IMMUNO TECHNOLOGY

LECTURE 01: OUTLINES OF IMMUNOLOGY

History of Immunology

Human fight against smallpox represents the first ever breakthrough in immunology. All of you know, it is the attempt of Edward Jenner, which laid the foundation for the process of immunization against this dreaded disease. In 1796 Jenner, collected pus from cowpox sores on the hands of milkmaid Sarah Nelmes and inoculated eight-year-old James Phipps. Phipps developed a fever but nothing more. Then Jenner inoculated Phipps with pus from active smallpox. The boy developed no reaction to the smallpox inoculation. Jenner conducted several similar successful experiments but met with resistance when trying to publish his work. Eventually Jenner had to publish his experimental results at his own expense. At first derided and laughed at, with the suggestion that cowpox inoculation would make people grow horns, a few enlightened physicians took up the idea. Their confirmation of Jenner's observations gradually led to acceptance of "vaccination". Cowpox is the result of the "vacca" virus. Hence the term "vaccination" (Vacca is Latin for cow).

Louis Pasteur was a scientist interested in fermentation of beer and wine and meat decay, which at the time was also regarded as fermentation. He was the first to isolate microorganisms from ferments. He was able to purify them and then introduce the microbes to fresh material to transfer the fermentation process. He also demonstrated that this transfer could be stopped by heating (pasteurization). He later became involved in examining silkworm blight that was seriously affecting France's silk industry in the 1850s. He was able to transfer his experiences in fermentation and demonstrate the presence of a microorganism in affected worms. He could show that transfer of the microbe from affected to unaffected worms transferred the condition. Amazingly from our point of view, this was still not recognised as being a possible mechanism of disease transfer in humans. Pasteur started studying anthrax in domestic animals, a significant cause of death for domesticated animals at the time.

By 1840 scientists were already aware of rod-shaped microbes in the blood of anthrax infected animals and Pasteur was able to recognise the similarities between these microbes and the ones he had seen at work in fermentation and decay processes. Once again Pasteur isolated the microbe and showed that injection into unaffected animals transferred the disease. By 1878 Pasteur had switched to examining chicken cholera. Chicken cholera is not the equivalent of human cholera but the general attributes were the same. It was a devastating disease for the poultry industry. Pasteur from his previous experience set to work isolating the microbe and demonstrating its presence by culturing the causative agent (what we now call *Pasteurella multocida*, a bacterium), and transferring it between affected and unaffected animals. However, he accidentally took this work a step further. Pasteur attempted in one experiment to transfer the microbe to unaffected animals as he had done before. But the culture he used was old and, unknown to him; the culture was what we describe as attenuated, weakened and limited in its infective capability. The chickens got sick but later recovered.

Realizing his mistake in using an attenuated culture he later reinjected the chickens using a fresh culture. However, the chickens did not die as he expected. He recognised that the old attenuated culture was a form of vaccine against chicken cholera.

Koch-Pasteur Germ Theorem

By this time Pasteur had a challenger to his crown in the shape of German scientist Robert Koch. Koch had been the first to isolate the anthrax microbe although it was Pasteur who demonstrated its ability to transfer disease. Koch, unaware of Pasteur's work, also demonstrated the ability of the anthrax microbe to transfer the disease. The competition between Koch and Pasteur became bitter and acrimonious. While Pasteur worked from the applied side of microbial science, Koch was the key theorizer, advancing much of the germ theory based on analysis of his and Pasteur's work. In 1881, following on from his experiments on chicken cholera, Pasteur produced an attenuated form of anthrax to use as a vaccine and added fuel to the fire. Pasteur went on to produce attenuated vaccines for swine erysipelas and rabies. Despite the fast and furious pace of development of a germ theory of disease by Koch and Pasteur, many were still reluctant to accept it applied to humans. Koch shook the medical world (and trumped Pasteur) by being the first to isolate the microbe that caused the human disease of tuberculosis in 1882. Now there could be no objection to the germ theory as applied to humans. Koch outlined the parameters required for identification of an etiologic agent, called "Koch's postulate", these requirements still stand when identifying infective organisms. Thus the field of immunology, and much of the basis for modern medicine was born from the work of just two people in the 1880s.

Most historians define the turning point as the publication of Pasteur's work on an attenuated chicken cholera vaccine (Pasteur L. De l'attenuation du virus du cholera des poules. C R Acad Sci (Paris) 1880: 101; 673-680). Smallpox may have been the spur towards a more analytical form of science and development of the understanding that infectious diseases could be manipulated and controlled, but the real driving force behind development of the immunological field were observations made on disease in animals. In 1888 Emile Roux and Alexandre Yersin isolated a soluble toxin from cultures of diphtheria. The bacterium itself is only found in the throat but its destructive effects are found throughout the body. Clearly to us the bacteria must be sending out an invisible factor, most likely chemical in nature, to cause the body wide destruction. This idea was the hypothesis of Roux and Yersin. They filtered diphtheria cultures to remove the bacteria and then used the remaining fluid filtrate (we call supernatant) to inject into healthy animals. As expected the animals showed diphtheria lesions but without any obvious presence of bacteria. Next on the podium were Emil von Behring and Shibasaburo Kitasato who took serum from animals infected with diphtheria and injected it into healthy animals. When these animals were later inoculated with diphtheria they were found to be resistant to infection. We now know this method of conferring infection resistance as "passive immunity". This first demonstration of defense against infection was revealed and described as mediated by "antitoxin". It was clear to Behring and Kitasato that the antitoxin was specific only for diphtheria; it did not confer any defense against other forms of infection. We now know this antitoxin to be antibodies

produced specifically against the diphtheria microbe. Rudolf Kraus in 1897 first visualized the reaction of antitoxins to bacteria by simply adding serum from infected animals to a culture of the bacteria and seeing a cloudy precipitate develop as the antibodies bound together the bacteria.Other scientists took different approaches and revealed serum based responses towards bacteria and their products. Initially these serum properties were given a range of different names such as precipitins, bacteriolysins, and agglutinins. Immunological research would have to wait until 1930 before these subtly different properties were unified and recognised as a single entity. Long before antibodies were actually isolated and identified in serum, Paul Erlich had put forward his hypothesis for the formation of antibodies. The words antigen and antibody (intentionally loose umbrella terms) were first used in 1900. It was clear to Erlich and others that a specific antigen elicited production of a specific antibody that apparently did not react to other antigens. The idea that there may be some unseen agent that spread through the human population and caused disease was not new. Several hypotheses had been put forward with this core idea. They were not accepted due again in part because of social immaturity. People were still very fatalistic about contracting disease. They had not developed the understanding that the factor that spread disease could be isolated and identified. The medical establishment was still embryonic. There was no research that might provide some basic evidence to support the hypotheses and identify a pathogenic organism. Several scientific advances had to be made before the idea of pathogens could be widely accepted. What was required was the understanding of germs. There was some evidence provided in the shape of Anton van Leeuwenhoeck's development of the microscope. With the microscope he was able to describe organisms not visible to the naked eye, but this still did not trigger the idea that similar organisms could be the cause of infectious disease.

Discovery of Major Histocompatibility Complexes (MHCs)

This is a major event in the history of immunology. Peter Gorer was working on blood group antigens of mice in 1930s. He identified 4 groups of genes encoding blood cell antigens A, B, A B and O, which he named as I, II, III, & IV respectively. During 1950s Gorer & George Snell II discovered that antigen encoded by gene II took part in the rejection of transplanted tumors (as skin grafts) among laboratory-bred mice. Snell called this gene as "Histocompatibility gene". He was awarded Nobel Prize in 1980 for this work. Raising congenic mice in the laboratory carried out further studies on these genes with reference to their inheritance and functional significance. In his experiment, selection of skin graft rejection is used to derive mice congenic for MHC. First you have to understand the term "congenic" in order to have a better perception of the experiment and its inferences. The term is basically used with reference to different strains of the same species. Two strains are said to be congenic if they are genetically identical except at a single genetic locus or region. Therefore, any phenotypic differences that can be detected between congenic strains are related and the genetic locus that distinguishes the strains. Using the following experiment, congenic strains of mice that are identical with each other except at the MHC locus were created.

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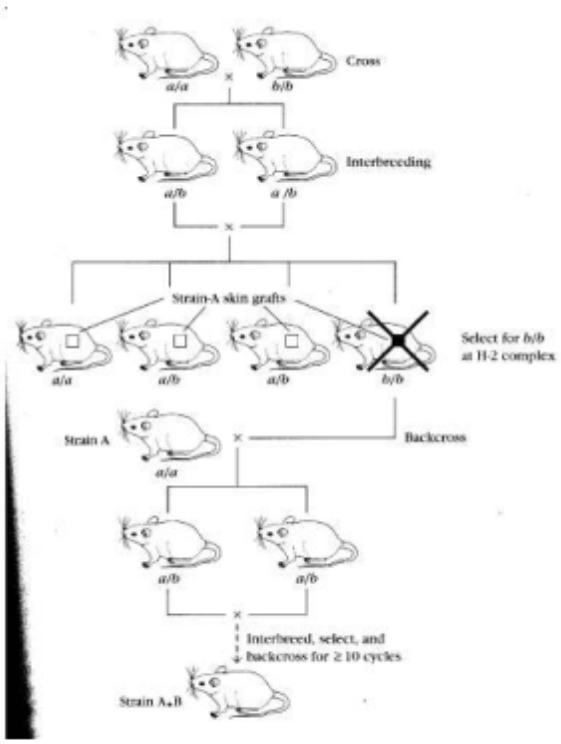

Fig.1. Production of congenic mouse strain A.B, which has the genetic background of parental strain A but the H-2 complex of strain B. Crossing inbred strain A (H-2a) with strain B (H-2b) generates F1 progeny that are heterozygous (a/*b)* at all H-2 loci. The F1 progeny are interbred to produce an F2 generation, which includes *a*/*a, a*/*b,* and *b*/*b* individuals. The F2 progeny homozygous for the B-strain H-2 Complex are selected by their ability to reject a skin graft from strain A; any progeny that accept an Astrain graft are eliminated from future breeding. The selected *b*/*b* homozygous mice are then backcrossed to strain A; the resulting progeny are again interbred and their offspring are again selected for *bib* homozygosity at the H-2 complex. This process of backcrossing to strain A, intercrossing, and selection for ability to reject an A-strain graft is repeated for at least 12 generations. In this way A-strain homozygosity is restored at all loci except the H-2 locus, which is homozygous for the B strain. The above experiment confirmed regulatory role of MHC locus on skin graft rejection in mice.

