18MBP301 ADVANCED IMMUNOLOGY 4H – 2C

Instruction Hours / Week: L: 4 T: 0P: 0 **Marks:** Internal: **40** External: **60**
Total: **100** **End Semester Exam**: 3 **Hours**

COURSE OBJECTIVES

Imparting advanced technological knowledge through a detailed study of topics such as immunodiagnosis, assessment of cell mediated immunity and current trends in immunology of diseases.

COURSE OUTCOME (CO'S)

To strengthen the knowledge of students through a detailed study on the immune system, their structure and classification, genetic control of antibody production, Types, structure of antigens and immunodiagnostics, Molecular immunology, hypersensitive immune reaction and Latest trends in immunology

UNIT – I

Immunity – types. Cells of the immune system - lymphoid cells, mononuclear cells, granulocytic cells and mast cells. T & B – cell maturation, activation and differentiation. Organs of the immune system - primary and secondary lymphoid organs – cutaneous / mucosal - associated lymphoid tissues

UNIT – II

Antigens - factor influence immunogenicity - Epitopes - Haptens - study of antigenicity. Immunoglobulins – structure – types and biological activities. Antigenic determinants. Monoclonal antibodies.

UNIT – III

Hypersensitive reactions – Type. Complement system - classical, alternative and lectin pathways, biological consequences. T - cell receptor. Cytokines – Structure, functions and receptors. Major Histocompatibility complex, classes, structure and its functions.

UNIT – IV

Autoimmune diseases: Antigen processing and presentation - Transplantation immunology - Transplantation antigens, HLA typing. Tumor immunology - treatment of tumors. Immune response to infectious disease.

UNIT – V

Antigen - Antibody reactions: Agglutination and precipitation. Immunoelectrophoresis, Complement fixation test, Immunofluorescence, ELISA, RIA, Immuno electron microscopy. Forensic serology, Immunohaematology – ABO, RH incompatibility.

SUGGESTED READINGS

TEXT BOOKS

1. Ananthanarayanan, R., and Panicker, C.K.J., (2007). *Text Book of Microbiology*. Orient Longman. New Delhi.
2. Coleman, R.M., Lombard, M.F., and Sicard, R.E., (2000). *Fundamental Immunology* (4nd ed.). Wm. C. Publishers. London.
3. Fathima, D., and Arumugam, N., (2005). *Immunology*. Saras Publications, Nagercoil.

REFERENCES

1. Coleman, R.M., Lombard, M.F., and Sicard, R.E., (2000). *Fundamentals of Immunology* (4th ed.). WMC Publications. London.
2. Goldsby, R.A., Barbara, T.J.K., and Osborne, A., (2006). *Kuby Immunology*. (6th ed.). W.H. Freeman and Company, New York.
3. Hyde, R.M. (2000). NMS - *Immunology*. (4th ed.). Lippincott Williams and Wilkins, Baltimore.
4. Janeway, Jr. C.A., Walport, P.T.M., and Shlomchick, M.J., (2001). *Immunobiology - The Immune System in Health and Disease*. (5th ed.). Churchill Livingstone - Garland Publishing Company, New York.
5. Pathaka, S., and Palan, U., (2005). *Immunology – Essentials and Fundamentals*. (2nd ed.). Capital Publishing Company, New Delhi.
6. Roitt, I.M., Brostaff, J.J., and Male, D.K., (2002). *Immunology*. (6th ed.). C.V. Mosby Publishers. St. Louis.
7. Delves, P., Martin, S., Burton, D., and Roitt, I., (2006). *Roitt's Essential Immunology*, Wiley-Blackwell, London

II –M.Sc Microbiology (Batch 2018-2020)

Lecture Plan

Unit - I

S. No	Duration	Topic	Reference
1.	1	Immunity –Definition, Concept of immunity and types of immunity.	R1: 4 to 13 T1: 64 to 79
2.	1	Cells of the immune system-lymphocytes and mononuclear cells-Types and functions.	R1: 24 to 38 R2: 297 to 312
3.	1	Cells of the immune system-Granulocytes and Mast cells-Types and functions.	R1: 38 to 43 R2: 297 to 312
4.	1	Maturation, activation and differentiation of T and B lymphocytes.	W1: page 2(7) R1: 39 to 40
5.	1	Organs of the Immune system-Primary lymphoid organs-Thymus and Bone marrow.	R1: 43 to 46 T1: 117 to 132
6.	1	Organs of the Immune system-Secondary lymphoid organs-cutaneous associated lymphoid organs-structure and function.	R1: 46 to 54
7.	1	Mucosal associated lymphoid organs-structure and function.	R2: 414 to 415
8.	1	Video presentation of immune system and their mechanism.	W1: page 1 to 9(7)
9.	1	Class Test I	-
		Total Hours	**9**

T1: Ananthanaryanan, R and C. K. J Panicker, 2004. Text book of Microbiology-Orient Longman, New Delhi. (7th Edition).

R1:Kuby, J., R.A. Goldsby, T.J.Kindt and B.A. Osborne, 2003.Immunology, 5th Edition. W.H. Freeman and Company, New York.

R2: Louise Hawley, Mary Rucbush, Don. J. Dunn, 2004. USMLE Step1, Lecture Notes- Microbiology and Immunology, Kaplan Medical Inc, USA.

W1: Human Physiology – Immune system; http://janux.ou.edu. (Created by University of Oklahoma; Dr. Heather).

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Lecture Plan

Unit - II

S. No	Duration	Topic	Reference
1.	2	Antigen-Definition, types and properties, Factors influencing immunogenicity, processing and presentation of antigens.	R1: 57 to 58 R2: 345 to 358 T1: 80 to 84
2.	2	Epitopes and Haptens-Role in antigenicity and their study.	R1: 68 to 70 R2: 403 to 419
3.	1	Immunoglobulins-Basic structure and derivation of fine structure	R1: 76 to 86 T1: 84 to 89
4.	1	Types of immunoglobulins and their functions.	R1: 87 to 94 W1: page 8(1)
5.	1	Antigenic determinants and their role in immunity.	R1: 95 to 96
6.	2	Monoclonal Antibodies –production and application.	R1: 95 to 96 R2 : 110 to 111
7.	1	Class test - II	-
		Total Hours	**10**

T1:Ananthanaryanan, R and C. K. J Panicker, 2004. Text book of Microbiology-Orient Longman, New Delhi. (7th Edition).

R1:Kuby, J., R.A. Goldsby, T.J.Kindt and B.A. Osborne, 2003.Immunology, 5th Edition. W.H. Freeman and Company, New York.

R2: Louise Hawley, Mary Rucbush, Don. J. Dunn, 2004. USMLE Step1, Lecture Notes- Microbiology and Immunology, Kaplan Medical Inc, USA.

W1: http://www.biologyexams4u.com/2011/09/immunology-notes.html.

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Lecture Plan

Unit - III

S. No	Duration	Topic	Reference
1.	1	Complements-Introduction, Properties- Classical and Alternative Pathways.	R1: 299 to 308 T1: 110 to 111
2.	1	Complement pathways-Lectin pathway and biological consequences.	R1: 309 to 316 T1: 113 to 114
3.	1	Hypersensitivity reactions: Introduction- Types of Hypersensitivity reaction –Type I and II- mechanisms	R1: 361 to 385
4.	1	Types of Hypersensitivity reaction –Type III and IV- mechanisms	R2: 422 to 492
5.	2	Cytokines –Structure, function and receptors.	T1: 159 to 168 R2: 464 to 466
6.	2	T- cell receptors and MHC types.	R1: 474 to 478
7.	1	Video presentation on hypersensitivity reaction	W1: page (1) W2: page 17(8)
8.	1	Class test III	-
		Total Hours	**10**

T1:Ananthanaryanan, R and C. K. J Panicker, 2004. Text book of Microbiology-Orient Longman, New Delhi. (7th Edition).

R1:Kuby, J., R.A. Goldsby, T.J.Kindt and B.A. Osborne, 2003.Immunology, 5th Edition. W.H. Freeman and Company, New York.

R2: Roitt, I.M., J.J.Brostaff and D.K. Male, 2002. Immunology, 6th edition, CV Mosby International Ltd. St. Louis.

W1: www.shomusbiology.com/immunology/hypersenitivity.html.

W2: www.harvard.edu/lecturenotes/imm-comp2add.index

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Lecture Plan

Unit –IV

S. No	Duration	Topic	Reference
1.	1	Introduction to autoimmune diseases – organ specific autoimmune diseases.	R1: 459 to 463 T1: 152 to 158
2.	1	Systemic auto immune diseases – Antigen processing and presentation.	R1: 464 to 478 T1:168 to 176
3.	2	Transplantation Immunology – Graft Vs Host reaction – Transplantation antigens.	R1: 478 to 493 R2: 342 to 365
4.	1	HLA typing.	W1: page 1-3(2)
5.	2	Tumor Immunology- Tumor antigens and treatment of tumors	R1: 504 to 518 R2: 401 to 411
6.	2	Immune response to infectious diseases.	R2: 414 to 421
7.	1	Class test IV	-
		Total Hours	**10**

T1:Ananthanaryanan, R and C. K. J Panicker, 2004. Text book of Microbiology-Orient Longman, New Delhi. (7th Edition).

R1:Kuby, J., R.A. Goldsby, T.J.Kindt and B.A. Osborne, 2003.Immunology, 5th Edition. W.H. Freeman and Company, New York.

R2: Roitt, I.M., J.J.Brostaff and D.K. Male, 2002. Immunology, 6th edition, CV Mosby International Ltd. St. Louis.

W1: www.shomusbiology.com/immunology/cancerbiology/part1to5.html.

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Lecture Plan

Unit - V

S. No	Duration	Topic	Reference
1.	1	Antigen-antibody interactions: principles and applications. Agglutination and precipitation reaction and its properties.	R1: 137 to 139 T1: 91 to 109 R1: 144 to 146 T1: 94 to 101
2.	1	Complement fixation test –Direct and Indirect methods.	R1: 146 to 147 R2: 3 to 105
3.	1	Immunofluorescence – ELISA and types.	R1: 149 to 150
4.	1	Radio Immuno assay principle and procedure.	R1: 152 to 154
5.	1	Immuno electron microscopy – principle and procedure.	W1: page 4(1)
6.	1	Forensic serology and application.	W1: page 6(8)
7.	1	Immunohaematology –ABO grouping and Rh Incompatibility.	T1: 184 to 191 W1: page 10(8)
8.	2	Revision of previous year question papers	-
		Total Hours	**9**

T1:Ananthanaryanan, R and C. K. J Panicker, 2004. Text book of Microbiology-Orient Longman, New Delhi. (7th Edition).

R1:Kuby, J., R.A. Goldsby, T.J.Kindt and B.A. Osborne, 2003.Immunology, 5th Edition. W.H. Freeman and Company, New York.

R2: Roitt, I.M., J.J.Brostaff and D.K. Male, 2002. Immunology, 6th edition, CV Mosby International Ltd. St. Louis.

W1: www.edx.org/books/immunohaematology/x:index.php

UNIT I

The Immune system is remarkably versatile defense system that has evolved to protect animals from invading pathogenic microorganisms and cancer. It is able to generate an enormous variety of cells and molecules capable of specifically recognizing and eliminating an apparently limitless variety of foreign invaders. These cells and molecules act together in a dynamic network whose complexity rivals that of the nervous system.

