus animals with tissues deficient in the necessary metabolites would selectively inhibit growth of microbes requiring such metabolites. High oxygen tension in normal tissues prevents proliferation of anaerobic microbes and in this way maintains passive resistance. Finally, certain species of animals possess tissues that are indifferent to bacterial exotoxins. For example,

frogs are entirely resistant to diphtheria and tetanus toxins. Whereas these toxins are lethal to most mammals.

Acquired Immunity

As already indicated, acquired immunity is an individual property, dependent on the host's previous exposure to or experience with the foreign agent. Nevertheless, the ability to respond immunologically is an inherent property and is genetically endowed within a given species.

Individuals may acquire immunity by either active or passive means. Natural active immunity may be acquired by a previous infection with a pathogenic microbe or by active immunization with bacterial and viral antigens (vaccination). Subsequent to these experiences, the individual develops antibodies specifically for the microorganism and thus acquires an active immune or resistant state to the microbe. This immune state may be of short or long duration, its length generally dictated by the inciting agent. For example, immunity to virus infections such as smallpox and yellow fever viruses may persist for years, whereas resistance developed against pneumococci or typhoid organisms is generally of short duration. The host response is dependent on the nature of the antigen and whether T or B cells or both have been activated (see Chapter 5).

Passive acquired immunity (borrowed immunity) may result from the transfer of human or animal serum that contains antibodies protective against a specific agent to another individual who has no protective antibodies. An example of this is the transfusion of neutralizing antibodies against measles virus to expectant mothers who have been exposed to the viruses in early pregnancy. Passive transfer to individuals of serum containing specific antibodies to certain infectious microbes was common practice prior to antibiotic therapy. It is generally no longer used for infectious diseases, but passive transfer of antivenoms or antitoxins is still employed against snakebites and other toxins. Transfer of immune cells is termed *adoptive immunity*. This type of passive transfer has been used therapeutically in immune deficiency diseases and in cancer patients (Chapters 15 and 16).

In the remainder of this text we shall discuss the details of the systems associated with innate and acquired immunity in man and animals.

Cellular Basis

Present immunology texts based on traditional teaching generally begin with antigens or immunogens. This is understandable since much information has been available regarding the nature of the immunogens. Early concepts of immunology and the study of immunity relied on the premise that the inoculation of a foreign substance (bacterial proteins, etc.) into an animal resulted in the

production and release of various kinds of antibodies into the serum of the host. Understandably, the physical and chemical analyses of these foreign substances, called *antigens* progressed far more rapidly than the analysis of the underlying biological response of the animals to the antigens. Thus, it was to be expected that the teaching of immunology emphasized and began with the antigens.

With the astounding advances due to new developments such as the use of radioisotopes, improved tissue culture techniques, and sophisticated assay procedures (see Chapters 4, 5, and 6), the nature of the responses of animals, including man, especially at the cellular and molecular levels, are better understood today. Therefore our emphasis will be on the host — the response of the host's tissues, cells, organelles, and macromolecules to the antigen (immunity). This section encompasses the description, characteristics, distribution, and ontogeny of the cellular system associated with the host defense or surveillance against deleterious agents, especially those leading to pathological processes. The tissues and cells to be described constitute the members of the two-component lymphoid system, the *T* and *B lymphocytes.*

Central Lymphoid System

Tissues associated with the lymphoid system are characterized as *central* or *peripheral.* Within these lymphoid tissues are distributed the lymphocytes that constitute the thymus-dependent (cell-mediated) and the thymus-independent or bursa-equivalent (humoral) systems. Unlike birds, which have an organ (*bursa of Fabricius*) consisting of B lymphocytes that is situated near the rectal region of the gastrointestinal tract (Figure 1-2), man has no such specific organ. An equivalent organ in mammals has not been found but the B lymphocytes in man are considered as *bursa-equivalent* or *thymus-independent* (Ti). Since the mammalian B lymphocytes perform the same functions as do the bursal lymphocytes of birds, the term *B cells* or *B lymphocytes* is retained. The bone marrow, liver, and the gut-associated lymphoid tissues (GALT), which include the appendix, tonsils, and lymphoid tissues of the intestine, have been implicated as likely candidates for bursa-equivalency in mammals. Thus the central lymphoid system comprises the *thymus* and *bursa of Fabricius* in birds and the *thymus* and *bursa-equivalent* tissues in mammals.