Milestones in the history of immunology

1798 Edward Jenner, Smallpox vaccination

1862 Ernst Haeckel, Recognition of phagocytosis

1877 Paul Erlich, recognition of mast cells

1879 Louis Pasteur, Attenuated chicken cholera vaccine development

1883 Elie Metchnikoff Cellular theory of vaccination

1885 Louis Pasteur, Rabies vaccination development

1888 Pierre Roux & Alexandre Yersin, Bacterial toxins

1888 George Nuttall, Bactericidal action of blood 1891 Robert Koch, Delayed type hypersensitivity

1894 Richard Pfeiffer, Bacteriolysis

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1895 Jules Bordet, Complement and antibody activity in bacteriolysis
1900 Paul Erlich, Antibody formation theory
1901 Karl Landsteiner, A, B and O blood groupings
1901-8 Carl Jensen & Leo Loeb, Transplantable tumors
1902 Paul Portier & Charles Richet, Anaphylaxis
1903 Almroth Wright & Stewart Douglas, Opsonization reactions
1906 Clemens von Pirquet, coined the word allergy
1907 Svante Arrhenius, coined the term immunochemistry
1910 Emil von Dungern, & Ludwik Hirszfeld, Inheritance of ABO blood groups
1910 Peyton Rous, Viral immunology theory
1914 Clarence Little, Genetics theory of tumor transplantation
1915-20 Leonell Strong & Clarence Little, Inbred mouse strains
1917 Karl Landsteiner, Haptens
1921 Carl Prausnitz & Heinz Kustner, Cutaneous reactions
1924 L Aschoff, Reticuloendothelial system
1926 Lloyd Felton & GH Bailey, Isolation of pure antibody preparation
1934-8 John Marrack, Antigen-antibody binding hypothesis
1936 Peter Gorer, Identification of the H-2 antigen in mice
1940 Karl Lansteiner & Alexander Weiner, Identification of the Rh antigens
1941 Albert Coons, Immunofluorescence technique
1942 Jules Freund & Katherine McDermott, Adjuvants
1942 Karl Landsteiner & Merill Chase, Cellular transfer of sensitivity in guinea pigs (anaphylaxis)
1944 Peter Medwar, Immunological hypothesis of all graft rejection
1948 Astrid Fagraeus, Demonstration of antibody production in plasma B cells
1948 George Snell, Congenic mouse lines
1949 Macfarlane Burnet & Frank Fenner, Immunological tolerance hypothesis
1950 Richard Gershon and K Kondo, Discovery of suppressor T cells
1952 Ogden and Bruton, discovery of agammagobulinemia (antibody immunodeficiency)
1953 Morton Simonsen and WJ Dempster, Graft-versus-host reaction
1953 James Riley & Geoffrey West, Discovery of histamine in mast cells
1953 Rupert Billingham, Leslie Brent, Peter Medwar, & Milan Hasek, Immunological tolerance hypothesis
1955-1959 Niels Jerne, David Talmage, Macfarlane Burnet, Clonal selection theory
1957 Ernest Witebsky et al., Induction of autoimmunity in animals
1957 Alick Isaacs & Jean Lindemann, Discovery of interferon (cytokine)
1958-62 Jean Dausset et al., Human leukocyte antigens
1959-62 Rodney Porter et al., Discovery of antibody structure
1959 James Gowans, Lymphocyte circulation
1961-62 Jaques Miller et al., Discovery of thymus involvement in cellular immunity
1961-62 Noel Warner et al., Distinction of cellular and humoral immune responses
1963 Jaques Oudin et al., antibody idiotypes
1964-8 Anthony Davis et al., T and B cell cooperation in immune response
1965 Thomas Tomasi et al., Secretory immunoglobulin antibodies

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1967 Kimishige Ishizaka et al., Identification of IgE as the reaginic antibody
1971 Donald Bailey, Recombinant inbred mouse strains
1974 Rolf Zinkernagel & Peter Doherty, MHC restriction
1975 Kohler and Milstein, Monoclonal antibodies used in genetic analysis
1984 Robert Good, Failed treatment of severe combined immunodeficiency (SCID, David the bubble boy) by bone marrow grafting.
1985 Tonegawa, Hood et al., Identification of immunoglobulin genes
1985-7 Leroy Hood et al., Identification of genes for the T cell receptor
1990 Yamamoto et al., Molecular differences between the genes for blood groups O and A and between those for A and B
1990 NIH team, Gene therapy for SCID using cultured T cells.
1993 NIH team, Treatment of SCID using genetically altered umbilical cord cells.
1985-onwards Rapid identification of genes for immune cells, antibodies, cytokines and other immunological structures.

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2. Types of immunity - Innate and acquired immunity

Immunity is a state of protection from infectious disease, which has both a less specific and a more specific component. The less specific component, innate immunity provides the first line of defense against infection. Most components of innate immunity are present before the onset of infection and constitute a set of disease resistant mechanisms that are not specific to a particular pathogen but that include cellular and molecular components that recognize classes of molecules peculiar to frequently encountered pathogens. Phagocytic cells, such as macrophages and neutophils, barriers such as skin, and a variety of antimicrobial compounds synthesized by the host all play important roles in innate immunity. In contrast to the broad reactivity of the innate immune system, which is uniform in all members of species, the specific component, adaptive immunity, does not come into play until there is an antigenic challenge to the organism. Adaptive immunity responds to the challenge with a high degree of specificity as well as the remarkable property of 'memory'.

Innate immunity includes four kinds of protective barriers such as anatomic, physiological, phagocytic and inflammatory. Following chart shows the activities of different barriers in mediating innate immune response.

TABLE 1-2 Summary of nonspecific host defenses

Type	Mechanism
Anatomic barriers	
Skin	Mechanical barrier retards entry of microbes. Acidic environment (pH 3–5) retards growth of microbes.
Mucous membranes	Normal flora compete with microbes for attachment sites and nutrients. Mucus entraps foreign microorganisms. Cilia propel microorganisms out of body.
Physiologic barriers	
Temperature	Normal body temperature inhibits growth of some pathogens. Fever response inhibits growth of some pathogens.
Low pH	Acidity of stomach contents kills most ingested microorganisms.
Chemical mediators	Lysozyme cleaves bacterial cell wall. Interferon induces antiviral state in uninfected cells. Complement lyses microorganisms or facilitates phagocytosis. Toll-like receptors recognize microbial molecules, signal cell to secrete immunostimulatory cytokines. Collectins disrupt cell wall of pathogen.
Phagocytic/endocytic barriers	Various cells internalize (endocytose) and break down foreign macromolecules. Specialized cells (blood monocytes, neutrophils, tissue macrophages) internalize (phagocytose), kill, and digest whole microorganisms.
Inflammatory barriers	Tissue damage and infection induce leakage of vascular fluid, containing serum proteins with antibacterial activity, and influx of phagocytic cells into the affected area.

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Inflammatory barrier: Tissue damage and infection include leakage of vascular fluid, containing serum proteins with antibacterial activity, and influx of phagocytic cells into the affected area.

Anatomical barrier: Skin and mucosal membranes lining various internal tracts constitute major components of the anatomical barrier of the body against invading foreign organisms.

Skin: Consists of two layers, thinner outer epidermis and thicker inner dermis. Epithelium consists of several layers of epithelial cells. Outer layer consists of dead cells filed with a waterproofing material called keratin. Dermis is formed of connective tissue, blood vessels, air follicles and glands called sweat and sebaceous glands. Sweat glands are concerned with sweating and sebaceous glands are concerned with the production of sebum. This is a mixture of lactic acid and fatty acids, which maintain a pH of 3 to 5. This acidic condition prevents the growth of most microorganisms. Because of this skin acts as a general barrier against a broad spectrum of infections. Any cuts or injury to the skin makes it prone to entry by microorganism that leads to infections.

Clinical dimensions: A group pf bacteria capable of metabolizing sebum live in the skin as commensals. But at times high populations of these cause acne. An acne drug, isotretinoin (Accutane) prevents the formation of sebum.

Mucosal membranes: Found on conjunctivae, internal epithelia of respiratory, digestive and urinogenital tracts. The membrane consists of an outer epithelial and inner connective tissue layer. This membrane is little more specialized in its dense strategies than the skin. Mucus membrane secretes materials like tears, saliva and mucous secretion, which contain certain antibacterial and antiviral substances like lysozyme. Some of the internal mucosal membranes are provide with cilia which entrap and eliminate the invading organisms through their beating activity. Normal flora is the microbe, mostly bacteria, which live in and on the body with, usually, no harmful effects to us. We have about 1013 cells in our bodies and 1014 bacteria, most of which live in the large intestine. There are 103–104 microbes per diphtheroids, streptococci, *Candida*, etc.). Various bacteria live in the nose and mouth. Lactobacilli live in the stomach and small intestine. The upper intestine has about 104 bacteria per gram; the large bowel has 1011 per gram, of which 95–99% are anaerobes (An anaerobe is a microorganism that can live without oxygen, while an aerobe requires oxygen.) or bacteroides. Various bacteria and diphtheroids lightly colonize the urogenitary tract. After puberty, the vagina is colonized by *Lactobacillus aerophilus* that ferment glycogen to maintain an acid pH however; some organisms have evolved ways of escaping this barrier (eg. Influenza virus).

Physiologic barriers: This consists of temperature, pH and various soluble and cell associated molecules.

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Temperature – Normal body temperature inhibits growth of some pathogens. Fever response inhibits growth of some more temperature resistant bacteria and hence a mechanism of body defense.

pH – Acidity of stomach kills most of the ingested microorganisms.

Soluble molecules – Lysozyme (mucous secretion & tears) cleaves bacterial cell wall. Interferon (protein produced by virus infected cells) induces antiviral state in uninfected cells. Complement (group of serum proteins which circulate in an inactive condition) lyses microorganisms or facilitates phagocytosis. Collectins (surfactant proteins) disrupt bacterial cell wall.

Cell associated Molecules – These molecules exhibit the phenomenon of *pattern recognition* (i.e. ability to recognize a given class of molecules). Examples-

Toll-like Receptors (TLRs) – recognize the lipopolysaccharide found on Gram-negative bacteria. Induces inflammatory response and eliminates bacteria.

Phagocytic Barrier: Phagocytosis involves general uptake of material by a cell from its external environment and its digestion.