Functionally, an immune response can be divided into two related activities—recognition and response. Immune recognition is remarkable for its specificity. The immune system is able to recognize subtle chemical differences that distinguish one foreign pathogen from another. Furthermore, the system is able to discriminate between foreign molecules and the body's own cells and proteins. Once a foreign organism has been recognized, the immune system recruits a variety of cells and molecules to mount an appropriate response, called an effector response, to eliminate or neutralize the organism. In this way the system is able to convert the initial recognition event into a variety of effector responses, each uniquely suited for eliminating a particular type of pathogen. Later exposure to the same foreign organism induces a memory response, characterized by a more rapid and heightened immune reaction that serves to eliminate the pathogen and prevent disease.

Elements of the primitive immune system persist in vertebrates as *innate immunity* along with a more highly evolved system of specific responses termed *adaptive immunity*. These two systems work in concert to provide a high degree of protection for vertebrate species. Finally, in some circumstances, the immune system fails to act as protector because of some deficiency in its components; at other times, it becomes an aggressor and turns its awesome powers against its own host.

Innate Immunity

Innate immunity can be seen to comprise four types of defensive barriers: anatomic, physiologic, phagocytic, and inflammatory

The skin and the mucosal surfaces provide protective barriers against infection

Physical and anatomic barriers that tend to prevent the entry of pathogens are an organism's first line of defense against infection. The skin and the surface of mucous membranes are included in this category because they are effective barriers to the entry of most microorganisms. The skin consists of two distinct layers: a thinner outer layer—the epidermis—and a thicker layer—the dermis. The epidermis contains several layers of tightly packed epithelial cells. The outer epidermal layer consists of dead cells and is filled with a waterproofing protein called keratin. The dermis, which is composed of connective tissue, contains blood vessels, hair follicles, sebaceous glands, and sweat glands. The sebaceous glands are associated with the hair follicles and produce an oily secretion called sebum. Sebum consists of lactic acid and fatty acids, which maintain the pH of the skin between 3 and 5; this pH inhibits the growth of most microorganisms. A few bacteria that metabolize sebum live as commensals on the skin and sometimes cause a severe form of acne. One acne drug, isotretinoin (Accutane), is a vitamin A derivative that prevents the formation of sebum.

Breaks in the skin resulting from scratches, wounds, or abrasion are obvious routes of infection. The skin may also be penetrated by biting insects (e.g., mosquitoes, mites, ticks, fleas, and sandflies); if these harbor pathogenic organisms, they can introduce the pathogen into the body as they feed. The protozoan

that causes malaria, for example, is deposited in humans by mosquitoes when they take a blood meal. Similarly, bubonic plague is spread by the bite of fleas, and Lyme disease is spread by the bite of ticks

The conjunctivae and the alimentary, respiratory, and urogenital tracts are lined by mucous membranes, not by the dry, protective skin that covers the exterior of the body. These membranes consist of an outer epithelial layer and an underlying layer of connective tissue. Although many pathogens enter the body by binding to and penetrating mucous membranes, a number of nonspecific defense mechanisms tend to prevent this entry. For example, saliva, tears, and mucous secretions act to wash away potential invaders and also contain antibacterial or antiviral substances. The viscous fluid called mucus, which is secreted by epithelial cells of mucous membranes, entraps foreign microorganisms. In the lower respiratory tract, the mucous membrane is covered by cilia, hairlike protrusions of the epithelial-cell membranes. The synchronous movement of cilia propels mucus-entrapped microorganisms from these tracts. In addition, nonpathogenic organisms tend to colonize the epithelial cells of mucosal surfaces. These *normal flora* generally outcompete pathogens for attachment sites on the epithelial cell surface and for necessary nutrients.

Some organisms have evolved ways of escaping these defense mechanisms and thus are able to invade the body through mucous membranes. For example, influenza virus (the agent that causes flu) has a surface molecule that enables it to attach firmly to cells in mucous membranes of the respiratory tract, preventing the virus from being swept out by the ciliated epithelial cells. Similarly, the organism that causes gonorrhea has surface projections that allow it to bind to epithelial cells in the mucous membrane of the urogenital tract. Adherence of bacteria to mucous membranes is due to interactions between hairlike protrusions on a bacterium, called fimbriae or pili, and certain glycoproteins or glycolipids that are expressed only by epithelial cells of the mucous membrane of particular tissues. For this reason, some tissues are susceptible to bacterial invasion, whereas others are not.

Physiologic barriers to infection include general conditions and specific molecules

The physiologic barriers that contribute to innate immunity include temperature, pH, and various soluble and cellassociated molecules.Many species are not susceptible to certain diseases simply because their normal body temperature inhibits growth of the pathogens. Chickens, for example, have innate immunity to anthrax because their high body temperature inhibits the growth of the bacteria. Gastric acidity is an innate physiologic barrier to infection because very few ingested microorganisms can survive the low pH of the stomach contents. One reason newborns are susceptible to some diseases that do not afflict adults is that their stomach contents are less acid than those of adults. A variety of soluble factors contribute to innate immunity, among them the soluble proteins lysozyme, interferon, and complement. Lysozyme, a hydrolytic enzyme found in mucous secretions and in tears, is able to cleave the peptidoglycan layer of the bacterial cell wall. Interferon comprises a group of proteins produced by virus-infected cells. Among the many functions of the interferons is the ability to bind to nearby cells and induce a generalized antiviral state.Complement, examined in detail in Chapter 13, is a group of serum proteins that circulate in an inactive state. A variety of specific and nonspecific immunologic mechanisms can convert the inactive forms of complement proteins into an active state with the ability to damage the membranes of pathogenic organisms, either destroying the pathogens or facilitating their clearance. Complement may function as an effector system that is triggered by binding of antibodies to certain cell surfaces, or it may be activated by reactions between complement molecules and certain components of microbial cell walls. Reactions between complement molecules or fragments of complement molecules and cellular receptors trigger activation of cells of the innate or adaptive immune systems. Recent studies on collectins indicate

that these surfactant proteins may kill certain bacteria directly by disrupting their lipid membranes or, alternatively, by aggregating the bacteria to enhance their susceptibility to phagocytosis. Many of the molecules involved in innate immunity have the property of pattern recognition, the ability to recognize a given class of molecules.Because there are certain types of molecules that are unique to microbes and never found in multicellular organisms, the ability to immediately recognize and combat invadersdisplaying such molecules is a strong feature of innate immunity.Molecules with pattern recognition ability may be soluble, like lysozyme and the complement components described above, or they may be cell-associated receptors. Among the class of receptors designated the toll-like receptors (TLRs), TLR2 recognizes the lipopolysaccharide (LPS) found on Gram-negative bacteria. It has long been recognized that systemic exposure of mammals to relatively small quantities of purified LPS leads to an acute inflammatory response. The mechanism for this response is via a TLR on macrophages that recognizes LPS and elicits a variety of molecules in the inflammatory response upon exposure.When the TLR is exposed to the LPS upon local invasion by a Gram-negative bacterium, the contained response results in elimination of the bacterial challenge.

Cells that ingest and destroy pathogens make up a phagocytic barrier to infection

Another important innate defense mechanism is the ingestion of extracellular particulate material by phagocytosis. Phagocytosis is one type of endocytosis, the general term for the uptake by a cell of material from its environment. In phagocytosis, a cell's plasma membrane expands around the particulate material, which may include whole pathogenic microorganisms, to form large vesicles called phagosomes. Most phagocytosis is conducted by specialized cells, such as blood monocytes, neutrophils, and tissue macrophages. Most cell types are capable of other forms of endocytosis, such as *receptor-mediated endocytosis,* in which extracellular 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.

Inflammation represents a complex sequence of events that stimulates immune responses

Tissue damage caused by a wound or by an invading pathogenic microorganism induces a complex sequence of events collectively known as the inflammatory response. As described above, a molecular component of a microbe, such as LPS, may trigger an inflammatory response via interaction with cell surface receptors. The end result of inflammation may be the marshalling of a specific immune response to the invasion or clearance of the invader by components of the innate immune system. Many of the classic features of the inflammatory response were described as early as 1600 BC, in Egyptian papyrus writings. In the first century AD, the Roman physician Celsus described the "four cardinal signs of inflammation" as *rubor* (redness), *tumor* (swelling), *calor* (heat), and *dolor* (pain). In the second century AD, another physician, Galen, added a fifth sign: *functio laesa* (loss of function). The cardinal signs of inflammation reflect the three major events of an inflammatory response:

1. *Vasodilation*—an increase in the diameter of blood vessels—of nearby capillaries occurs as the vessels that carry blood away from the affected area constrict, resulting in engorgement of the capillary network. The engorged capillaries are responsible for tissue redness (*erythema*) and an increase in tissue temperature.

2. An *increase in capillary permeability* facilitates an influx of fluid and cells from the engorged capillaries into the tissue. The fluid that accumulates (exudate) has much higher protein content than fluid

normally released from the vasculature. Accumulation of exudate contributes to tissue swelling (edema).

3. *Influx of phagocytes* from the capillaries into the tissues is facilitated by the increased permeability of the capillaries. The emigration of phagocytes is a multistep process that includes adherence of the cells to the endothelial wall of the blood vessels (margination), followed by their emigration between the capillaryendothelial cells into the tissue (diapedesis or extravasation), and, finally, their migration through the tissue to the site of the invasion (chemotaxis). As phagocytic cells accumulate at the site and begin to phagocytose bacteria, they release lytic enzymes, which can damage nearby healthy cells. The accumulation of dead cells, digested material, and fluid forms a substance called pus. 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. Some of these mediators are derived from invading microorganisms, some are released from damaged cells in response to tissue injury, some are generated by several plasma enzyme systems, and some are products of various white blood cells participating in the inflammatory response.Among the chemical mediators released in response to tissue damage are various serum proteins called acute-phase proteins. The concentrations of these proteins increase dramatically in tissue-damaging infections. C-reactive protein is a major acute-phase protein produced by the liver in response to tissue damage. Its name derives from its pattern recognition activity: C-reactive protein binds to the C- polysaccharide cell-wall component found on a variety of bacteria and fungi. This binding activates the complement system, resulting in increased clearance of the pathogen either by complement-mediated lysis or by a complement mediated increase in phagocytosis. One of the principal mediators of the inflammatory response is histamine, a chemical released by a variety of cells in response to tissue injury. Histamine binds to receptors on nearby capillaries and venules, causing vasodilation and increased permeability. Another important group of inflammatory mediators, small peptides called kinins, are normally present in blood plasma in an inactive form. Tissue injury activates these peptides, which then cause vasodilation and in- creased permeability of capillaries. A particular kinin, called bradykinin, also stimulates pain receptors in the skin. This effect probably serves a protective role, because pain normally causes an individual to protect the injured area.

Vasodilation and the increase in capillary permeability in an injured tissue also enable enzymes of the blood-clotting system to enter the tissue. These enzymes activate an enzyme cascade that results in the deposition of insoluble strands of fibrin, which is the main component of a blood clot. The fibrin strands wall off the injured area from the rest of the body and serve to prevent the spread of infection. Once the inflammatory response has subsided and most of the debris has been cleared away by phagocytic cells, tissue repair and regeneration of new tissue begins. Capillaries grow into the fibrin of a blood clot. New connective tissue cells, called fibroblasts, replace the fibrin as the clot dissolves. As fibroblasts and capillaries accumulate, scar tissue forms.