Ontogeny, Immune System

The genesis and development of immunocompetent T and B lymphocytes and their relationship to each other and to various lymphoid tissues of the body are shown in Figure 1-3. As indicated in the figure, T and B lymphocytes originate from the same sources (although not illustrated, both the yolk sac and embryonic liver in addition to bone marrow have been implicated as stem cell sources, especially during embryogenesis).

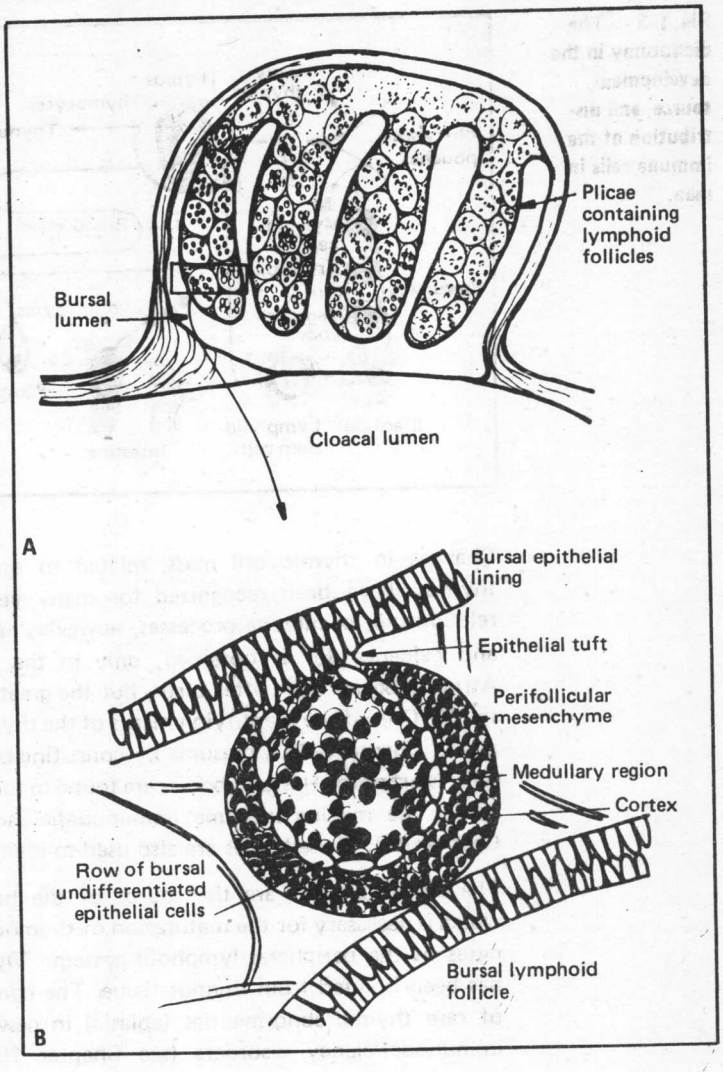


Fig. 1-2 : A. An avian bursa of Fabricius with plicae containing the lymphoid follicles. B. Cellular details of the lymphoid follicle found in the plicae.

THYMUS: CENTRAL LYMPHOID SYSTEM (T CELL). The thymus (Figure 1-4) is formed from the III and IV pharyngeal pouches (Figure 1-3), which contribute the mesenchymal (epithelial) cells, and from the stem cells of the bone marrow, which contribute the prethymocytes (pre-T cells).

Earlier literature on the morphology of the thymus stated that the thymus showed atrophy with age, and the immunological functions associated with the T cells are now known to be related to these morphological changes. The thymus consists of the cortex and medulla with (interspersed) connective tissues. There are no fixed germinal centers or plasma cells.