The process involves following steps-

- Molecule or organism gets attached to membrane evaginations called pseudopodia formed by the phagocytic cell.
- Material is ingested in the cell forming phagosome.
- Fusion of phagosome with lysome. Digestion of the material by lysosomal enzymes.
- Products of digestion are eliminated from the cell by exocytosis. Monocytes, neutrophils and macrophages play major role in this respect.

Another important innate defense mechanism is the ingestion of extra cellular particulate material by phagocytosis. Phagocytosis is one type of endocytosis, the general term for the uptake by a cell of material from its environment.

- Bacterium gets attached to membrane evaginations called pseudopodia.
- Bacterium is ingested-forming phagosome.
- Phagosome fuses with lysosome.
- Lysosomal enzymes digest captured material.
- Digestion products are released from cell.

In phagocytosis a cell's plasma membrane expands around the particulate material, which may include whole pathogenic microorganisms, to form large vesicles called phagosomes. Specialized cells, such as blood monocytes, neutrophils, and tissue macrophage, conduct phagocytosis. Most cell types are capable of other forms of endocytosis, such as *receptor-mediated endocytosis,* in which extra cellular molecules are internalized after binding by specific cellular receptors, and *pinocytosis*, the process by which cells take up fluid from the surrounding medium along with any molecules contained in it.

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Inflammatory Barrier: Inflammation refers to the complex sequence of events that induce immune response against invading pathogen or injury to the tissue. A first century physician called Celsus identified four consequences like *rubor* (rednes), *tumor* (swelling), *calor* (heat) and *dolor* (pain) as signs of inflammation.

The major events of inflammatory response are listed below:

***Vasodilation*:** The term means increase in the diameter of blood vessels. This happens with blood vessels neighboring the site of injury as a result of the constriction of vessels carrying blood away from the site. This results in engorgement of capillary network at the site giving redness and increase in temperature.

***Increase in capillary permeability*:** This brings about influx of fluid and cells from the engorged capillaries into the tissue. The accumulated fluid (exudate) has higher protein content. The fluid accumulation results in edema or swelling.

***Influx of phagocytes*:** Increased permeability of capillaries allows migration of phagocytic cells from the blood to the tissue. This takes place in following steps –

- Margination- adherence of the cells into the endothelial wall.
- Diapedesis or Extravasation – emigration of phagocytes from capillaries to the tissue.

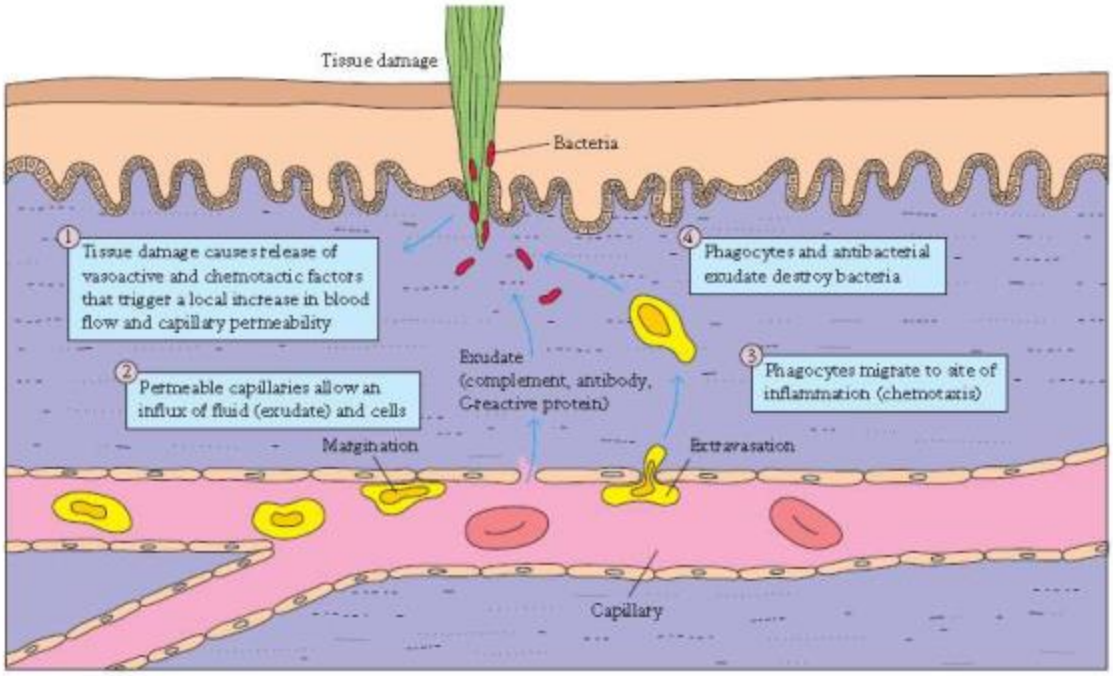

FIGURE 1-4 Major events in the inflammatory response. A bacterial infection causes tissue damage with release of various vasoactive and chemotactic factors. These factors induce increased blood flow to the area, increased capillary permeability, and an influx of white blood cells, including phagocytes and lymphocytes, from the blood into the tissues. The serum proteins contained in the exudate have antibacterial properties, and the phagocytes begin to engulf the bacteria, as illustrated in Figure 1-3.

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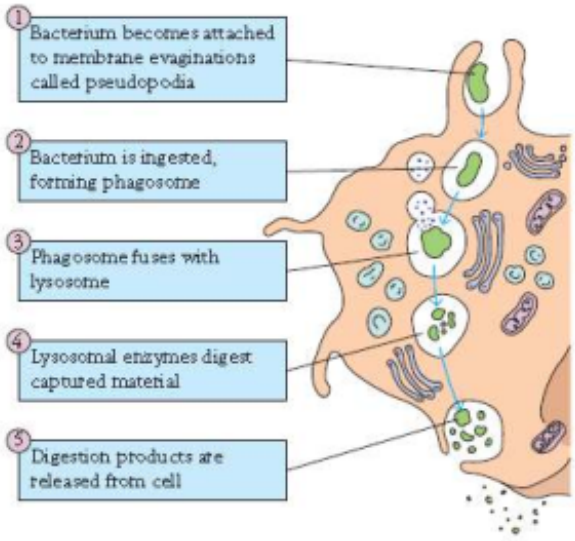
Chemotaxis: Migration of phagocytes through the tissue to the site of invasion or injury.



Phagocytosis: Intake of bacteria or other foreign molecules by phagocytes and digestion followed by exocytosis of the digested material. Meanwhile, activity of lytic enzymes released by the phagocytes damage neighbouring cells. The accumulation of dead cells, digested material and fluid forms a substance called pus. Blood clotting factors also attracted to the site of inflammation and result in the development of clot, followed by tissue repair after inflammation subsides. Inflammatory response explained above is being mediated by several chemical molecules released by invading organism, damaged cells, WBCs participating in the process etc. Some of the important molecules, their origin and activities are listed below.

Histamines – produced by a variety of cells. Causes vasodilation and increase in capillary permeability.

Kinins- a type of protein normally present in blood plasma in an inactive form and gets activated by tissue injury. Causes vasodilation and increases capillary permeability. A kind of kinin called bradykinin stimulates pain receptors on skin.

Acute-phase proteins – These are serum proteins released in response to tissue damage. Eg. C-reactive protein produced by liver. Recognises and bind to C-polysaccharide cell-wall component found on variety of bacteria and fungi. This binding activates complement system resulting in lysis of the pathogen.

Collectins – a group of carbohydrate binding proteins resembling complement C1q. Found on cell surface of microorganisms. Receptors for collectins on macrophages recognize collectin bound microorganisms and kill them.

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The events in the inflammatory response are initiated by a complex series of events involving a variety of chemical mediators whose interactions are only partly understood. There are two kinds of mediators:

I. Cell derived mediators (produced by white blood cells), which include Arachidonic acid derivatives (Prostaglandins and Leukotrines) involved in blood clotting which are produced rapidly local to the site of infection. They have a short range of action and degenerate spontaneously.

Cytokines, Lymphokines and Monokines One of the most important of these is a substance known as interleukin. This stimulates lymphocytes and other white blood cells into action. Also promotes fever by resetting the body's thermostat located in the hypothalamus. In addition it induces drowsiness (somnolence).

Platelet Activating Factor (PAF) Produces vasodilation. Makes blood and lymph vessels more permeable. Stimulates production of prostaglandin.

Histamine Effects are vasodilation and increased vascular permeability. Appears to be effective in the early stages as evidenced by the fact that antihistamines have no effect upon vascular permeability 1 hour after the onset of nflammation.

II. Plasma derived mediators (found in blood plasma), which include Complement

An extremely important complex of over 20 proteins found in the blood plasma. They work as follows:

a. Increases vascular permeability. Opsonisation basically coating invading cells in order to make them more appetising to phagocytic cells.
b. Chemotaxis producing chemicals, which attract important elements of the specific, and non-specific defence mechanisms to the site of infection.
c. Direct lysis of organisms.

Interferon
These are chemical messages from virally infected cells that are borne in the plasma. They stimulate other cells to produce anti-viral proteins. Virus particles produced in cells exposed to interferon are less effective at infecting other cells. Also stimulate Natural Killer Cells into action. They are not viral specific and cannot save the infected cell. Appear to be most effective in short-term viral infections such as colds and flu.

Adaptive immunity has evolved to fight against specific pathological conditions.

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Adaptive Immunity Acquired (Acquired Immunity)

This is specific towards a particular pathogen or molecule. It can recognize and selectively eliminate its target factor. Adaptive immunity exhibits 4 characteristic attributes such as Antigenic specificity: ability to distinguish even minute difference between antigens. eg. Abs can distinguish between antigens differing by a single amino acid. The specificity of adaptive immunity is ensured by antibodies and t cell receptors.

Diversity – enables immense diversity through innumerable antibodies T cell receptors.

Immunologic memory – once the immune system has recognized and reacted to an antigen, the memory will be available throughout the life of the individual and a second encounter will be warded with intense and immediate reaction.

Self – non-self discrimination – capable of distinguishing self and non-self and respond only to non-self molecules.

Sources of Adaptive Immunity

Basically the entire range of immune response coming under adaptive immunity is dependent on B and T lymphocytes. They operate through humoral and cell mediated responses respectively. Humoral response is best suited for elimination of exogenous antigens while cell-mediated responds is useful in elimination of endogenous antigens.