Adaptive Immunity

Adaptive immunity is capable of recognizing and selectively eliminating specific foreign microorganisms and molecules (i.e., foreign antigens). Unlike innate immune responses, adaptive immune responses are not the same in all members of a species but are reactions to specific antigenic challenges.

Adaptive immunity displays four characteristic attributes:

- Antigenic specificity
- Diversity
- Immunologic memory
- Self/nonself recognition

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.

The adaptive immune system requires cooperation between lymphocytes and antigen-presenting cells.

An effective immune response involves two major groups of cells: *T lymphocytes* and *antigen-presenting cells.* Lymphocytes are one of many types of white blood cells produced in the bone marrow by the process of hematopoiesis (see Chapter 2). Lymphocytes leave the bone marrow, circulate in the blood and lymphatic systems, and reside in various lymphoid organs. Because they produce and display antigenbinding cell-surface receptors, lymphocytes mediate the defining immunologic attributes of specificity, diversity, memory, and self/nonself recognition. The two major populations of lymphocytes—B lymphocytes (B cells) and T lymphocytes (T cells).

B LYMPHOCYTES

B lymphocytes mature within the bone marrow; when they leave it, each expresses a unique antigen-binding receptor on its membrane. This antigen-binding or B-cell receptor is a membrane-bound antibody molecule. Antibodies are glycoproteins that consist of two identical heavy polypeptide chains and two identical light polypeptide chains. Each heavy chain is joined with a light chain by disulfide bonds, and additional disulfide bonds hold the two pairs together. The amino-terminal ends of the pairs of heavy and light chains form a cleft within which antigen binds. When a naive B cell (one that has not previously encountered antigen) first encounters the antigen that matches its membrane bound antibody, the binding of the antigen to the antibody causes the cell to divide rapidly; its progeny differentiate into memory B cells and effector B cells called plasma cells. Memory B cells have a longer life span than naive cells, and they express the same membrane-bound antibody as their parent B cell. Plasma cells produce the antibody in a form that can be secreted and have little or no membrane-bound antibody. Although plasma cells live for only a few days, they secrete enormous amounts of antibody during this time. It has been estimated that a single plasma cell can secrete more than 2000 molecules of antibody per second. Secreted antibodies are the major effector molecules of humoral immunity.

T LYMPHOCYTES

T lymphocytes also arise in the bone marrow. Unlike B cells, which mature within the bone marrow, T cells migrate to the thymus gland to mature. During its maturation within the thymus, the T cell comes to express a unique antigen-binding molecule, called the T-cell receptor, on its membrane.Unlike membrane-bound antibodies on B cells,which can recognize antigen alone, T-cell receptors can recognize only antigen that is bound to cell-membrane proteins called major histocompatibility complex (MHC) molecules. MHC molecules that function in this recognition event,which is termed "antigen presentation," are polymorphic (genetically diverse) glycoproteins found on cell membranes. There are two major types of MHC molecules: Class I MHC molecules, which are expressed by nearly all nucleated cells of vertebrate species, consist of a heavy chain linked to a small invariant protein called _2-microglobulin. Class II MHC molecules, which consist of an alpha and a beta glycoprotein chain, are expressed only by antigen-presenting cells.When a naive T cell encounters antigen combined with a MHC molecule on a cell, the T cell proliferates and differentiates into memory T cells and various effector T cells. There are two well-defined subpopulations of T cells: T helper (TH) and T cytotoxic (TC) cells.Although a third type of T cell, called a T suppressor (TS) cell, has been postulated, recent evidence suggests that it may not be distinct from TH and TC subpopulations. T helper and T cytotoxic cells can be distinguished from one another by the presence of either CD4 or CD8 membrane glycoproteins on their surfaces. T cells displaying CD4 generally function as TH cells, whereas those displaying CD8 generally function as TC cells.

After a TH cell recognizes and interacts with an antigen– MHC class II molecule complex, the cell is activated—it becomes an effector cell that secretes various growth factors known collectively as cytokines. The secreted cytokines play an important role in activating B cells, TC cells, macrophages, and various other cells that participate in the immune response. Differences in the pattern of cytokines produced by activated TH cells result in different types of immune response. Under the influence of TH-derived cytokines, a TC cell that recognizes an antigen–MHC class I molecule complex proliferates and differentiates into an effector cell called a cytotoxic T lymphocyte (CTL). In contrast to the TC cell, the CTL generally does not secrete many cytokines and instead exhibits cell-killing or cytotoxic activity. The CTL has a vital function in monitoring the cells of the body and eliminating any that display antigen, such as virus-infected cells, tumor cells, and cells of a foreign tissue graft. Cells that display foreign antigen complexed with a class I MHC molecule are called altered self-cells; these are targets of CTLs.

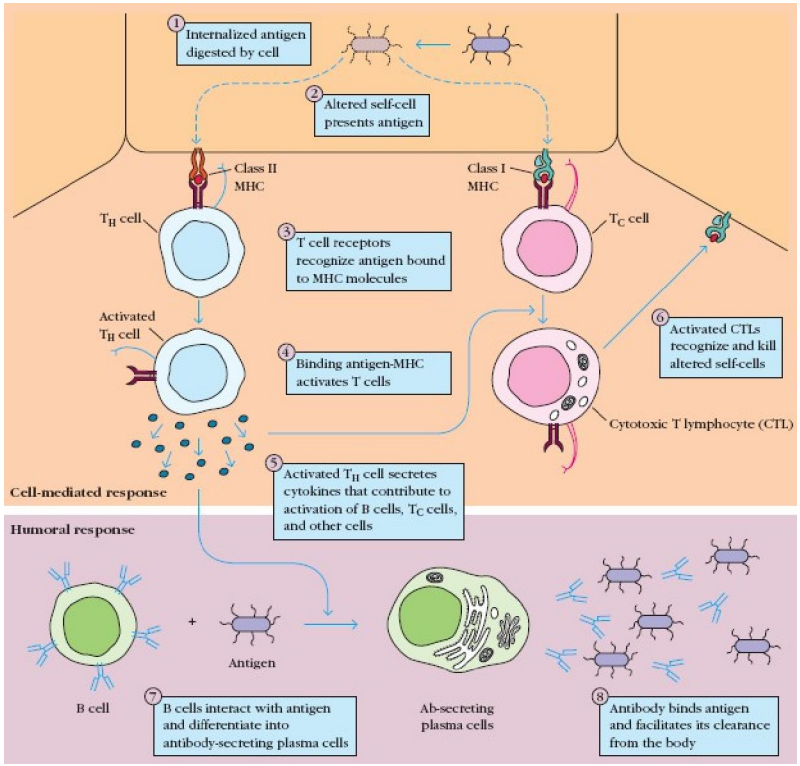
ANTIGEN-PRESENTING CELLS

Activation of both the humoral and cell-mediated branches of the immune system requires cytokines produced by TH cells. It is essential that activation of TH cells themselves be carefully regulated, because an inappropriate T-cell response to self-components can have fatal autoimmune consequences. To ensure carefully regulated activation of TH cells, they can recognize only antigen that is displayed together with class MHC II molecules on the surface of antigen-presenting cells (APCs). These specialized cells, which include macrophages, B lymphocytes, and dendritic cells, are distinguished by two properties:

(1) they express class II MHC molecules on their membranes, and

(2) they are able to deliver a co-stimulatory signal that is necessary for TH-cell activation.

Antigen-presenting cells first internalize antigen, either by phagocytosis or by endocytosis, and then display a part of that antigen on their membrane bound to a class II MHC molecule. The TH cell recognizes and interacts with the antigen–class II MHC molecule complex on the membrane of the antigen-presenting cell. An additional costimulatory signal is then produced by the antigen-presenting cell, leading to activation of the TH cell.



Overview of thehumoral and cell- mediated branches of the immune system. In the humoral response, B cells interact with antigen and then differentiate into antibody-secreting plasma cells. The secreted antibody binds to the antigen and facilitates its clearance from the body. In the cell-mediated response, various subpopulations of T cells recognize antigen presented on self-cells. TH cells respond to antigen by producing cytokines. TC cells respond to antigen by developing into cytotoxic T lymphocytes (CTLs), which mediate killing of altered self-cells (e.g., virus-infected cells).

The immune system

The immune system consists of many different organs and tissues that are found throughout the body. These organs can be classified functionally into two main groups. The *primary lymphoid organs* provide appropriate microenvironments for the development and maturation of lymphocytes. The *secondary lymphoid organs* trap antigen from defined tissues or vascular spaces and are sites where mature lymphocytes can interact effectively with that antigen. Blood vessels and lymphatic systems connect these organs, uniting them into a functional whole. 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

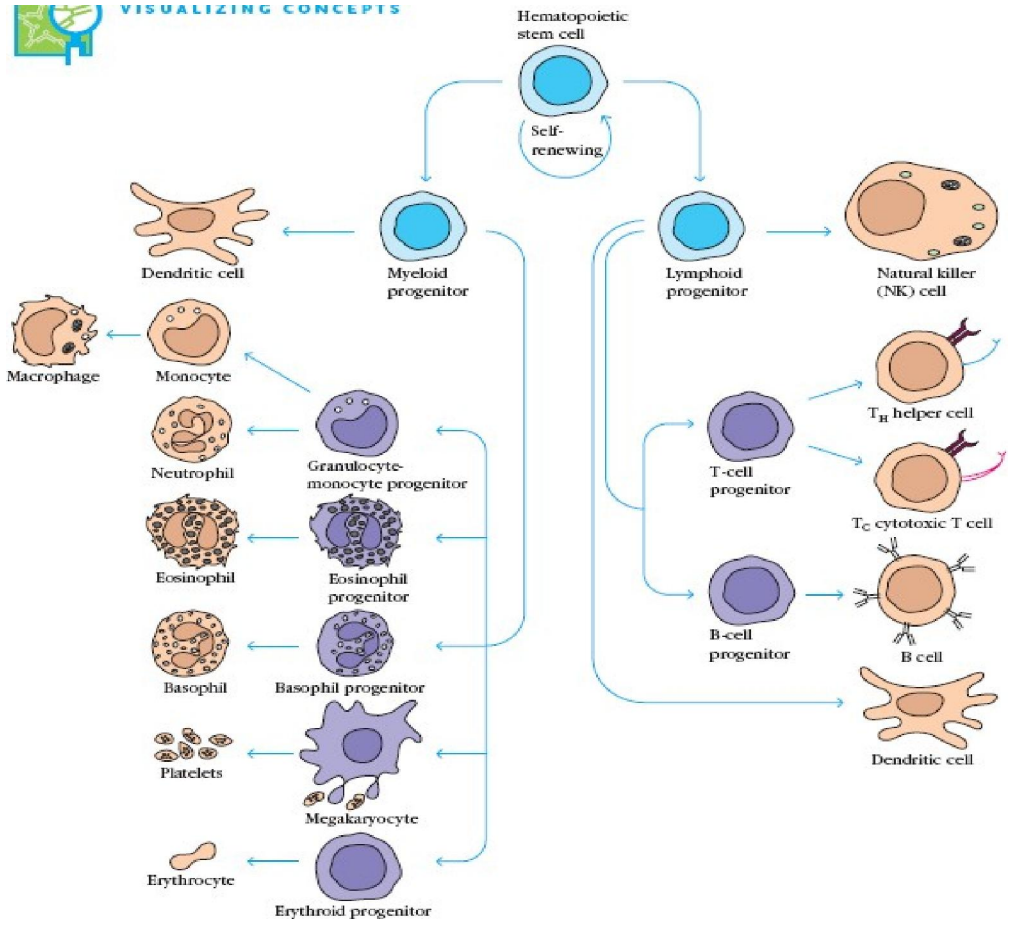
functions of the lymphoid organs.