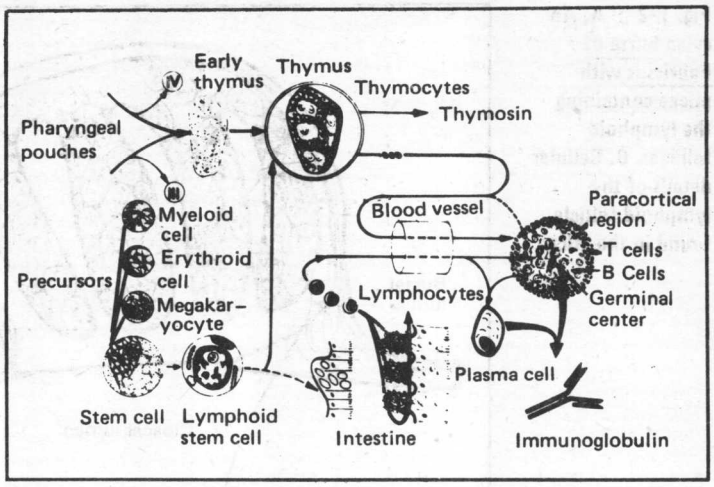
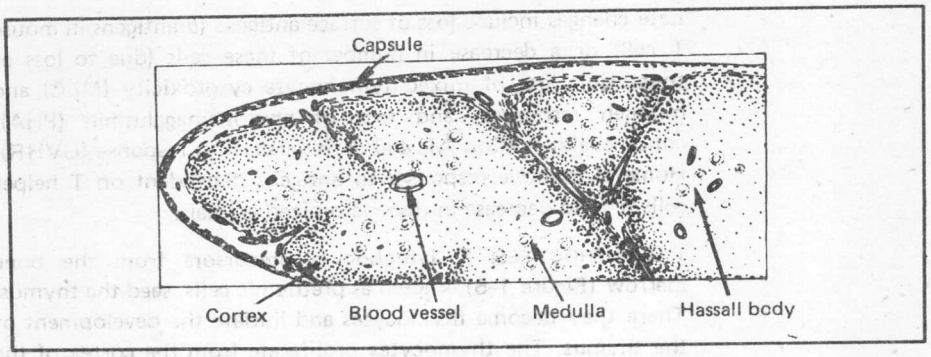


Fig. 1-3 : The dichotomy in the development, source, and distribution of the immune cells in man.

Changes in thymic cell mass, related to age and area of the thymus, have been recognized for many years. The thymus's relationship to immune processes, however, has been recognized, and evidence for it obtained, only in the past two decades. Atrophy begins soon after birth, but the greatest changes start at age 12. One of the identifying marks of the thymus is Hassall's corpuscle (Figure 1-4A), presumably consisting of atrophied thymic cells. Most of the Hassall's bodies are found in the medulla. Hassall's bodies are missing in some immunodeficiency syndromes (see Chapter 15). These bodies are also used to identify thymic tissues.

The epithelial cells are the source of the hormone *thymosin,* which is necessary for the maturation of thymocytes to T lymphocytes of the peripheral lymphoid system. *Thymosin,* a protein, has been isolated from thymus tissue. The congenital occurrence of rare thymic abnormalities (aplasia) in newborns with T cell immunodeficiency disorders (see Chapter 15) has led to the suggestion that the thymus is related to the development of the immune system. Additional evidence from neonatal thymectomy in the mouse has shown the following: (1) a decrease in circulating lymphocytes (lymphopenia); (2) severe impairment of the animals' ability to reject graft; (3) reduced humoral antibody responses to some (those antigens requiring T helper cells, i.e., T-dependent antigens) but not all antigens; (4) wasting disease at 1 to 3 months after thymectomy, probably the result of inability to combat infections effectively, since a germ-free environment or use of antibiotics can minimize or prevent *wasting* disease; and (5) loss of cell-mediated immunity.

The deficit initiated by neonate thymectomy can be restored with transplant of whole thymus, crude extracts of thymus, or with the purified hormone *thymosin.*



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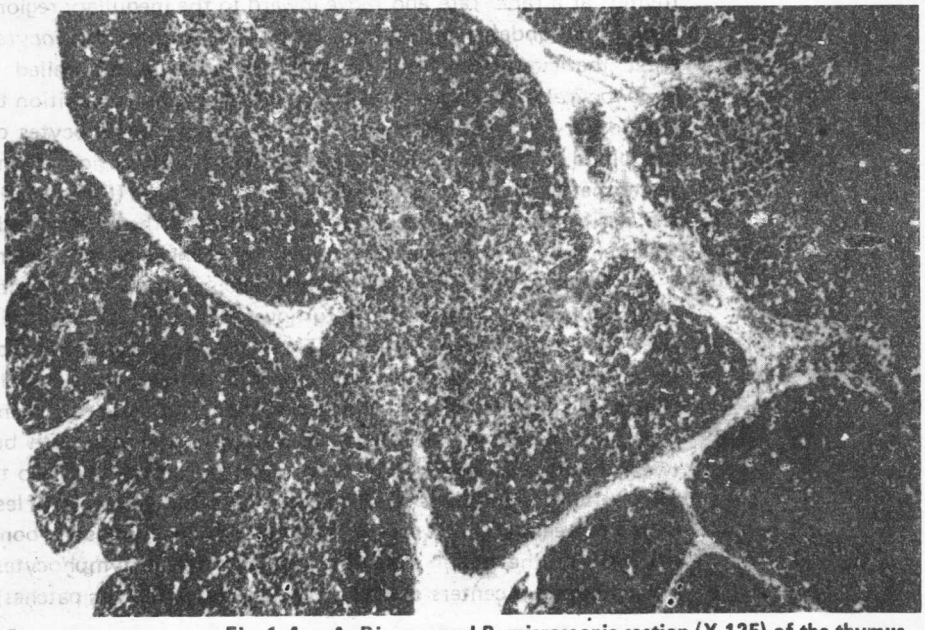