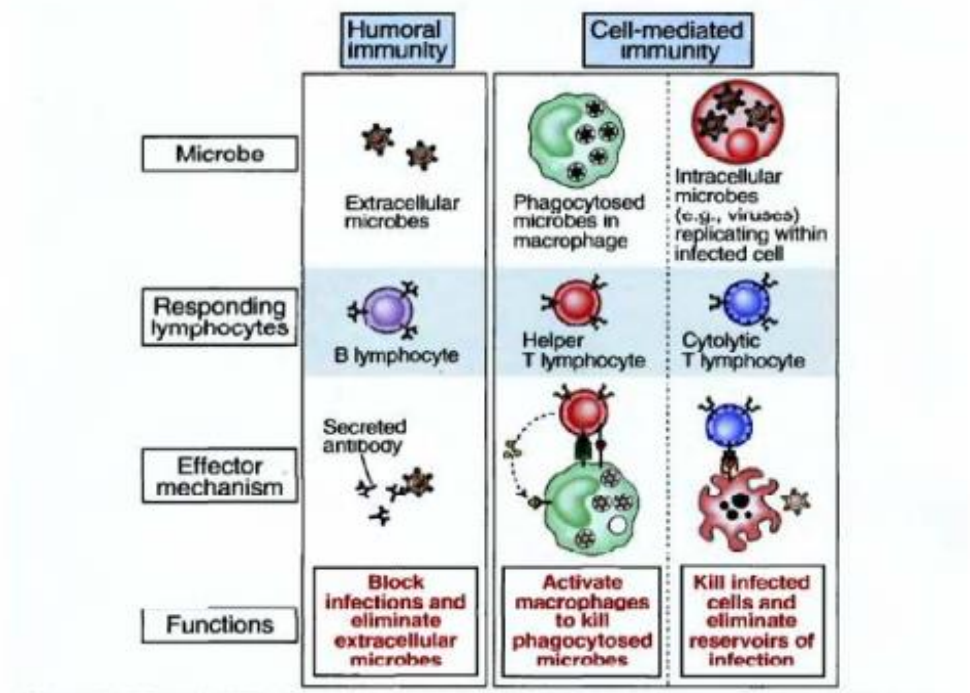

Figure 1–4 Types of adaptive immunity. In humoral immunity, B lymphocytes secrete antibodies that eliminate extracellular microbes. In cell-mediated immunity, T lymphocytes either activate macrophages to destroy phagocytosed microbes or kill infected cells.

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The components and mechanisms involved in adaptive immunity. An outline of the two adaptive immune mechanisms is provided below.

a. Humoral Immune Response: Exhibited by B cells. On maturation, these cells leave bone marrow and express unique antigen binding receptors (Ab molecules) on their surface. With the help of these molecules, B cells interact with antigens and differentiate into AB secreting plasma cells. Antibodies bind with antigen and executes its elimination from the body.

b. Cell mediated response: Exhibited by T cells. Unlike B cells these cells cannot recognize antigens wherever they are, but when presented by MHC molecules of the self-cells.

The process of cell-mediated immune response can be outlined in the following steps:

- Internalised Ag digested by the cell.
- Altered self cell presents self cell (all nucleated cells possess class I MHCs and APCs possess class II MHCs.).
- TH cell receptors recognize Ags bound to class II MHC molecules.
- Binding of Ag activates TH cells activates them. Activated cells secrete cytokines, which induce B cells, and Tc cells.
- Ags bound to class I MHCs are recognized and bound by Tc cells.
- Ag bound Tc cells get altered to CTLs with the help of cytokines released by TH cells.
- Activated CTLs recognize and kill the altered self cells presenting antigen.

3. Specificity

The **antigenic specificity** of the immune system permits it to distinguish subtle differences among antigens. Antibodies can distinguish between two protein molecules that differ in only a single amino acid. The immune system is capable of generating tremendous *diversity* in its recognition molecules, allowing it to recognize billions of unique structures on foreign antigens. Once the immune system has recognized and responded to an antigen, it exhibits *immunologic memory;* that is, a second encounter with the same antigen induces a heightened state of immune reactivity. Because of this attribute, the immune system can confer life-long immunity to many infectious agents after an initial encounter. Finally, the immune system normally responds only to foreign antigens, indicating that it is capable of *self/nonself recognition.* The ability of the immune system to distinguish self from nonself and respond only to nonself molecules is essential, for, as described below, the outcome of an inappropriate response to self molecules can be fatal. Adaptive immunity is not independent of innate immunity. The phagocytic cells crucial to nonspecific immune responses are intimately involved in activating the specific immune response.Conversely, various soluble factors produced by a specific immune response have been shown to augment the activity of these phagocytic cells. As an inflammatory response develops, for example, soluble mediators are produced that attract cells of the immune system. The immune response will, in turn, serve to regulate the intensity of the inflammatory response. Through the carefully regulated interplay of adaptive and innate immunity, the two systems work together to eliminate a foreign invader.

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4. Immunological memory of immune system

Immunological memory can be considered broadly as any alteration in the response to an antigen induced by previous exposure to the same antigen. Strictly speaking, memory would include other phenomena such as partial or complete tolerance induction. However, this chapter focuses on the more classical view of memory as an enhanced (or primed) state of the immune system after exposure to antigen. By this definition, immune memory includes continued expression of effector activity, particularly antibody production, as well as the persistence of immune ("memory") T and B cells. To a large extent, immunological memory is a reflection of a greatly increased frequency of specifically reactive T and B cells in relation to unprimed animals. In addition, memory cells possess intrinsic functional differences from naïve cells that contribute to the enhanced nature of the secondary response. In this chapter, we discuss the current understanding of how immunological memory is generated and maintained as well as the cellular and molecular parameters contributing to memory.

LONGEVITY OF IMMUNOLOGICAL MEMORY

It is clear that immune-mediated protection against disease can be extremely long-lived after infection or vaccination. This is evident from the fact that lifelong resistance results from infections with childhood diseases such as chicken pox, mumps, and measles. However, because reexposure to the viruses that cause these diseases is a common occurrence, immunity might hinge on repeated boosting of memory cells through subsequent subclinical infection. Nevertheless, at least for certain viruses, a single exposure is sufficient to confer lifelong immunity.

Perhaps the best example in which this was shown directly was the detection of vaccinia virus–specific CD4 + and CD8 + T cells in individuals vaccinated up to 50 years before. Because there was virtually no chance that these people were subsequently exposed to vaccinia (or the cross-reacting smallpox virus, for which the vaccine was given), these observations implied that T-cell memory can persist for many decades after a single virus infection. These data indicate that immunological memory in humans can be very long-lived at both the T-cell and B-cell level. These findings are supported by a large body of evidence derived from experimental animals (mainly mice) showing lifelong antibody production and persistence of antigen-specific CD4 + and CD8 + T cells at high frequencies after infection. Although long-term memory is apparently not dependent on reinfection, it remains possible that intermittent contact between the immune system and antigen serves to boost the intensity of the secondary response.

GENERATION OF MEMORY

There are two main outcomes of a typical immune response:
(a) generation of effector cells that act to clear the acute infection and
(b) generation of immune memory, which provides long-term protection against reinfection. Although the distinction between these two phases of the immune response is not absolute

(for example, long-term expression of effector activity probably contributes to immune memory, as outlined later), effector and memory responses typically exhibit considerable quantitative, qualitative, and temporal differences. Hence, the effector response is characterized by the generation of extremely high numbers of antigen-specific T and B cells that are in a highly activated state and have direct effector activity; the majority of these cells disappear once the infection is cleared (usually within 1 to 2 weeks of initiating the response).

Conversely, the frequency of antigen-specific lymphocytes among memory cells is much lower than that found during the acute response; moreover, most memory cells are in a less activated state than are effector cells. In addition, unlike the majority of effector lymphocytes, memory cells persist long after the infection is cleared.

Although the precise mechanisms involved in generating immune memory remain poorly understood, it is clear that both memory and effector cells are produced as a result of the activation of initially naïve lymphocytes in the specialized environment of secondary lymphoid tissues.

Generation of Memory T Cells

T cells are generated from immature precursors through a complex series of selection events in the thymus. During this process, immature thymocytes that lack T-cell receptor (TCR) specificity for self-peptides bound to major histocompatibility complex (MHC) molecules fail to receive a survival signal and die by apoptosis, whereas cells expressing a TCR with high affinity for self-MHC/self-peptide complexes are signaled to die. The outcome of these positive and negative selection events is the generation of a population of mature T cells expressing TCR with low but significant affinity for self-MHC/self-peptide complexes; cells with overt reactivity to these ligands are deleted.

Mature T cells are released from the thymus into the bloodstream in low numbers: approximately 1 to 2 × 10 6 cells per day (about 1% of total thymocytes) in young (<2-month-old) mice. Thymic output of T cells decreases considerably in older mice and humans, because of atrophy of the thymus at puberty in both species. Recent thymic emigrants are considered to be immunologically naïve, exposure to foreign antigens in the thymus being negligible. Naïve T cells recirculate continuously between blood and lymph, entering into lymph nodes (LNs) via specialized high endothelial venules (HEV) before returning to the bloodstream through thoracic duct lymph. Naïve T cells maintain this pattern of recirculation through expression of a specific combination of adhesion molecules and chemokine receptors. In particular, naïve T cells express high levels of two LN homing receptors: (a) CD62L, which allows cells to adhere to specific ligands (vascular addressins) expressed in HEV, and (b) the CCR7 chemokine receptor, which controls responsiveness to chemokines (e.g., ELC) expressed in LN at sites of lymphocyte entry. Conversely, because of limited expression of other adhesion molecules and chemokine receptors, naïve T cells are unable to extravasate into peripheral, nonlymphoid tissues. This pattern of recirculation through the lymphoid tissues is of key importance because it brings naïve T cells into continuous contact

with specialized antigen-presenting cells (APCs), especially dendritic cells (DCs), which are present in the T-cell areas of lymph into secondary lymphoid organs via blood (i.e., in the spleen) or afferent lymph (in LNs).