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. It is remarkable that every functionally specialized, mature blood cell is derived from the same type of stem cell. In contrast to a *unipotent* cell, which differentiates into a single cell type, 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 _ 104 cells in the bone marrow. The study of hematopoietic stem cells is difficult both because of their scarcity and because they are hard to grow in vitro.As a result, little is known about how their proliferation and differentiation are regulated. By virtue of their capacity for self-renewal, hematopoietic stem cells are maintained at stable levels throughout adult life; however, when there is an increased demand for hematopoiesis, HSCs display an enormous proliferative capacity. This can be demonstrated in mice whose hematopoietic systems have been completely destroyed by a lethal dose of x-rays (950 rads; one rad represents the absorption by an irradiated target of an amount of radiation corresponding to 100 ergs/gram of target). Such irradiated mice will die within 10 days unless they are infused with normal bone-marrow cells from a syngeneic (genetically identical) mouse. Although a normal mouse has 3 X 10^8 bone-marrow cells, infusion of only 10^4–10^5 bone-marrow cells (i.e., 0.01%–0.1% of the normal amount) from a donor is sufficient to completely restore the hematopoietic system, which demonstrates the enormous proliferative and differentiative capacity of the stem cells.

Early in hematopoiesis, a multipotent stem cell differentiates along one of two pathways, giving rise to either a common lymphoid progenitor cell or a common myeloid progenitor cell. The types and amounts of growth factors in the microenvironment of a particular stem cell or progenitor cell control its differentiation. During the development of the lymphoid and myeloid lineages, stem cells differentiate into progenitor cells, which have lost the capacity for self-renewal and are committed to a particular cell lineage. Common lymphoid progenitor cells give rise to B, T, and NK (natural killer) cells and some dendritic cells.Myeloid stem cells generate progenitors of red blood cells (erythrocytes), many of the various white blood cells (neutrophils, eosinophils, basophils, monocytes, mast cells, dendritic cells), and platelets.Progenitor commitment depends on the acquisition of responsiveness to particular growth factors and cytokines. When the appropriate factors and cytokines are present, progenitor cells proliferate and differentiate into the corresponding cell type, either a mature erythrocyte, a particular type of leukocyte, or a platelet-generating cell (the megakaryocyte). Red and white blood cells pass into bonemarrow channels, from which they enter the circulation. In bone marrow, hematopoietic cells grow and mature on a meshwork of stromal cells, which are nonhematopoietic cells that support the growth and differentiation of hematopoietic cells. Stromal cells include fat cells, endothelial cells, fibroblasts, and macrophages. Stromal cells influence the differentiation of hematopoietic stem cells by providing a hematopoietic-inducing microenvironment (HIM) consisting of a cellular matrix and factors that promote growth and

differentiation. Many of these hematopoietic growth factors are soluble agents that arrive at their target cells by diffusion, others are membrane-bound molecules on the surface of stromal cells that require cell-to-cell contact between the responding cells and the stromal cells. During infection, hematopoiesis is stimulated by the production of hematopoietic growth factors by activated macrophages and T cells.



Cells of the Immune System

Lymphocytes are the central cells of the immune system, responsible for adaptive immunity and the immunologic attributes of diversity, specificity, memory, and self/nonself recognition. The other types of white blood cells play important roles, engulfing and destroying microorganisms, presenting antigens, and secreting cytokines.

Lymphoid Cells

Lymphocytes constitute 20%–40% of the body's white blood cells and 99% of the cells in the lymph. There are approximately 1011 (range depending on body size and age: ~1010–1012) lymphocytes in the human body. These lymphocytes continually circulate in the blood and lymph and are capable of migrating into the tissue spaces and lymphoid organs, thereby integrating the immune system to a high degree.

The lymphocytes can be broadly subdivided into three populations—B cells, T cells, and natural killer cells—on the basis of function and cell-membrane components. Natural killer cells (NK cells) are large, granular lymphocytes that do not express the set of surface markers typical of B or T cells. Resting B and T lymphocytes are small, motile, nonphagocytic cells, which cannot be distinguished morphologically. B and T lymphocytes that have not interacted with antigen— referred to as naive, or unprimed—are resting cells in the G0 phase of the cell cycle. Known as small lymphocytes, these cells are only about 6 _m in diameter; their cytoplasm forms a barely discernible rim around the nucleus. Small lymphocytes have densely packed chromatin, few mitochondria, and a poorly developed endoplasmic reticulum and Golgi apparatus. The naive lymphocyte is generally thought to have a short life span. Interaction of small lymphocytes with antigen, in the presence of certain cytokines discussed later, induces these cells to enter the cell cycle by progressing from G0 into G1 and subsequently into S, G2, and M. As they progress through the cell cycle, lymphocytes enlarge into 15 _m-diameter blast cells, called lymphoblasts; these cells have a higher cytoplasm: nucleus ratio and more organellar complexity than small lymphocytes.

Lymphoblasts proliferate and eventually differentiate into effector cells or into memory cells. Effector cells function in various ways to eliminate antigen. These cells have short life spans, generally ranging from a few days to a few weeks. Plasma cells—the antibody-secreting effector cells of the Bcell lineage—have a characteristic cytoplasm that contains abundant endoplasmic reticulum (to support their high rate of protein synthesis) arranged in concentric layers and also many Golgi vesicles. The effector cells of the T-cell lineage include the cytokine-secreting T helper cell (TH cell) and the T cytotoxic lymphocyte (TC cell). Some of the progeny of B and T lymphoblasts differentiate into memory cells. The persistence of this population of cells is responsible for life-long immunity to many pathogens. Memory cells look like small lymphocytes but can be distinguished from naive cells by the presence or absence of certain cellmembrane molecules. Different lineages or maturational stages of lymphocytes can be distinguished by their expression of membrane molecules recognized by particular monoclonal antibodies (antibodies that are specific for a single epitope of an antigen; see Chapter 4 for a description of monoclonal antibodies).All of the monoclonal antibodies that react with a particular membrane molecule are grouped together as a cluster of differentiation (CD). Each new monoclonal antibody that recognizes a leukocyte membrane molecule is analyzed for whether it falls within a recognized CD designation; if it does not, it is given a new CD designation reflecting a new membrane molecule. Although the CD nomenclature was originally developed for the membrane molecules of human leukocytes, the homologous membrane molecules of other species, such as mice, are commonly referred to by the same CD designations.

BLYMPHOCYTE

The B lymphocyte derived its letter designation from its site of maturation, in the bursa of Fabricius in birds; the name turned out to be apt, for *b*one marrow is its major site of maturation in a number of mammalian species, including humans and mice. Mature B cells are definitively distinguished from other lymphocytes by their synthesis and display of membrane-bound immunoglobulin (antibody) molecules, which serve as receptors for antigen. Each of the approximately 1.5 _ 105 molecules of antibody on the membrane of a single B cell has an identical binding site for antigen**.** Among the other molecules expressed on the membrane of mature B cells are the following: B220 (a form of CD45) is frequently used as a marker for B cells and their precursors. However, unlike antibody, it is not expressed uniquely by B-lineage cells.

Class II MHC molecules permit the B cell to function as an antigen-presenting cell (APC).CR1 (CD35) and CR2 (CD21) are receptors for certain complement products. FcγRII (CD32) is a receptor for IgG, a type of antibody.B7-1 (CD80) and B7-2 (CD86) are molecules that interact with CD28 and CTLA-4, important regulatory molecules on the surface of different types of T cells, including TH cells.CD40 is a molecule that interacts with CD40 ligand on the surface of helper T cells.In most cases this interaction is critical for the survival of antigenstimulated B cells and for their development into antibody-secreting plasma cells or memory B cells. Interaction between antigen and the membrane-bound antibody on a mature naive B cell, as well as interactions with T cells and macrophages, selectively induces the activation and differentiation of B-cell clones of corresponding specificity. In this process, the B cell divides repeatedly and differentiates over a 4- to 5-day period, generating a population of plasma cells and memory cells. Plasma cells, which have lower levels of membrane-bound antibody than B cells, synthesize and secrete antibody. All clonal progeny from a given B cell secrete antibody molecules with the same antigen-binding specificity. Plasma cells are terminally differentiated cells, and many die in 1 or 2 weeks.

T LYMPHOCYTES

T lymphocytes derive their name from their site of maturation in the thymus. Like B lymphocytes, these cells have membrane receptors for antigen. Although the antigenbinding T-cell receptor is structurally distinct from immunoglobulin, it does share some common structural features with the immunoglobulin molecule, most notably in the structure of its antigen-binding site. Unlike the membrane- bound antibody on B cells, though, the T-cell receptor (TCR) does not recognize free antigen. Instead the TCR recognizes only antigen that is bound to particular classes of self-molecules. Most T cells recognize antigen only when it is bound to a self-molecule encoded by genes within the major histocompatibility complex (MHC). Thus, as explained in Chapter 1, a fundamental difference between the humoral and cell-mediated branches of the immune system is that the B cell is capable of binding soluble antigen,whereas the T cell is restricted to binding antigen displayed on self-cells. To be recognized by most T cells, this antigen must be displayed together with MHC molecules on the surface of antigen-presenting cells or on virus-infected cells, cancer cells, and grafts. The T-cell system has developed to eliminate these altered self-cells,which pose a threat to the normal functioning of the body. Like B cells, T cells express distinctive membrane molecules. All T-cell subpopulations express the T-cell receptor, a complex of polypeptides that includes CD3; and most can be distinguished by the presence of one or the other of two membrane molecules, CD4 and CD8. In addition, most mature T cells express the following membrane molecules CD28, a receptor for the co-stimulatory B7 family of molecules present on B cells and other antigenpresenting cell CD45, a signal-transduction molecule T cells that express the membrane glycoprotein molecule CD4 are restricted to recognizing antigen bound to class II MHC molecules, whereas T cells expressing CD8, a dimeric membrane glycoprotein, are restricted to recognition of antigen bound to class I MHC molecules. Thus the expression of CD4 versus CD8 corresponds to the