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Fig. 1-4 : A. Diagram and B. microscopic section (X 125) of the thymus, with details of the major areas and cells. (Histological section courtesy of Dr. E. Yanagihara).

Impairment of the immune system can also be demonstrated in the adult thymectomized mouse. In this case impairment is attributed to loss of *thymosin,* which is synthesized by the residual epithelial cells of the hypertrophied adult thymus. The changes observed in the *adult* thymectomized mouse can be divided into two phases, early and late.

Early changes are the loss of E-rosette-forming T cells, due to deletion of or membrane changes in, surgace receptors; loss of RNA synthesis; loss of serum thymosin; and decrease in T suppressor cell. Hence there is a tendency toward formation of autoantibodies and an increase in autoimmune diseases, especially in the older thymectomized animals.

Late changes include loss of surface antigens (θ-antigens in mouse T cell) or a decrease in number of these cells (due to loss of thymosin); loss of mixed lymphocyte cytotoxicity (MLC) and mitogen responses; and loss of phytohemagglutinin (PHA), concanavalin A (Con A), and graft-versus-host response (GVHR). Humoral immune responses to antigens dependent on T helper cells tend to decrease in thymectomized animals.

T LYMPHOCYTES. T lymphocyte precursors from the bone marrow (Figure 1-5), known as prethymic cells, seed the thymus. There they become thymocytes and initiate the development of the thymus. The thymocytes proliferate from the cortex of the thymus at a rapid rate and move inward to the medullary region, where they undergo maturation. When these *mature thymocytes* enter the circulating pool of lymphocytes, they are called *T lymphocytes.* They have a relatively long half-life. In addition to composing 70 to 80 percent of the circulating lymphocytes of the blood, T cells are found in the perivascular region of the white matter of the spleen (Malpighian corpuscles) (Figure 1-6), the perifollicular and deep cortical regions of the lymph node (Figure 1-7), the thoracic duct, and the bone marrow (Figure 1-5). The T lymphocytes are a heterogeneous group of cells with diverse functions. They differ in turnover rates, circulating routes, and surface antigenic markers. There are two populations of small lymphocytes in the blood and the thoracic duct lymph. One population is formed at a slower rate, is long-lived (having a half-life of 100 to 200 days), and moves back and forth between blood and lymph. The other is produced rapidly (two to three mitotic divisions per day) and has a circulating life span of less than two weeks ($T_{½}$ of 3 to 4 days). Although the thymus and bone marrow are the major sites for these short-lived lymphocytes, other lymphoid centers can also produce them (Peyer's patches).

As indicated earlier, an organ equivalent to the bursa of Fabricius of birds has not been found in man. The embryonic liver, bone marrow, spleen, and gut-associated lymphoid tissues have all been implicated. Neither has a hormone equivalent to thymosin been defined or found. The embryonic liver and especially the bone marrow (Figure 1-5A), however, are sources of stem cells. This is true for the bone marrow. In addition to stem cells of lymphoid T and B cells the bone marrow contains precursor cells of the myeloid, megakaryocytic, erythroid, and monocytic series. Whether all these cells originate from a single pluripotential cell (monophyletic theory) or each of these cells originate from individual (polyphyletic theory) remains a controversial question. According to the monophyletic concept, presumably a single undifferentiated and uncommitted bone marrow stem cell may become committed through the poietins. For example, stem cell conversion to the erythroid series is via erythropoietin. The