In addition, immature DCs present in peripheral tissues, such as Langerhans cells in skin, are induced to migrate to the T-cell areas of lymphoid organs after antigen capture. This homing property of antigen-bearing DCs allows naïve T cells to scan the entire body for the presence of foreign antigens. The fact that DCs are the cell type scrutinized by naïve T cells in this surveillance operation is also highly significant, because DCs are the major, if not the only, APCs able to initiate the activation of naïve T cells. T cells become activated in secondary lymphoid tissues upon recognition of MHC/peptide complexes to which their TCRs have high affinity. Optimal T-cell activation is dependent not only on triggering of the TCR but also on the delivery of a "second" signal, usually referred to as co-stimulation, by the APC. The best characterized co-stimulatory signal is that mediated by the binding of CD28 on the T cell to B7-1 (CD80) or B7-2 (CD86) molecules on APCs. This interaction has been shown to be crucially important in T-cell activation in vivo through a variety of studies of mice deficient in CD28 function. However, some T-cell responses can occur in the absence of CD28, which implies either that alternate co-stimulatory pathways are available for the activation of naïve T cells or that activation can occur in the absence of co-stimulation under certain circumstances. Other molecules on naïve T cells that are capable of delivering co-stimulatory signals include leukocyte function–associated antigen 1 (LFA-1) (CD11a/CD18), which binds to intercellular adhesion molecule (ICAM)–1, ICAM-2, or ICAM-3, and CD2, which binds to CD58 (humans) or CD48 (mouse). The heat-stable antigen (HSA) (CD24), expressed on APCs, has also been shown to provide co-stimulation for T-cell activation, although its receptor on T cells has not been identified. In addition, a number of other co-stimulatory molecules are up-regulated after activation of T cells and APCs; as discussed later, these molecules may play an important role in amplifying or prolonging the response rather than in initiating T-cell activation.

Generation of Memory B Cells

B cells can be divided broadly into a minority population of B-1 cells and a major population of conventional B-2 cells. The B-1 subset is located primarily in the pleural and peritoneal cavities, whereas B-2 cells are found mainly in defined B-cell zones in the spleen and LNs. This section focuses on the current understanding of memory generation among B-2 cells.

B cells are produced throughout life in the bone marrow in an IL-7–dependent manner. It has been estimated that 10% to 20% of the immature B cells produced in the bone marrow enter the mature peripheral pool and that most of the loss occurs either in the bone marrow or during the migration of cells from the bone marrow to the spleen. Upon exiting the bone marrow, immature B cells enter the T-cell zones of secondary lymphoid organs before undergoing final maturation and entering B-cell follicles; newly produced B cells make up between 5% and 10% of splenic cells.

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Differences in the usage of immunoglobulin (Ig) variable region genes between peripheral B cells and pre–B cells in the bone marrow has been taken as evidence that, as in positive and negative selection of T cells in the thymus, the peripheral B cell repertoire is generated through ligand-mediated selection processes. In his regard, self-reactive B cells can either be deleted or undergo a change in their specificity through a process of receptor editing; whether the cells die or change their specificity seems to depend on when the cells first encounter antigen and whether the antigen is cell associated or soluble. Whether B cells undergo positive selection to self-ligands is unclear, although B-cell deficiencies in a variety of mutant mouse strains suggest that the transition of immature splenic B cells into follicular B cells is an active process. Of note is that relatively normal generation of immature B cells but poor production of mature B cells are evident in mice deficient for a number of molecules associated with signaling through the B-cell receptor (BCR), including the tyrosine kinase Syk, Bruton's tyrosine kinase (Btk), Iga, CD45, and CD22. The implication is therefore that B cells must be triggered through the BCR before completing their maturation process, although it is possible that selection is not ligand driven but is simply dependent on proper assembly of all signaling components. If an external ligand is involved, it appears to be independent of foreign antigen, inasmuch as a stable pool of peripheral B cells is generated in germ-free mice. Furthermore, entry into the mature peripheral pool is not accompanied by somatic mutations of Ig variable region genes, which distinguishes this process from an overt B-cell response to foreign antigens.

Interestingly, immature B cells predominate in mice deficient for B-cell activation factor (BAFF), a member of the tumor necrosis family, or its receptor on B cells, which implies that BAFF may guide the final stages of B-cell maturation in the spleen. As for T cells, naïve B cells recirculate continuously between blood and lymph, and B-cell responses are initiated in secondary lymphoid organs. In LNs and the spleen, naïve B cells are anatomically segregated from T cells and localize primarily in follicles. Although it is unclear where naïve B cells first encounter antigen, antigen-binding proliferating B cells can be detected in the outer T-cell zones of LNs association with class II MHC to activated CD4 + helper T (Th) cells. Cell membrane–associated and soluble signals delivered from Th cells promote further B cell activation. Subsequently, some of the B cells proliferate and differentiate to form foci of antibody-forming cells (AFCs) in the area adjacent to the T-cell zone (the red pulp in the spleen or the medullary cords in LNs). These cells do not mutate their Ig variable region genes and are short-lived, dying by apoptosis within 2 weeks of immunization. At the same time, other B cells migrate to follicles and initiate the germinal center (GC) reaction. It is within the GC that both memory B cells and AFCs secreting high-affinity, isotype-switched antibodies are generated.

IDENTIFYING MEMORY CELLS

Identification of memory cells is clearly of crucial importance for understanding how these cells are generated and maintained and for examining their functional characteristics. The assumption that these cells can be identified as a discrete subset is based on the idea that memory cells carry a permanent imprint of having previously responded to antigen. For this

reason, many of the cellular characteristics that have been employed as indicators for memory cells are surface marker changes that occur in response to lymphocyte activation. Although these markers have been useful for enriching memory cell populations, the discovery of definitive memory cell markers has so far been elusive.

Five main facts have contributed to the difficulty in identifying memory cells:
(a) Many of the markers that are expressed by previously activated lymphocytes do not distinguish between recently activated effectors and long-lived memory cells;
(b) phenotypic changes that occur upon lymphocyte activation may be transient, with the result that memory cells revert to a naïve phenotype with time;
(c) some cells may fail to acquire typical activation markers when stimulated with antigen;
(d) naïve cells may express markers of activation without having responded to antigen;
(e) memory cells appear to be heterogeneous with regard to both phenotype and function.

Identification of Memory T Cells

Attempts to discover markers for memory T cells have focussed on cell surface molecules that differ in expression between bona fide naïve T cells and previously activated T cells. By comparing the phenotypes of T cells that are presumed not to have encountered antigen (e.g., in umbilical cord blood or germ-free mice), T cells that have been acutely activated with antigen, and T cells that mediate a recall response in previously immunized individuals, a number of molecules have been identified as putative markers of memory cells. Extensive work over many years has shown that the utility of these markers varies with animal species, CD4 + versus CD8 + T cells, and even the specific immune response being studied.

Many of the molecules reported to be up-regulated on the surface of memory T cells are adhesion molecules. These include ß1 (CD49d, CD49e, CD29) and ß2 (CD11a, CD11b, CD18) integrins; CD2; CD44; CD54; and CD58. To a degree, the detection of increased levels of adhesion molecules on “memory” T cells may reflect the presence of cells that have recently responded to antigen. In accordance with this idea, human memory-phenotype T cells express some markers of activation and are slightly larger than typical naïve T cells. However, at least one adhesion molecule, CD44, appears to be a long-term marker of memory T cells. Thus, murine memory CD8 + T cells retain a CD44 hi phenotype indefinitely after adoptive transfer to recipient mice in the absence of antigen. For this reason, high surface expression of CD44 is commonly used as a marker of memory-phenotype CD8 + T cells in the mouse. CD44 is also used to distinguish between naïve and memory CD4 + T cells, although the stability of CD44 expression on memory CD4 + T cells is less certain; in at least one report, these cells reverted to a CD44 lo phenotype.

Identification of Memory B Cells

Identification of memory B cells is more straightforward than for memory T cells for two main reasons. First, memory B cells are clearly distinct from fully differentiated plasma cells. Thus, as described previously, these two cell types represent the products of separate

differentiation pathways. Furthermore, memory B cells and plasma cells are phenotypically very different: Memory B cells are small and express surface immunoglobulin and class II MHC, whereas plasma cells are large and generally lack surface immunoglobulin and MHC class II.

Second, memory B cells are easily distinguished from naïve B cells by multiple parameters. For example, somatic hypermutation of Ig genes is prominent in memory B cells but largely undetectable in naïve B cells [although in some species, such as sheep, hypermutation is involved in generating the primary Ig repertoire in an antigen-independent manner]. Clearly, there are practical limitations to isolating memory B cells on the basis of sequencing rearranged Ig genes. Nevertheless, the fact that somatic mutation is a bona fide distinction between memory and naïve B cells is useful for retrospective analysis of memory markers—that is, as evidence for the presence of memory B cells in cell populations separated on the basis of putative cell surface memory markers. One caveat to this approach, however, is that some cells exiting GC may bear unmutated Ig (BCR) genes, presumably because some germline-encoded BCR have sufficient affinity for antigen to compete successfully with mutated BCR.

FACTORS CONTRIBUTING TO MEMORY

The factors contributing to the intensity of memory responses can be grouped into three categories:
(a) continued expression of effector activity,
(b) systemic differences between the memory and naïve state
(c) altered properties of memory cells on a per-cell basis.

a. Continued Expression of Effector Activity

Antibody secretion can continue indefinitely after infection or vaccination.In addition, although most direct effector T-cell activity disappears after the resolution of the acute immune response, some T cells exhibiting the characteristics of effector T cells can be detected long after the infection has been cleared. As discussed earlier, these cells are now commonly referred to as effector memory cells and are particularly prominent at mucosal sites. Whether such continued expression of effector activity should be classified as a "memory" function depends in part on the type of memory being considered. In terms of protection against reinfection, preexisting effector activity is of obvious importance for inducing an immediate response to the pathogen.

b. Systemic Differences between the Memory and Naïve State

Two systemic features of memory cells cause secondary responses to pathogens to be more effective than primary responses. First, the frequency of antigen-specific T and B memory cells is much higher than that of naïve cells. This increase in frequency confers a strong kinetic advantage during the secondary response; thus, in contrast to the primary response,

only a few cell divisions are needed to generate large numbers of effector cells in the secondary response. Second, unlike naïve T cells, effector memory T cells make rapid contact with pathogens, through their ability to migrate into inflamed tissues, and accumulate at mucosal sites. In this regard, it is well documented that subpopulations of memory T cells possess the capacity to migrate preferentially to different tissues: for example, the gut versus skin. This tissue-specific homing ability is linked to the expression of unique combinations of adhesion molecules and chemokine receptors and may be related to the initial conditions of activation.

c. Altered Properties of Memory Cells on a Per-Cell Basis

In addition to the systemic differences in frequency and distribution just discussed, for T cells there is considerable evidence that memory cells respond to antigen in a qualitatively different way than do naïve cells. The responses of naïve- and memory-phenotype T cells to mitogens, mitogenic antibodies, or allogeneic stimuli; use of these surrogate antigens was necessary at the time because of the very low frequency of naïve T cells against any particular antigen. In general, these studies showed that memory-phenotype T cells were more easily activated, were less dependent on co-stimulation, and secreted a much broader range of cytokines than did naïve-phenotype cells.