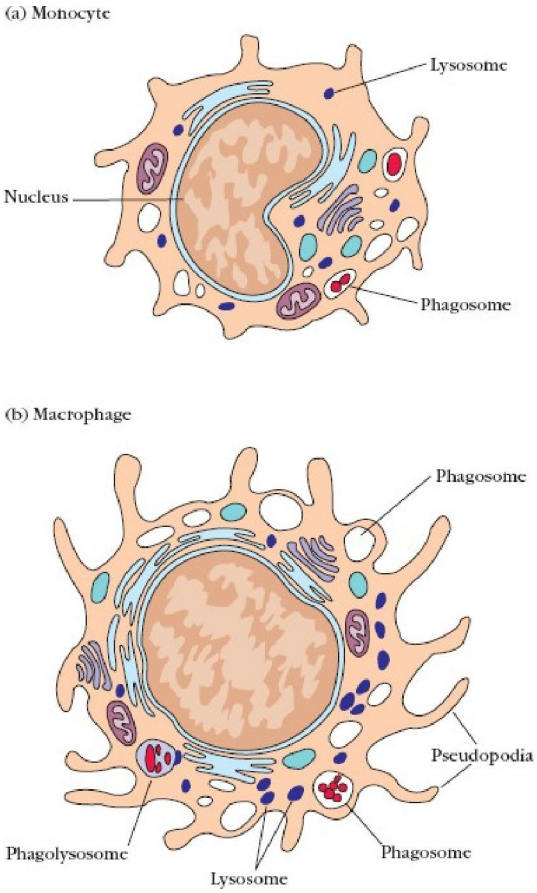
MHC restriction of the T cell. In general, expression of CD4 and of CD8 also defines two major functional subpopulations of T lymphocytes. CD4_ T cells generally function as T helper (TH) cells and are class-II restricted; CD8_ T cells generally function as T cytotoxic (TC) cells and are class-I restricted. Thus the ratio of TH to TC cells in a sample can be approximated by assaying the number of CD4_ and CD8_ T cells. This ratio is approximately 2:1 in normal human peripheral blood, but it may be significantly altered by immunodeficiency diseases, autoimmune diseases, and other disorders. The classification of CD4_ class II–restricted cells as TH cells and CD8_ class I–restricted cells as TC cells is not absolute. Some CD4_ cells can act as killer cells. Also, some TC cells have been shown to secrete a variety of cytokines and exert an effect on other cells comparable to that exerted by TH cells. The distinction between TH and TC cells, then, is not always clear; there can be ambiguous functional activities. However, because these ambiguities are the exception and not the rule, the generalization of T helper (TH) cells as being CD4_ and class-II restricted and of T cytotoxic cells (TC) as being CD8_ and class-I restricted is assumed throughout, unless otherwise specified.

TH cells are activated by recognition of an antigen–class II MHC complex on an antigen-presenting cell. After activation, the TH cell begins to divide and gives rise to a clone of effector cells, each specific for the same antigen–class II MHC complex. These TH cells secrete various cytokines, which play a central role in the activation of B cells, T cells, and other cells that participate in the immune response. Changes in the pattern of cytokines produced by TH cells can change the type of immune response that develops among other leukocytes. The TH1 response produces a cytokine profile that supports inflammation and activates mainly certain T cells and macrophages, whereas the TH2 response activates mainly B cells and immune responses that depend upon antibodies. TC cells are activated when they interact with an antigen–class I MHC complex on the surface of an altered self-cell (e.g., a virus-infected cell or a tumor cell) in the presence of appropriate cytokines. This activation, which results in proliferation, causes the TC cell to differentiate into an effector cell called a cytotoxic T lymphocyte (CTL). In contrast to TH cells, most CTLs secrete few cytokines. Instead, CTLs acquire the ability to recognize and eliminate altered self-cells. Another subpopulation of T lymphocytes—called T suppressor (TS) cells—has been postulated. It is clear that some T cells help to suppress the humoral and the cell-mediated branches of the immune system, but the actual isolation and cloning of normal TS cells is a matter of controversy and dispute among immunologists. For this reason, it is uncertain whether TS cells do indeed constitute a separate functional subpopulation of T cells. Some immunologists believe that the suppression mediated by T cells observed in some systems is simply the consequence of activities of TH or TC subpopulations whose end results are suppressive.

NATURAL KILLER CELLS

The natural killer cell was first described in 1976, when it was shown that the body contains a small population of large, granular lymphocytes that display cytotoxic activity against a wide range of tumor cells in the absence of any previous immunization with the tumor. NK cells were subsequently shown to play an important role in host defense both against tumor cells and against cells infected with some, though not all, viruses. These cells, which constitute 5%–10% of lymphocytes in human peripheral blood, do not express the membrane molecules and receptors that distinguish T- and B-cell lineages.

Although NK cells do not have T-cell receptors or immunoglobulin incorporated in their plasma



membranes, they can recognize potential target cells in two different ways. In some cases, an NK cell employs NK cell receptors to distinguish abnormalities, notably a reduction in the display of class I MHC molecules and the unusual profile of surface antigens displayed by some tumor cells and cells infected by some viruses.Another way in which NK cells recognize potential target cells depends upon the fact that some tumor cells and cells infected by certain viruses display antigens against which the immune system has made an antibody response, so that antitumor or antiviral antibodies are bound to their surfaces. Because NK cells express CD16, a membrane receptor for the carboxyl-terminal end of the IgG molecule, called the Fc region, they can attach to these antibodies and subsequently destroy the targeted cells. This is an example of a process known as antibody-dependent cellmediated cytotoxicity (ADCC). The exact mechanism of NK-cell cytotoxicity, the focus of much current experimental study, is described further in Chapter 14. Several observations suggest that NK cells play an important role in host defense against tumors. For example, in humans the Chediak-Higashi syndrome—an autosomal recessive disorder—is associated with impairment in neutrophils, macrophages, and NK cells and an increased incidence of lymphomas. Likewise, mice with an autosomal mutation called beige lack NK cells; these mutants are more susceptible than normal mice to tumor growth following injection with live tumor cells. There has been growing recognition of a cell type, the NK1-T cell, that has some of the characteristics of both T cells and NK cells. Like T cells, NK1-T cells have T cell receptors (TCRs).Unlike most T cells, the TCRs of NK1-T cells interact with MHC-like

molecules called CD1 rather than with class I or class II HC molecules. Like NK cells, they have variable levels of CD16 and other receptors typical of NK cells, and they can kill cells. A population of triggered NK1-T cells can rapidly secrete large amounts of the cytokines needed to support antibody production by B cells as well as inflammation and the development and expansion of cytotoxic T cells. Some immunologists view this cell type as a kind of rapid response system that has evolved to provide early help while conventional TH responses are still developing.

Mononuclear Phagocytes

The mononuclear phagocytic system consists of monocytes circulating in the blood and macrophages in the tissues (Figure 2-8). During hematopoiesis in the bone marrow, granulocyte-monocyte progenitor cells differentiate into promonocytes, which leave the bone marrow and enter the blood, where they further differentiate into mature monocytes. Monocytes circulate in the bloodstream for about 8 h, during which they enlarge; they then migrate into the tissues and differentiate into specific tissue macrophages or, as discussed later, into dendritic cells.

Differentiation of a monocyte into a tissue macrophage involves a number of changes: The cell enlarges five- to tenfold; its intracellular organelles increase in both number and complexity; and it acquires increased phagocytic ability, produces higher levels of hydrolytic enzymes, and begins to secrete a variety of soluble factors. Macrophages are dispersed throughout the body. Some take up residence in particular tissues, becoming fixed macrophages, whereas others remain motile and are called free, or wandering, macrophages. Free macrophages travel by amoeboid movement throughout the tissues.Macrophage-like cells serve different functions in different tissues and are named according to their tissue location:

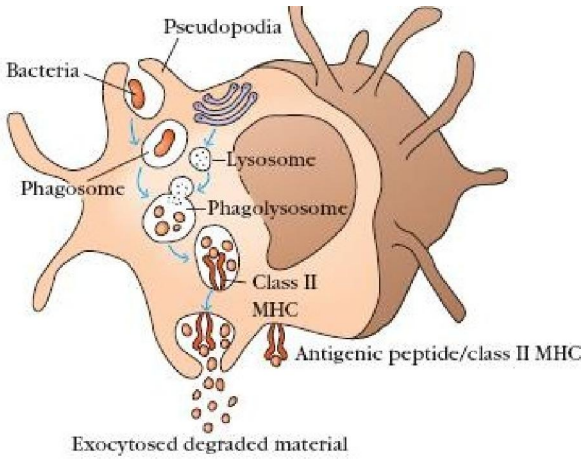
Alveolar macrophages in the lung
Histiocytes in connective tissues
Kupffer cells in the liver
Mesangial cells in the kidney
Microglial cells in the brain
Osteoclasts in bone

Although normally in a resting state, macrophages are activated by a variety of stimuli in the course of an immune response. Phagocytosis of particulate antigens serves as an initial activating stimulus. However, macrophage activity can be further enhanced by cytokines secreted by activated TH cells, by mediators of the inflammatory response, and by components of bacterial cell walls. One of the most potent activators of macrophages is interferon gamma (IFN-γ) secreted by activated TH cells. Activated macrophages are more effective than resting ones in eliminating potential pathogens, because they exhibit greater phagocytic activity, an increased ability to kill ingested microbes, increased secretion of inflammatory mediators, and an increased ability to activate T cells. In addition, activated macrophages, but not resting ones, secrete various cytotoxic proteins that help them eliminate a broad range of pathogens, including virus-infected cells, tumor cells, and intracellular bacteria. Activated macrophages also express higher levels of class II MHC molecules, allowing them to function more effectively as antigen-presenting cells. Thus, macrophages and TH cells facilitate each other's activation during the immune response.

Phagocytosis

Macrophages are capable of ingesting and digesting exogenous antigens, such as whole microorganisms and insoluble particles, and endogenous matter, such as injured or dead host cells, cellular debris, and

activated clotting factors. In the first step in phagocytosis, macrophages are attracted by and move toward a variety of substances generated in an immune response; this process is called chemotaxis. The next step in phagocytosis is adherence of the antigen to the macrophage cell membrane. Complex antigens, such as whole bacterial cells or viral particles, tend to adhere well and are readily phagocytosed; isolated proteins and encapsulated bacteria tend to adhere poorly and are less readily phagocytosed. Adherence induces membrane protrusions, called pseudopodia, to extend around the attached material. Fusion of the pseudopodia encloses the material within a membrane-bounded structure called a phagosome, which then enters the endocytic processing pathway. In this pathway, a phagosome moves toward the cell interior, where it fuses with a lysosome to form a phagolysosome. Lysosomes contain lysozyme and a variety of other hydrolytic enzymes that digest the ingested material. The digested contents of the phagolysosome are then eliminated in a process called exocytosis. The macrophage membrane has receptors for certain classes of antibody. If an antigen (e.g., a bacterium) is coated with the appropriate antibody, the complex of antigen and antibody binds to antibody receptors on the macrophage membrane more readily than antigen alone and phagocytosis is enhanced. In one study, for example, the rate of phagocytosis of an antigen was 4000-fold higher in the presence of specific antibody to the antigen than in its absence. Thus, antibody functions as an opsonin, a molecule that binds to both antigen and macrophage and enhances phagocytosis. The process by which particulate antigens are rendered more susceptible to phagocytosis is called opsonization.



ANTIMICROBIAL AND CYTOTOXIC ACTIVITIES

A number of antimicrobial and cytotoxic substances produced by activated macrophages can destroy phagocytosed microorganisms.