LIFE SPAN AND TURNOVER OF MEMORY CELLS

Life Span of Memory T Cells

At a population level, mature T cells can survive for long periods of time in situations in which there is no possibility for the input of newly generated T cells: for example, in thymectomized animals or after adoptive transfer to T cell–deficient recipients. Under these conditions, memory T cells persist indefinitely. For naïve T cells, the life span of these cells in mice has been estimated to be approximately 6 months to a year, on the basis of the rate at which responsiveness to neoantigens is lost after thymectomy. However, in addition to death, the slow disappearance of naïve T cells may reflect conversion to memory cells as the result of exposure to environmental antigens. Nevertheless, it is clear that both naïve and memory T cells are relatively long-lived at a population level.

Life Span of Memory B Cells

In vivo DNA labeling studies have shown that B cells in the periphery have an average life span of several weeks to months. These cells can be divided into a minor (10% to 15%) population of HSA hi cells with a rapid turnover and a longer lived HSA lo/int subset. The short-lived cells correspond to the transitional, immature B cells of the spleen; these cells either are selected into the peripheral recirculating pool or die within a few days of export from the bone marrow (see previous discussion). Their brief life span may be related to low expression of the antiapoptotic molecule A1, which is expressed at tenfold higher levels in the long-lived peripheral B cell pool.

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MAINTENANCE OF MEMORY

Maintenance of T-Cell Memory

Since 1990, considerable progress has been made toward understanding how T cell memory is maintained. During this time, much debate has centered on the issue of whether maintenance of memory requires periodic contact with antigen. Because many pathogens can persist at low levels in the host, and because even nonreplicating antigens can remain trapped for substantial lengths of time on the surface of FDCs, it seems possible and perhaps even likely that persisting antigen could affect the behavior of memory cells. Thus, if memory T cells can gain access to antigen in recognizable form (i.e., as peptide/MHC complexes expressed on cells encountered during normal T-cell migration), intermittent contact with antigen may induce some degree of T-cell activation, possibly affecting cell life span, migration, and effector function. Furthermore, because memory T cells appear to be more easily triggered by cross-reactive antigens than are naïve T cells, this type of stimulation might not even require the original priming antigen.

Maintenance of B-Cell Memory

There is less consensus concerning the possible role of persisting antigen in maintaining B-cell memory. As with T cells, it seems likely that intermittent contact with antigen by memory B cells modifies the behavior of these cells, but whether such contact is an absolute requirement for memory maintenance is a matter of debate. The ability of antigen to persist in an area that is accessible to B cells [i.e., on FDCs], combined with the apparent short life span of many AFCs, contributed to the view that continued production of antibody is strictly dependent on persistence of antigen. Similarly, B cell memory appears to wane after adoptive transfer of cells from immune to naïve animals in the absence of antigen, which implicates a role for antigen in prolonging the survival of memory B cells. However, these long-held views have been challenged by two key findings. First, the discovery of a long-lived population of AFCs that can persist in the absence of antigen implies that at least a proportion of long-term antibody production is independent of persisting antigen.

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5. Erythropoiesis, of Cell lineage of immune cells

Carried within the blood and lymph and populating the lymphoid organs are various white blood cells, or **leukocytes,** that participate in the immune response. Of these cells, only the lymphocytes possess the attributes of diversity, specificity, memory, and self/nonself recognition, the hallmarks of an adaptive immune response. All the other cells play accessory roles in adaptive immunity, serving to activate lymphocytes, to increase the effectiveness of antigen clearance by phagocytosis, or to secrete various immune-effector molecules. Some leukocytes, especially T lymphocytes, secrete various protein molecules called cytokines. These molecules act as immunoregulatory hormones and play important roles in the regulation of immune responses. This chapter describes the formation of blood cells, the properties of the various immune-system cells, and the functions of the lymphoid organs.

Hematopoiesis

All blood cells arise from a type of cell called the **hematopoietic stem cell (HSC). Stem cells** are cells that can differentiate into other cell types; they are self-renewing—they maintain their population level by cell division. In humans, **hematopoiesis,** the formation and development of red and white blood cells, begins in the embryonic yolk sac during the first weeks of development. Here, yolk-sac stem cells differentiate into primitive erythroid cells that contain embryonic hemoglobin. In the third month of gestation, hematopoietic stem cells migrate from the yolk sac to the fetal liver and then to the spleen; these two organs have major roles in hematopoiesis from the third to the seventh months of gestation. After that, the differentiation of HSCs in the bone marrow becomes the major factor in hematopoiesis, and by birth there is little or no hematopoiesis in the liver and spleen.

A hematopoietic stem cell is *multipotent,* or *pluripotent,*able to differentiate in various ways and thereby generate erythrocytes, granulocytes, monocytes, mast cells, lymphocytes, and megakaryocytes. These stem cells are few, normally fewer than one HSC per 5 X 104 cells in the bone marrow. The following chart explains how the stem cell differentiates into different lineages and what are the factors responsible for that.

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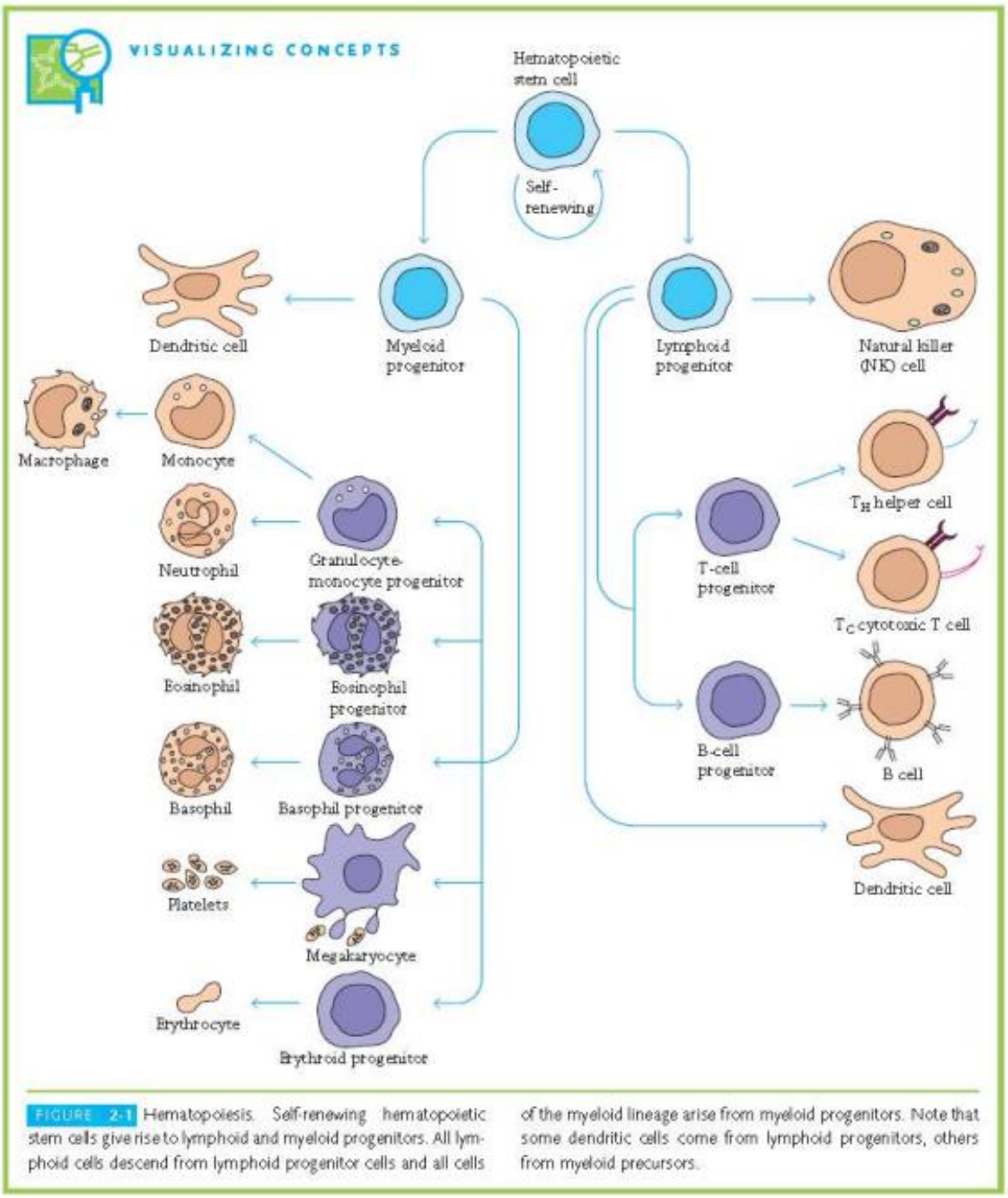

FIGURE 2-1 Hematopoiesis. Self-renewing hematopoietic stem cells give rise to lymphoid and myeloid progenitors. All lymphoid cells descend from lymphoid progenitor cells and all cells of the myeloid lineage arise from myeloid progenitors. Note that some dendritic cells come from lymphoid progenitors, others from myeloid precursors.

All the various types of blood cells are produced in the bone marrow (some 1011 of them each day in an adult human!).They arise from a single type of cell called a pleuripotent stem cell. These stem cells are very rare (only about one in 10,000 bone marrow cells). They are attached to osteoblasts lining the inner surface of bone cavities; and they express a surface protein designated CD34 and produce, by mitosis, two kinds of progeny: more stem cells and cells that begin to differentiate along the paths leading to the various kinds of blood cells. Which path is taken is regulated by the need for more of that type of blood cell, which is, in turn, controlled by appropriate cytokines and/or hormones. Examples:

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Lymphocytes

Interleukin-7 (IL-7) It is a major factor in stimulating bone marrow stem cells to start down the path leading to the various lymphocytes (mostly B cells and T cells). The hormone thrombopoietin (TPO), secreted by the liver, starts cells down the path leading to megakaryocytes and red blood cells.

Red blood cells.

Erythropoietin (EPO), produced by the kidneys, enhances the production of RBCs.

Platelets.

Thrombopoietin (TPO), assisted by Interleukin-11 (IL-11), stimulates the production of megakaryocytes and their fragmentation into platelets.

Granulocytes and Monocytes

Granulocyte-monocyte colony-stimulating factor (GM-CSF), as its name suggests, sends cells down the path leading to both those cell types. In due course, one path or the other is taken. Under the influence of granulocyte colony-stimulating factor (G-CSF), they differentiate into neutrophils. Further stimulated by interleukin-5 (IL-5) they develop into eosinophils. Interleukin-3 (IL-3) participates in the differentiation of most of the white blood cells but plays a particularly prominent role in the formation of basophils (responsible for some allergies). Stimulated by macrophage colony- stimulating factor (M-CSF) the granulocyte/macrophage progenitor cells differentiate into monocytes, the precursors of macrophages.

Microenvironment of Hemopoiesis

In bone marrow, HSCs grow and mature on a meshwork of stromal cells, which support the growth and differentiation process. Stromal cells include fat cells, endothelial cells, fibroblasts, and macrophages. Stromal cells provide hemopoietic inducing microenvironment (HIM) consisting of cellular matrix, and factors promoting growth and distribution. These factors are different for different lineages of blood cells as shown in the above list. Chemically these factors are glycoproteins and collectively called as cytokines. Apart from the HIM and cytokines, the process of hemopoiesis is also regulated by genetic factors. This is achieved through expression of different sets of genes (i.e. different lineage determining and lineage specific genes) at appropriate time and in correct order. Proteins expressed by these genes are called as transcription factors, which regulate the process of differentiation. Following table gives examples of transcription factors essential for hemopoiesis. After differentiation, red and white blood cells pass into bone marrow channels and from there they enter into circulation. Now you are equipped with enough information on how blood cells originate and differ from each other. Now you should know how the population of each lineage of blood cells is maintained in the blood. This is achieved through a balancing act called homeostasis of hemopoiesis.

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Abundance, Distribution and Types of Lymphocytes

Lymphocytes constitute 20 – 40% of the WBCs and 99% of the cells in lymph. They circulate in blood, lymph, tissue spaces, and lymphoid organs. They are of 3 types – T cells, B cells and Natural killer cells (NK cells). This classification is done on the basis of their function and cell membrane components (surface markers). B and T cells have a common precursor called naïve or unprimed or resting or small lymphocyte. They measure about 6mm in diameter. These cells have little amount of cytoplasm, densely packed chromatin, few mitochondria, poorly developed ER and Golgi complex. They are at G0 phase of the cell cycle. On encounter with an antigen, they resume cell cycle, enlarge in size and proliferate onto different lineages. During the course of differentiation, small lymphocytes resume cell cycle and attain a prominent form at S phase called lymphoblasts, which proliferate to give rise to functional forms called effector and memory cells.

Effector Cells

They function in various ways to eliminate antigen. They have short life span of few days to few weeks. Effector B cells are antibody producing cells and possess abundant ER and Golgi vesicles. Effector T cells include cytokine secreting TH cells and cytotoxic T (Tc) cells. Memory cells of both B and T cell lineages are concerned with immunological memory.

Maturation and distinguishing of cells of different lineages:

This is done on the basis of cell surface molecules being expressed by them. To detect these individual groups of molecules, monoclonal antibodies (i.e. antibodies specific for a single epitope of an antigen) are made use. In order to designate the monoclonal antibodies (Mabs) a system of naming is followed, where similar Mabs are designated as CDs.

CD (Cluster of Differentiation) and CD Markers

A collection of Mabs that all recognise an antigen found on a particular differentiated cell type or types are called as a CD. Each of the antigens recognised by a particular CD is called a CD marker and assigned a unique identifying number (eg. CD4, CD8, CD2, CD6, etc.). More than 200 CD markers have been described so far.

B – Lymphocytes

They are produced and get matured in bone marrow (primary lymphoid organ) in all vertebrates, except birds. In birds maturation takes place in bursa fabricius.

Maturation of B Cells

During B cell development, sequential Ig –gene rearrangements transform a precursor B cell into an immature B cell expressing membrane bound IgM (mIgM) with a single antigenic specificity. Further development yields mature naïve B cells expressing both mIgM and mIgD molecules. They are distinguished by the presence of antibodies (immunoglobulins) on them. These act as receptors or binding sites for antigens specific to B cells.

· B cells possess following receptors on their surface.
· II MHC molecules: These molecules present antigens to TH cells.

· CD 35 (CR 1): complement receptor.
· CD 21 (CR 2): complement receptor.
· CD 32 (Fc & R II): Binds to IgG antigen complexes.
· CD 80/86 (B7 – 1,2): Bind CD 28 on T cells to trigger T cells activation. CD 28 & CTLA – 4 are important regulatory molecules on surface of different types T cells (all types).
· CD 40: Interacts with CD 40 ligand on the surface of TH cells and signal the activation of the B cells.
· Iga & Igb: CD 79a/b, mediate cellular activation on binding of antigen to cell surface antibody.
· Cell surface antibody: IgM and IgD on mature B cells.
· CD19: Co-receptor sub unit.
· CD20: Ca2+ channel.
· CDB1: TAPA, coreceptor.
· ICAM – 1: binds to LFA -1.
· LFA -3: Binds to CD2.

Effector B Cells
All clonal progeny of given B cell posses same antibodies (same specificity). Longevity of the cells is 1 to 2 weeks.

Memory Cells
Produce Abs only to express on their surface. They remain able to respond to antigen if it is reintroduced. Memory B cells are classified into 2 based on their mechanism of action.

B1 cells Arise early during ontogeny; mainly express IgM (encoded by germline Ab genes). They mature independently in the bone marrow. Mechanism of their action is independent of T cells. They recognize multimeric sugar/lipid antigens of microbes.

B2 cells (Conventional B cells)- Primarily responsible for humoral immunity. T cell dependent activity. Produce IgG, IgA, and IgE with the help of TH cells.

T Lymphocytes
Maturation takes place in thymus.

Maturation of T Cells
T cell progenitors undergo 8-9 weeks of gestation in bone marrow before migrating to thymus. Now the cells are called as thymocytes. Maturation involves rearrangements of the germ-line TCR genes and expression of various membrane markers. In most of the thymocytes, rearrangement of the genes encoding a and b chains of TCRs to produce *ab* T cells. A small population of the precursor undergo rearrangement of gd genes to produce gd T cells. The earliest thymocytes lack CD4 and CD8 and are referred to as double-negative cells. During maturation they develop into CD4+CD8- ab T cells and CD4-CD8+ T cells. This is achieved through two processes called positive and negative selections in thymus.

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Now the description of the above processes:

Positive Selection

This ensures MHC restriction of the T cells. This process takes place at the cortical region of the thymus. During positive selection, the RAG-1, RAG-2, and TdT proteins required for gene rearrangement and modification continue to be expressed. Thus each of the immature thymocytes in a clone expressing given b chain have an opportunity to rearrange different TCR a chain sequences, and are then selected for self-MHC recognition, i.e. only those cells whose ab TCR heterodimer recognizes a self- MHC molecule are selected for survival. Clones of cells, which fail to recognize the self- MHC will be eliminated by apoptosis within 3 to 4 days.

Negative Selection

The population of thymocytes that survive positive selection comprise some cells with low affinity for self-antigens presented by self-MHC molecules and other cells with high affinity receptors. The latter group undergoes negative selection by an interaction with thymic stromal cells. During negative selection, dendritic cells and macrophages, bearing class I and class II MHC molecules interact with thymocytes bearing high-affinity receptors for self-antigen plus self-MHC molecules or for self- MHC molecules alone. Cells that undergo negative selection are subjected to apoptosis. Tolerance to self-antigens encountered in the thymus is thereby brought about by elimination of T cells that arte reactive to these antigens. T cells also possess specialized surface receptors. But T cell receptors (TCRs) cannot recognize or bind to soluble antigens (antibodies), but antigens presented by MHC encoded protein. This happens in special kinds of cells called antigen-presenting cells (APCs), virus infected cells or grafts. T cells are meant for elimination of cancer or virus infected cells.

Membrane Receptors of T cells:

· CD 3

· CD 4 : Membrane glycoprotein molecule . Recognise Ags bound to class II MHC molecules.

· CD 8: Dimeric membrane glycoprotein. Recognise Ags bound to class I MHC molecules.

· CD 28: Receptor for co-stimulatory B 7 family of molecules present on B cells and other APCs.

· Cd45: Signal transduction molecule.

T cells possessing CD4 receptors are called CD4+ and they function as TH cells and are class II restricted. CD8+ cells are class I restricted and act as Tc cells. Ratio of TH : Tc is 2:1 in normal human beings in peripheral blood. Alterations occur in case of immunodeficiency diseases, autoimmune diseases or other disorders.

Natural Killer Cells (NK cells). They are large, granular lymphocytes, which lack surface markers of T or B cells. They have 3 other kinds of surface receptors.

· Fc receptors: Recognise IgG (Fc g R III).

· Killer activator receptors (KARs).
· Killer inhibitory receptors (KIRs).

TABLE 2-5 Common CD markers used to distinguish functional lymphocyte subpopulations

CD designation*	Function	B cell	T CELL T_H	T CELL T_C	NK cell
CD2	Adhesion molecule; signal transduction	–	+	+	+
CD3	Signal-transduction element of T-cell receptor	–	+	+	–
CD4	Adhesion molecule that binds to class II MHC molecules; signal transduction	–	+ (usually)	– (usually)	–
CD5	Unknown	+	+ (subset)	+	–
CD8	Adhesion molecule that binds to class I MHC molecules; signal transduction	–	– (usually)	+ (usually)	+ (variable)
CD16 (FcγRIII)	Low-affinity receptor for Fc region of IgG	–	–	–	+
CD21 (CR2)	Receptor for complement (C3d) and Epstein-Barr virus	+	–	–	–
CD28	Receptor for co-stimulatory B7 molecule on antigen-presenting cells	–	+	+	–
CD32 (FcγRII)	Receptor for Fc region of IgG	+	–	–	–
CD35 (CR1)	Receptor for complement (C3b)	+	–	–	–
CD40	Signal transduction	+	–	–	–
CD45	Signal transduction	+	+	+	+
CD56	Adhesion molecule	–	–	–	+

*Synonyms are shown in parentheses.