OXYGEN-DEPENDENT KILLING MECHANISMS

Activated phagocytes produce a number of reactive oxygen intermediates (ROIs) and reactive nitrogen intermediates that have potent antimicrobial activity. During phagocytosis, a metabolic process known as

the respiratory burst occurs in activated macrophages. This process results in the activation of a membrane-bound oxidase that catalyzes the reduction of oxygen to superoxide anion, a reactive oxygen intermediate that is extremely toxic to ingested microorganisms. The superoxide anion also generates other powerful oxidizing agents, including hydroxyl radicals and hydrogen peroxide. As the lysosome fuses with the phagosome, the activity of myeloperoxidase produces hypochlorite from hydrogen per oxide and chloride ions. Hypochlorite, the active agent of household bleach, is toxic to ingested microbes. When macrophages are activated with bacterial cell-wall components such as lipopolysaccharide (LPS)

or, in the case of mycobacteria, muramyl dipeptide (MDP), together with a T-cell–derived cytokine (IFN-γ), they begin to express high levels of nitric oxide synthetase (NOS), an enzyme that oxidizes L-arginine to yield L-citrulline and nitric oxide (NO), a gas:

L-arginine + O2 + NADPH →NO + L-citrulline + NADP

Nitric oxide has potent antimicrobial activity; it also can combine with the superoxide anion to yield even more potent antimicrobial substances. Recent evidence suggests that much of the antimicrobial activity of macrophages against bacteria, fungi, parasitic worms, and protozoa is due to nitric oxide and substances derived from it.

OXYGEN-INDEPENDENT KILLING MECHANISMS

Activated macrophages also synthesize lysozyme and various hydrolytic enzymes whose degradative activities do not require oxygen. In addition, activated macrophages produce a group of antimicrobial and cytotoxic peptides, commonly known as defensins. These molecules are cysteine-rich cationic peptides containing 29–35 amino-acid residues. Each peptide, which contains six invariant cysteines, forms a circular molecule that is stabilized by intramolecular disulfide bonds. These circularized defensin peptides have been shown to form ion-permeable channels in bacterial cell membranes. Defensins can kill a variety of bacteria, including Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli, Pseudomonas aeruginosa, and Haemophilus influenzae. Activated
macrophages also secrete tumor necrosis factor α (TNF-α), a cytokine that has a variety of effects and is cytotoxic for some tumor cells.

ANTIGEN PROCESSING AND PRESENTATION

Although most of the antigen ingested by macrophages is degraded and eliminated, experiments with radiolabeled antigens have demonstrated the presence of antigen peptides on the macrophage membrane. Phagocytosed antigen is digested within the endocytic processing pathway into peptides that associate with class II MHC molecules; these peptide–class II MHC complexes then move to the macrophage membrane. Activation of macrophages induces increased expression of both class II MHC molecules and the co-stimulatory B7 family of membrane molecules, thereby rendering the macrophages more effective in activating TH cells.

SECRETION OF FACTORS

A number of important proteins central to development of immune responses are secreted by activated macrophages. These include a collection of cytokines, such as interleukin 1 (IL-1), TNF-α and interleukin 6 (IL-6), that promote inflammatory responses. Typically, each of these agents has a variety of effects. For example, IL-1 activates lymphocytes; and IL-1, IL-6, and TNF-α promote fever by affecting the

thermoregulatory center in the hypothalamus. Activated macrophages secrete a variety of factors involved in the development of an inflammatory response. The complement proteins are a group of proteins that assist in eliminating foreign pathogens and in promoting the ensuing inflammatory reaction. The major site of synthesis of complement proteins is the liver, although these proteins are also produced in macrophages. The hydrolytic enzymes contained within the lysosomes of macrophages also can be secreted when the cells are activated. The buildup of these enzymes within the tissues contributes to the inflammatory response and can, in some cases, contribute to extensive tissue damage. Activated macrophages also secrete soluble factors, such as TNF-α, that can kill a variety of cells. The secretion of these cytotoxic factors has been shown to contribute to tumor destruction by macrophages. Finally, as mentioned earlier, activated macrophages secrete a number of cytokines that stimulate inducible hematopoiesis.

Granulocytic Cells

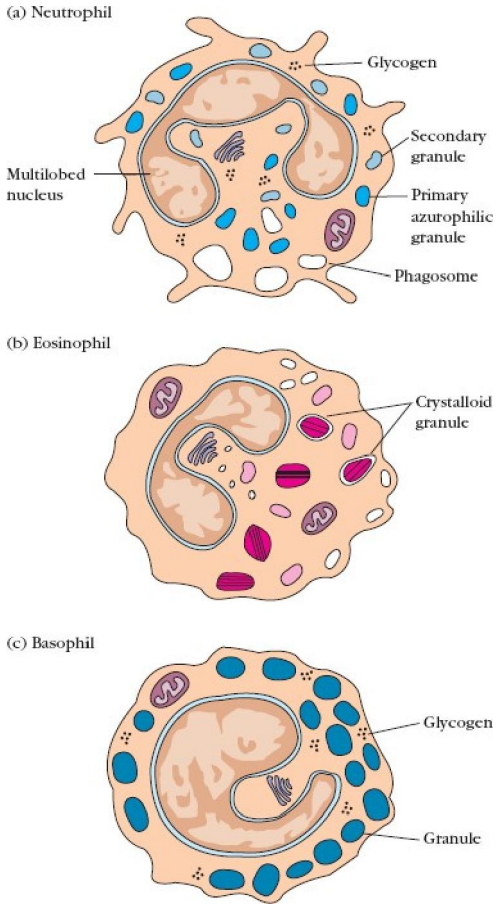
The granulocytes are classified as neutrophils, eosinophils, or basophils on the basis of cellular morphology and cytoplasmic staining characteristics. The neutrophil has a multilobed nucleus and a granulated cytoplasm that stains with both acid and basic dyes; it is often called a polymorphonuclear leukocyte (PMN) for its multilobed nucleus. The eosinophil has a bilobed nucleus and a granulated cytoplasm that stains with the acid dye eosin red (hence its name). The basophil has a lobed nucleus and heavily granulated cytoplasm that stains with the basic dye methylene blue. Both neutrophils and eosinophils are phagocytic, whereas basophils are not. Neutrophils, which constitute 50%–70% of the circulating white blood cells, are much more numerous than eosinophils (1%–3%) or basophils (<1%).

NEUTROPHILS

Neutrophils are produced by hematopoiesis in the bone marrow. They are released into the peripheral blood and circulate for 7–10 h before migrating into the tissues, where they have a life span of only a few days. In response to many types of infections, the bone marrow releases more than the usual number of neutrophils and these cells generally are the first to arrive at a site of inflammation. The resulting transient increase in the number of circulating neutrophils, called leukocytosis, is used medically as an indication of infection. Movement of circulating neutrophils into tissues, called extravasation, takes several steps: the cell first adheres to the vascular endothelium, then penetrates the gap between adjacent endothelial cells lining the vessel wall, and finally penetrates the vascular basement membrane, moving out into the tissue spaces. A number of substances generated in an inflammatory reaction serve as chemotactic factors that promote accumulation of neutrophils at an inflammatory site. Among these chemotactic factors are some of the complement components, components of the blood-clotting system, and several cytokines secreted by activated TH cells and macrophages.

Like macrophages, neutrophils are active phagocytic cells. Phagocytosis by neutrophils is similar to that described for macrophages, except that the lytic enzymes and bactericidal substances in neutrophils are contained within primary and secondary granules. The larger, denser primary granules are a type of lysosome containing peroxidase, lysozyme, and various hydrolytic enzymes. The smaller secondary granules contain collagenase, lactoferrin, and lysozyme. Both primary and secondary granules fuse with phagosomes, whose contents are then digested and eliminated much as they are in macrophages. Neutrophils also employ both oxygen-dependent and oxygen-independent pathways to generate antimicrobial substances. Neutrophils are in fact much more likely than macrophages to kill ingested microorganisms. Neutrophils exhibit a larger respiratory burst than macrophages and consequently are

able to generate more reactive oxygen intermediates and reactive nitrogen intermediates. In addition, neutrophils express higher levels of defensins than macrophages do.



EOSINOPHILS

Eosinophils, like neutrophils, are motile phagocytic cells that can migrate from the blood into the tissue spaces. Their phagocytic role is significantly less important than that of neutrophils, and it is thought that they play a role in the defense against parasitic organisms. The secreted contents of eosinophilic granules may damage the parasite membrane.

BASOPHILS

Basophils are nonphagocytic granulocytes that function by releasing pharmacologically active substances from their cytoplasmic granules. These substances play a major role in certain allergic responses.

MAST CELLS

Mast-cell precursors, which are formed in the bone marrow by hematopoiesis, are released into the blood as undifferentiated cells; they do not differentiate until they leave the blood and enter the tissues.Mast cells can be found in a wide variety of tissues, including the skin, connective tissues of various organs, and mucosal epithelial tissue of the respiratory, genitourinary, and digestive tracts. Like circulating

basophils, these cells have large numbers of cytoplasmic granules that contain histamine and other harmacologically active substances. Mast cells, together with blood basophils, play an important role in the development of allergies.

DENDRITIC CELLS

The dendritic cell (DC) acquired its name because it is covered with long membrane extensions that resemble the dendrites of nerve cells. Dendritic cells can be difficult to isolate because the conventional procedures for cell isolation tend to damage their long extensions. The development of isolation techniques that employ enzymes and gentler dispersion has facilitated isolation of these cells for study in vitro. There are many types of dendritic cells, although most mature dendritic cells have the same major function, the presentation of antigen to TH cells. Four types of dendritic cells are known: Langerhans cells, interstitial dendritic cells, myeloid cells, and lymphoid dendritic cells. Each arises from hematopoietic stem cells via different pathways and in different locations. Despite their differences, they all constitutively express high levels of both class II MHC molecules and members of the co-stimulatory B7 family. For this reason, they are more potent antigen-presenting cells than macrophages and B cells, both of which need to be activated before they can function as antigen-presenting cells (APCs). Immature or precursor forms of each of these types of dendritic cells acquire antigen by phagocytosis or endocytosis; the antigen is processed, and mature dendritic cells present it to TH cells. Following microbial invasion or during inflammation, mature and immature forms of Langerhans cells and interstitial dendritic cells migrate into draining lymph nodes, where they make the critical presentation of antigen to TH cells that is required for the initiation of responses by those key cells.

Another type of dendritic cell, the follicular dendritic cell, does not arise in bone marrow and has a different function from the antigen-presenting dendritic cells described above. Follicular dendritic cells do not express class II MHC molecules and therefore do not function as antigenpresenting cells for TH-cell activation. These dendritic cells were named for their exclusive location in organized structures of the lymph node called lymph follicles, which are rich in B cells. Although they do not express class II molecules, follicular dendritic cells express high levels of membrane receptors for antibody, which allows the binding of antigen-antibody complexes. The interaction of B cells with this bound antigen can have important effects on B cell responses.

ELL MATURATION, ACTIVATION, AND DIFFERENTIATION

T-Cell Maturation and the Thymus Progenitor T cells from the early sites of hematopoiesis begin to migrate to the thymus at about day 11 of gestation in mice and in the eighth or ninth week of gestation in humans. In a manner similar to B-cell maturation in the bone marrow, Tcellmaturation involves rearrangements of the germ-line TCR genes and the expression of various membrane markers.In the thymus, developing T cells, known as thymocytes, proliferate and differentiate along developmental pathways that generate functionally distinct subpopulations of mature T cells.