They constitute 5-10 % of lymphocytes in peripheral blood. They attack tumour cells and cells infected with some but all viruses. NK cells use novel mechanisms for recognizing their target cells. Distinguishing normal and abnormal (i. e. infected/altered/tumor). KIR receptors biding class1 MHC molecules perform this. Once they bind class1 MHC they give negative signal for NK cell and preventing its cytotoxic steps. Infection by some viruses and mutation in tumour cells reduce class1MHC molecules on cells concerned and that makes them prone to the attack of cytotoxic activity of NK cells. KAR molecules are associated with other molecules, which contain ITAMS (immuno receptor tyrosin based action motifs). On activation by KAR binding ITAMS containing proteins initiate release of cytotoxic molecules from NK cells.

TABLE 2-1 Some transcription factors essential for hematopoietic lineages

Factor	Dependent lineage
GATA-1	Erythroid
GATA-2	Erythroid, myeloid, lymphoid
PU.1	Erythroid (maturational stages), myeloid (later stages), lymphoid
BM11	Myeloid, lymphoid
Ikaros	Lymphoid
Oct-2	B lymphoid (differentiation of B cells into plasma cells)

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6. Primary and secondary lymphoid organs – bone marrow, thymus, spleen, lymph nodes, CALT, MALT.

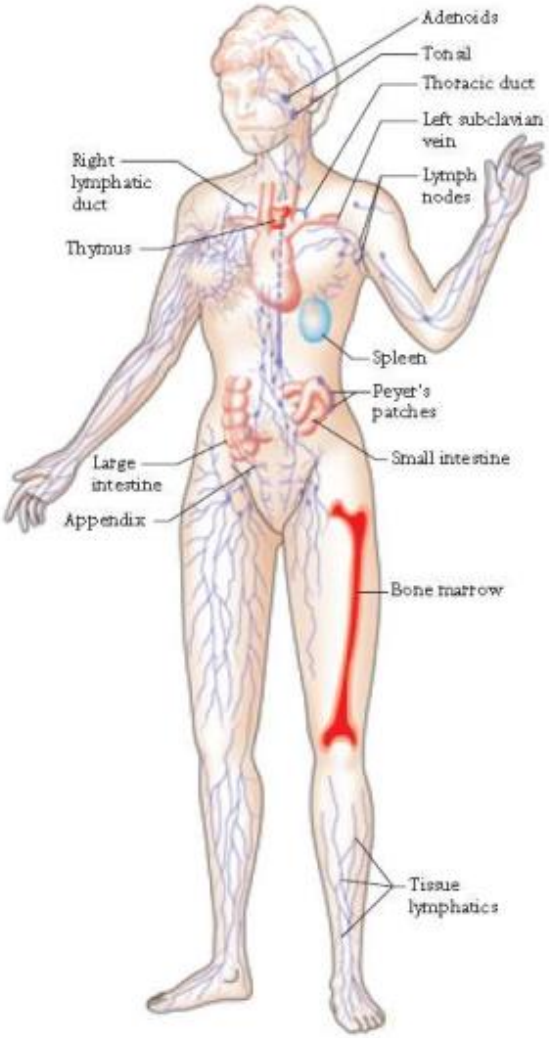

FIGURE 2-13 The human lymphoid system. The primary organs (bone marrow and thymus) are shown in red; secondary organs and tissues, in blue. These structurally and functionally diverse lymphoid organs and tissues are interconnected by the blood vessels (not shown) and lymphatic vessels (purple) through which lymphocytes circulate. Only one bone is shown, but all major bones contain marrow and thus are part of the lymphoid system. [*Adapted from H. Lodish et al., 1995, Molecular Cell Biology, 3rd ed., Scientific American Books.*]

Organs of the Immune System

A number of morphologically and functionally diverse organs and tissues have various functions in the development of immune responses. These can be distinguished by function as the **primary** and **secondary lymphoid organs**. The thymus and bone marrow are the primary

(or central) lymphoid organs, where maturation of lymphocytes takes place. The lymph nodes, spleen, and various mucosal associated lymphoid tissues (MALT) such as gut-associated lymphoid tissue (GALT) are the secondary (or peripheral) lymphoid organs, which trap antigen and provide sites for mature lymphocytes to interact with that antigen. In addition, **tertiary lymphoid tissues,** which normally contain fewer lymphoid cells than secondary lymphoid organs, can import lymphoid cells during an inflammatory response. Most prominent of these are cutaneous-associated lymphoid tissues. Once mature lymphocytes have been generated in the primary lymphoid organs, they circulate in the blood and **lymphatic system,** a network of vessels that collect fluid that has escaped into the tissues from capillaries of the circulatory system and ultimately return it to the blood.

Primary Lymphoid Organs

Immature lymphocytes generated in hematopoiesis mature and become committed to a particular antigenic specificity within the primary lymphoid organs. Only after a lympho cyte has matured within a primary lymphoid organ is the cell **immunocompetent** (capable of mounting an immune response). T cells arise in the **thymus,** and in many mammals—humans and mice for example—B cells originate in **bone marrow.**

THYMUS

The thymus is the site of T-cell development and maturation. It is a flat, bilobed organ situated above the heart. Each lobe is surrounded by a capsule and is divided into lobules, which are separated from each other by strands of connective tissue called trabeculae. Each lobule is organized into two compartments: the outer compartment, or *cortex,* is densely packed with immature T cells, called thymocytes, whereas the inner compartment, or *medulla,* is sparsely populated with thymocytes. Both the cortex and medulla of the thymus are crisscrossed by a three-dimensional stromal-cell network composed of epithelial cells, dendritic cells, and macrophages, which make up the framework of the organ and contribute to the growth and maturation of thymocytes. Many of these stromal cells interact physically with the developing thymocytes (Figure 2-14). Some thymic epithelial cells in the outer cortex, called **nurse cells,** have long membrane extensions that surround as many as 50 thymocytes, forming large multicellular complexes. Other cortical epithelial cells have long interconnecting cytoplasmic extensions that form a network and have been shown to interact with numerous thymocytes as they traverse the cortex. The function of the thymus is to generate and select a repertoire of T cells that will protect the body from infection. As thymocytes develop, an enormous diversity of T-cell receptors is generated by a random process that produces some T cells with receptors capable of recognizing antigen-MHC complexes.However, most of the T-cell receptors produced by this random process are incapable of recognizing antigen-MHC complexes and small portion react with combinations of self antigen-MHC complexes. The thymus induces the death of those T cells that cannot recognize antigen- MHC complexes and those that react with self-antigen MHC and pose a danger of causing autoimmune disease. More than 95% of all thymocytes die by apoptosis in the thymus without ever reaching maturity.

lymphatic capillaries and then into a series of progressively larger collecting vessels called **lymphatic vessels**.

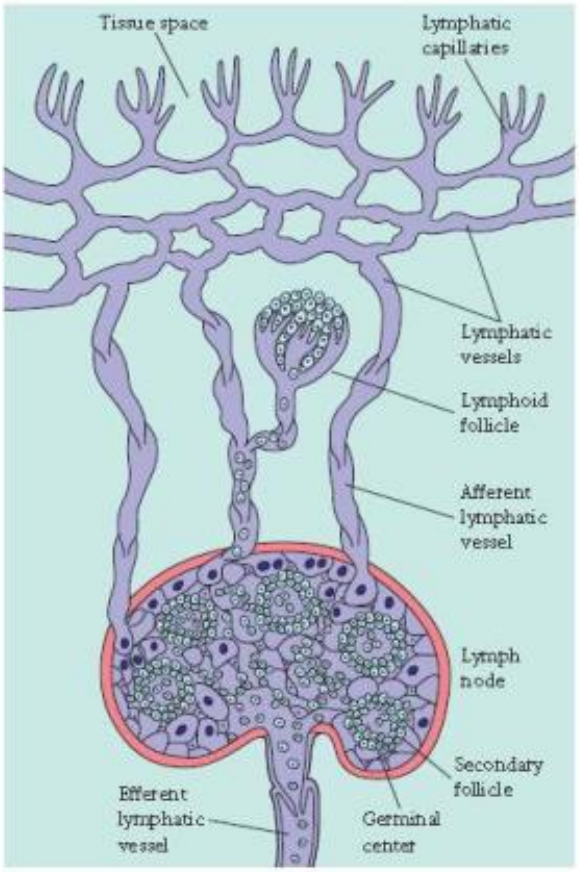

FIGURE 2-16 Lymphatic vessels. Small lymphatic capillaries opening into the tissue spaces pick up interstitial tissue fluid and carry it into progressively larger lymphatic vessels, which carry the fluid, now called lymph, into regional lymph nodes. As lymph leaves the nodes, it is carried through larger efferent lymphatic vessels, which eventually drain into the circulatory system at the thoracic duct or right lymph duct (see Figure 2-13).

The largest lymphatic vessel, the **thoracic duct,** empties into the left subclavian vein near the heart. In this way, the lymphatic system captures fluid lost from the blood and returns it to the blood, thus ensuring steady-state levels of fluid within the circulatory system. The heart does not pump the lymph through the lymphatic system; instead the flow of lymph is achieved as the lymph vessels are squeezed by movements of the body's muscles. A series of one-way valves along the lymphatic vessels ensures that lymph flows only in one direction. When a foreign antigen gains entrance to the tissues, it is picked up by the lymphatic system (which drains all the tissues of the body) and is carried to various organized lymphoid tissues such as lymph nodes, which trap the foreign antigen. As lymph passes from the tissues to lymphatic vessels, it becomes progressively enriched in lymphocytes. Thus, the lymphatic system also serves as a means of transporting lymphocytes and antigen from the connective tissues to organized lymphoid tissues where the lymphocytes may interact with the trapped antigen and undergo activation.

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Lymph nodes and the **spleen** are the most highly organized of the secondary lymphoid organs; they comprise not only lymphoid follicles, but additional distinct regions of Tcell and B-cell activity, and they are surrounded by a fibrous capsule. Less-organized lymphoid tissue, collectively called mucosal-associated lymphoid tissue (MALT), is found in various body sites. MALT includes Peyer's patches (in the small intestine), the tonsils, and the appendix, as well as numerous lymphoid follicles within the lamina propria of the intestines and in the mucous membranes lining the upper airways, bronchi, and genital tract.