The thymus occupies a central role in T-cell biology.Aside from being the main source of all T cells, it is where T cells diversify and then are shaped into aneffective primary T-cell repertoire by an extraordinary pair of selection processes. One of these, positive selection, permits the survival of only those T cells whose TCRs are capable ofrecognizing self-MHC molecules. It is thus responsible for the creation of a self-MHC-restricted repertoire of T cells. The other, negative selection, eliminates T cells that react too strongly with self-MHC or with self-MHC plus selfpeptides. It is an extremely important factor in generating a primary T-cell repertoire that is self-tolerant.

When T-cell precursors arrive at the thymus, they do not express such signature surface markers of T cells as the T-cell receptor, the CD3 complex, or the coreceptors CD4 and CD8. In fact, these progenitor cells have not yet rearranged their TCR genes and do not express proteins, such as RAG-1 and RAG-2, that are required for rearrangement. After arriving at the thymus, these T-cell precursors enter theouter cortex and slowly proliferate.During approximately three weeks of development in the thymus, the differentiating T cells progress through a series of stages that are marked by characteristic changes in their cellsurface phenotype. For example, as mentioned previously, thymocytes early in development lack detectable CD4 and CD8. Because these cells are CD4_CD8_, they are referred to as double-negative (DN) cells. Even though these coreceptors are not expressed during the DN early stages, the differentiation program is progressing and is marked by changes in the expression of such cell surface molecules as c-Kit, CD44, and CD25. The initial thymocyte population displays c-Kit, the receptor for stem-cell growth factor, and CD44, an adhesion molecule involved in homing; CD25, the _-chain of the IL-2 receptor, also appears on early-stage DN cells. During this period, the cells are proliferating but the TCR genes remain unrearranged. Then the cells stop expressing c-Kit, markedly reduce CD44 expression, turn on expression of the recombinase genes *RAG-1* and *RAG-2* and begin to rearrange their TCR genes. Although it is not shown in Figure 10-1, a small percentage (_5%) of thymocytes productively rearrange the γ- and δ-chain genes and develop into double-negative $CD3^+$ $\gamma\delta$T cells. In mice, this thymocyte subpopulation can be detected by day 14 of gestation, reaches maximal numbers between days 17 and 18, and then declines until birth.

TH-Cell Activation

The central event in the generation of both humoral and cellmediated immune responses is the activation and clonal expansion of TH cells. Activation of TC cells, which is generally similar to TH-cell activation. THcell activation is initiated by interaction of the TCR-CD3 complex with a processed antigenic peptide bound to a class II MHC molecule on the surface of an antigen-presenting cell. This interaction and the resulting activating signals also involve various accessory membrane molecules on the TH cell and the antigen-presenting cell. Interaction of a TH cell with antigen initiates a cascade of biochemical events that induces the resting TH cell to enter the cell cycle, proliferating and differentiating into memory cells or effector cells.Many
of the gene products that appear upon interaction with antigencan be grouped into one of three categories depending on how early they can be detected after antigen recognition .

- *Immediate genes,* expressed within half an hour of antigen recognition, encode a number of transcription factors, including c-Fos, c-Myc, c-Jun, NFAT, and NF-κB
- *Early genes,* expressed within 1–2 h of antigen recognition, encode IL-2, IL-2R (IL-2 receptor), IL-3, IL-6, IFN-γ, and numerous other proteins
- *Late genes,* expressed more than 2 days after antigen recognition, encode various adhesion molecules

These profound changes are the result of signal-transduction pathways that are activated by the encounter between the TCR and MHC-peptide complexes. An overview of some of the basic strategies of cellular signaling will be useful backgroundfor appreciating the specific signaling pathways used by T cells.

T cells in the periphery. T cells that have not yet encountered antigen (naive T cells) are characterized by condensed chromatin, very little cytoplasm, and little transcriptional activity. Naïve T cells continually recirculate between the blood and lymph systems. During recirculation, naïve T cells reside in secondary

lymphoid tissues such as lymph nodes. If a naive cell does not encounter antigen in a lymph node, it exits through the efferent lymphatics, ultimately draining into the thoracic duct and rejoining the blood. It is estimated that each naive T cell recirculates from the blood to the lymph nodes and back again every 12–24 hours. Because only about 1 in 10^5 naive T cells is specific for any given antigen, this large-scale recirculation increases the chances that a naive T cell will encounter appropriate antigen.

Activated T Cells Generate Effector and Memory T Cells

If a naive T cell recognizes an antigen-MHC complex on an appropriate antigen-presenting cell or target cell, it will be activated, initiating a primary response. About 48 hours after activation, the naive T cell enlarges into a blast cell and begins undergoing repeated rounds of cell division. As described earlier, activation depends on a signal induced by engagement of the TCR complex and a co-stimulatory signal induced by the CD28-B7 interaction. These signals trigger entry of the T cell into the G1 phase of the cell cycle and, at the same time, induce transcription of the gene for IL-2 and the _ chain of the high-affinity IL-2 receptor. In addition, the co-stimulatory signal increases the half-life of the IL-2 mRNA. The increase in IL-2 transcription, together with stabilization of the IL-2 mRNA, increases IL-2 production by 100-fold in the activated T cell. Secretion of IL-2 and its subsequent binding to the high-affinity IL-2 receptor induces the activated naive T cell to proliferate and differentiate. T cells activated in this way divide 2–3 times per day for 4–5 days, generating a large clone of progeny cells, which differentiate into memory or effector T-cell populations. The various *effector T cells* carry out specialized functions such as cytokine secretion and B-cell help (activated CD4_ TH cells) and cytotoxic killing activity (CD8_ CTLs). Effector cells are derived from both naive and memory cells after antigen activation. Effector cells are short-lived cells, whose life spans range from a few days to a few weeks. The effector and naive populations express different cell-membrane molecules, which contribute to different recirculation patterns. $CD4^+$effector

T cells form two subpopulations distinguished by the different panels of cytokines they secrete. One population, called the **TH1 subset,** secretes IL-2, IFN-α, and TNF-β. The TH1 subset is responsible for classic cell-mediated functions, such as delayed-type hypersensitivity and the activation of cytotoxic T lymphocytes. The other subset, called the **TH2 subset,** secretes IL-4, IL-5, IL-6, and IL-10. This subset functions more effectively as a helper for B-cell activation. The *memory T-cell* population is derived from both naïve T cells and from effector cells after they have encountered antigen. Memory T cells are antigen-generated, generally long-lived, quiescent cells that respond with heightened reactivity to a subsequent challenge with the same antigen, generating a secondary response. An expanded population of memory T cells appears to remain long after the population of effector T cells has declined. In general, memory T cells express many of the same cell-surface markers as effector T cells; no cell-surface markers definitively identify them as memory cells.

Like naive T cells, most memory T cells are resting cells in the G0 stage of the cell cycle, but they appear to have less stringent requirements for activation than naive T cells do. For example, naive TH cells are activated only by dendritic cells, whereas memory TH cells can be activated by macrophages, dendritic cells, and B cells. It is thought that the expression of high levels of numerous adhesion molecules by memory TH cells enables these cells to adhere to a broad spectrum of antigen-presenting cells. Memory cells also display recirculation patterns that differ from those of naive or effector T cells.

B-CELL GENERATION, ACTIVATION, AND DIFFERENTIATION

B-Cell Maturation The generation of mature B cells first occurs in the embryo and continues throughout life. Before birth, the yolk sac, fetal liver, and fetal bone marrow are the major sites of B-cell maturation; after birth, generation of mature B cells occurs in the bone marrow.

Progenitor B Cells Proliferate in Bone Marrow

B-cell development begins as lymphoid stem cells differentiate into the earliest distinctive B-lineage cell—the progenitor B cell (pro-B cell)—which expresses a transmembrane tyrosine phosphatase called CD45R (sometimes called B220 in mice). Pro-B cells proliferate within the bone marrow, filling the extravascular spaces between large sinusoids in the shaft of a bone. Proliferation and differentiation of pro-B cells into precursor B cells (pre-B cells) requires the microenvironment provided by the bone-marrow stromal cells. If pro-B cells are removed from the bone marrow and cultured in vitro, they will not progress to more mature B-cell stages unless stromal cells are present. The stromal cells play two important roles: they interact directly with pro-B and pre-B cells, and they secrete various cytokines, notably IL-7, that support the developmental process.

At the earliest developmental stage, pro-B cells require direct contact with stromal cells in the bone marrow. This interaction is mediated by several cell-adhesion molecules, including VLA-4 on the pro-B cell and its ligand, VCAM-1, on the stromal cell. After initial contact is made, a receptor on the pro-B cell called c-Kit interacts with a stromal-cell surface molecule known as stem-cell factor (SCF). This interaction activates c-Kit, which is a tyrosine kinase, and the pro-B cell begins to divide and differentiate into a pre-B cell and begins expressing a receptor for IL-7. The IL-7 secreted by the stromal cells drives the maturation process, eventually inducing down-regulation of the adhesion molecules on the pre-B cells, so that the proliferating cells can detach from the stromal cells. At this stage, pre-B cells no longer require direct contact with stromal cells but continue to require IL-7 for growth and maturation.

Ig-Gene Rearrangment Produces Immature B Cells

B-cell maturation depends on rearrangement of the immunoglobulin DNA in the lymphoid stem cells. The mechanisms of Ig-gene rearrangement occur in the pro-B cell stage is a heavy-chain DH-to-JH gene rearrangement; this is followed by a VH-to-DHJH rearrangement. If the first heavy-chain rearrangement is not productive, then VH-DH-JH rearrangement continues on the other chromosome. Upon completion of heavy-chain rearrangement, the cell is classified as a pre-B cell. Continued development of a pre-B cell into an immature B cell requires a productive light-chain gene rearrangement. Because of allelic exclusion, only one light-chain isotype is expressed on the membrane of a B cell. Completion of a productive light-chain rearrangement commits the now immature B cell to a particular antigenic specificity determined by the cell's heavy-chain VDJ sequence and light-chain VJ sequence. Immature B cells express mIgM (membrane IgM) on the cell surface. As would be expected, the recombinase enzymes RAG-1 and RAG-2, which are required for both heavy-chain and light-chain gene rearrangements, are expressed during the pro-B and pre-B cell stages. The enzyme terminal deoxyribonucleotidyl transferase (TdT), which catalyzes insertion of N-nucleotides at the DH-JH and VH-DHJH coding joints, is active during the pro-B cell stage and ceases to be active early in the pre–B-cell stage. Because TdT expression is turned off during the part of the pre–B-cell stage when light-chain rearrangement occurs,N-nucleotides are not usually found in the VL-JL coding joints.

The bone-marrow phase of B-cell development culminates in the production of an IgM-bearing immature B cell.At this stage of development the B cell is not fully functional, and antigen induces death or unresponsiveness (anergy) rather than division and differentiation. Full maturation is signaled by the co-expression of IgD and IgM on the membrane.This progression involves a change in RNA processing

of the heavy-chain primary transcript to permit production of twomRNAs, one encoding the membrane form of the μ chain and the other encoding the membrane form of the γchain.

The Pre–B-Cell Receptor Is Essential for B-Cell Development

During one stage in T-cell development,
the β chain of the T-cell receptor associates with pre-Tα to form the pre–T-cell receptor A parallel situation occurs during B-cell development. In the pre-B cell, the membrane μchain is associated with the **surrogate light chain,** a complex consisting of two proteins: a V-like sequence called **Vpre-B** and a C-like sequence called λ5, which associate noncovalently to form a light chain–like structure. The membrane-bound complex of heavy chain and surrogate light chain appears on the pre-B cell associated with the Ig-α/Ig-β heterodimer to form the pre–B-cell receptor. Only pre-B cells that are able to express membranebound heavy chains in association with surrogate light chains are able to proceed along the maturation pathway. There is speculation that the pre–B-cell receptor recognizes a not-yet-identified ligand on the stromal-cell membrane, thereby transmitting a signal to the pre-B cell that prevents VH to DHJH rearrangement of the other heavy-chain allele, thus leading to allelic exclusion. Following the establishment of an effective pre–B-cell receptor, each pre-B cell undergoes multiple cell divisions, producing 32 to 64 descendants. Each of these progeny pre-B cells may then rearrange different light-chain gene segments, thereby increasing the overall diversity of the antibody repertoire. The critical role of the pre–B-cell receptor was demonstrated with knockout mice in which the gene encoding the 5 protein of the receptor was disrupted. B-cell development in these mice was shown to be blocked at the pre-B stage, which suggests that a signal generated through the receptor is necessary for pre-B cells to proceed to the immature B-cell stage.

B-Cell Activation and Proliferation

After export of B cells from the bone marrow, activation, proliferation, and differentiation occur in the periphery and require antigen.Antigen-driven activation and clonal selection of naive B cells leads to generation of plasma cells and memory B cells. In the absence of antigen-induced activation, naive B cells in the periphery have a short life span, dying within a few weeks by apoptosis.Thymus-Dependent and Thymus- Independent Antigen Have Different Requirements for Response Depending on the nature of the antigen, B-cell activation proceeds by two different routes, one dependent upon TH cells, the other not. The B-cell response to thymus-dependent (TD) antigens requires direct contact with TH cells, not simply exposure to TH-derived cytokines.Antigens that can activate B cells in the absence of this kind of direct participation by TH cells are known as thymus-independent (TI) antigens. TI antigens are divided into types 1 and 2, and they activate B cells by different mechanisms. Some bacterial cell-wall components, including lipopolysaccharide (LPS), function as type 1 thymus- independent (TI-1) antigens. Type 2 thymus-independent (TI-2) antigens are highly repetitious molecules such as polymeric proteins (e.g., bacterial flagellin) or bacterial cell-wall polysaccharides with repeating polysaccharide units.

Most TI-1 antigens are polyclonal B-cell activators (mitogens); that is, they are able to activate B cells regardless of their antigenic specificity. At high concentrations, some TI-1 antigens will stimulate proliferation and antibody secretion by as many as one third of all B cells. The mechanism by which TI-1 antigens activate B cells is not well understood. When B cells are exposed to lower concentrations of TI-1 antigens, only those B cells specific for epitopes of the antigen will be activated. These antigens can stimulate antibody production in nude mice (which lack a thymus and thus are greatly deficient in T cells), and the response is not greatly augmented by transferring T cells into these athymic mice, indicating that TI-1 antigens are truly T-cell independent. The prototypic TI-1 antigen is

lipopolysaccharide (LPS), a major component of the cell walls of gram-negative bacteria. At low concentrations, LPS stimulates the production of antibodies specific for LPS. At high concentrations, it is a polyclonal B-cell activator. Two Types of Signals Drive B Cells into and Through the Cell Cycle Naive, or resting, B cells are nondividing cells in the G0 stage of the cell cycle. Activation drives the resting cell into the cell cycle, progressing through G1 into the S phase, in which DNA is replicated. The transition from G1 to S is a critical restriction point in the cell cycle. Once a cell has reached S, it completes the cell cycle, moving through G2 and into mitosis (M). The B-Cell–Coreceptor Complex Can Enhance B-Cell Responses Stimulation through antigen receptors can be modified significantly by signals through coreceptors. Recall that costimulation through CD28 is an essential feature of effective positive stimulation of T lymphocytes, while signaling through CTLA-4 inhibits the response of the T cell. In cells. a component of the B-cell membrane, called the **B cell coreceptor,** provides stimulatory modifying signals.The B-cell coreceptor is a complex of three proteins: CD19, CR2 (CD21), and TAPA-1 (CD81). CD19, a member of the immunoglobulin superfamily, has a long cytoplasmic tail and three extracellular domains. The CR2 component is a receptor of C3d, a breakdown product of the complement system, which is an important effector mechanism for destroying invaders. The delivery of these signaling molecules to the BCR complex contributes to the activation process, and the coreceptorcomplex serves to amplify the activating signal transmitted through the BCR. In one experimental in vitro system, for example, 104 molecules of mIgM had to be engaged by antigen for B-cell activation to occur when the coreceptor was not involved. But when CD19/CD2/TAPA-1 coreceptor was crosslinked to the BCR, only 102 molecules of mIgM had to be engaged for B-cell activation.Another striking experiment highlights the role played by the B-cell coreceptor.Mice were immunized with either unmodified lysozyme or a hybrid protein in which genetic engineering was used to join hen's egg lysozyme to C3d. The fusion protein bearing 2 or 3 copies of C3d produced anti-lysozyme responses that were 1000 to 10,000 times greater than those to lysozyme alone. Perhaps coreceptor phenomena such as these explain how naïve B cells that often express mIg with low affinity for antigen are able to respond to low concentrations of antigen in a primary response. Such responses, even though initially of low affinity, can play a significant role in the ultimate generation of high-affinity antibody.

TH Cells Play Essential Roles in Most B-Cell Responses

As noted already, activation of B cells by soluble protein antigens requires the involvement of TH cells. Binding of antigen to B-cell mIg does not itself induce an effective competence signal without additional interaction with membrane molecules on the TH cell. In addition, a cytokine-mediated progression is required for B-cell proliferation. Figure 11-10 outlines the probable sequence of events in B-cell activation by a thymus-dependent (TD) antigen. This process is considerably more complex than activation induced by thymusindependent (TI) antigens.

FORMATION OF T-B CONJUGATE

After binding of antigen by mIg on B cells, the antigen is internalized by receptor-mediated endocytosis and processed within the endocytic pathway into peptides.Antigen binding also initiates signaling through the BCR that induces the B cell to up-regulate a number of cell-membrane molecules, including class II MHC molecules and the co-stimulatory ligand B7. Increased expression of both of these membrane proteins enhances the ability of the B cell to function as an antigen-presenting cell in TH-cell activation. B-cells could be regarded as helping their helpers because the antigenic peptides produced within the endocytic processing pathway associate with class II MHC molecules and are presented on the B-cell membrane to the TH cell, inducing its activation. It generally takes 30–60 min after internalization

of antigen for processed antigenic peptides to be displayed on the B-cell membrane in association with class II MHC molecules. Because a B cell recognizes and internalizes antigen specifically, by way of its membrane-bound Ig, a B cell is able to present antigen to TH cells at antigen concentrations that are 100 to 10,000 times lower than what is required for presentation by macrophages or dendritic cells. When antigen concentrations are high, macrophages and dendritic cells are effective antigen-presenting cells, but, as antigen levels drop, B cells take over as the major presenter of antigen to TH cells. Once a TH cell recognizes a processed antigenic peptide displayed by a class II MHC molecule on the membrane of a B cell, the two cells interact to form a T-B conjugate.

CONTACT-DEPENDENT HELP MEDIATED BY CD40/CD40L INTERACTION

Formation of a T-B conjugate not only leads to the directional release of TH-cell cytokines, but also to the up-regulation of CD40L (CD154), a TH-cell membrane protein that then interacts with CD40 on B cells to provide an essential signal for T-cell–dependent B-cell activation. CD40 belongs to the tumor necrosis factor (TNF) family of cell-surface proteins and soluble cytokines that regulate cell proliferation and programmed cell death by apoptosis. CD40L belongs to the TNF receptor (TNFR) family. Interaction of CD40L with CD40 on the B cell delivers a signal (signal 2) to the B cell that, in concert with the signal generated by mIg crosslinkage (signal 1), drives the B cell into G1.

The signals from CD40 are transduced by a number of intracellular signaling pathways, ultimately resulting in changes in gene expression. Studies have shown that although CD40 does not have kinase activity, its crosslinking is followed by the activation of protein tyrosine kinases such as Lyn and Syk. Crosslinking of CD40 also results in the activation of phospholipase C and the subsequent generation of the second messengers IP3 and DAG, and the activation of a number of transcription factors. *Ligation* of CD40 also results in its association with members of the TNFR-associated factor (TRAF) family.A consequence of this interaction is the activation of the transcription factor NF-6B.

SIGNALS PROVIDED BY TH-CELL CYTOKINES

Although B cells stimulated with membrane proteins from activated TH cells are able to proliferate, they fail to differentiate unless cytokines are also present; this finding suggests that both a membrane-contact signal and cytokine signals are necessary to induce B-cell proliferation and differentiation. As noted already, electron micrographs of T-B conjugates reveal that the antigen-specific interaction between a TH and a B cell induces a redistribution of TH-cell membrane proteins and cytoskeletal elements that results in the polarized release of cytokines toward the interacting B cell.

Once activated, the B cell begins to express membrane receptors for various cytokines, such as IL-2, IL-4, IL-5, and others. These receptors then bind the cytokines produced by the interacting TH cell. The signals produced by these cytokine-receptor interactions support B-cell proliferation and can induce differentiation into plasma cells and memory B cells, class switching, and affinity maturation. Each of these events is described in a later section.

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- they all constitutively express high levels of both class II MHC molecules and members of the co-stimulatory B7 family.For this reason, they are more potent antigen-presenting cells than macrophages and B cells, both of which need to be activated before they can function as antigen-presenting cells (APCs). Immature or precursor forms of each of these types of dendritic cells acquire antigen by phagocytosis or endocytosis; the antigen is processed, and mature dendritic cells present it to TH cells. Following microbial invasion or duringinflammation, mature and immature forms of Langerhans cells and interstitial dendritic cells migrate into draining lymph nodes, where they make the critical presentation of antigen to TH cells that is required for the initiation of responses by those key cells. Another type of dendritic cell, the **follicular dendritic cell**, does not arise in bone marrow and has a different function from the antigen-presenting dendritic cells described above. Follicular dendritic cells do not express class II MHC molecules and therefore do not function as antigenpresenting cells for TH-cell activation. These dendritic cells were named for their exclusive location in organized structures of the lymph node called lymph follicles,which are rich in B cells. Although they do not express class II molecules, follicular dendritic cells express high levels of membrane receptors for antibody,which allows the binding of antigen-an 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 (see Chapter 9) 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 a small portion react with combinations of self antigen-MHC complexes. Using mechanisms that are discussed in Chapter 10, 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.

THE THYMUS AND IMMUNE FUNCTION

The role of the thymus in immune function can be studied in mice by examining the effects of neonatal thymectomy, a procedure in which the thymus is surgically removed from newborn mice. These thymectomized mice show a dramatic decrease in circulating lymphocytes of the T-cell lineage and an absence of cell-mediated immunity. Other evidence of the importance of the thymus comes from studies of a congenital birth defect in humans **(DiGeorge's syndrome)** and in certain mice **(nude mice)** in which the thymus fails to develop. In both cases, there is an absence of circulating T cells and of cell-mediated immunity and an increase in infectious disease. Aging is accompanied by a decline in thymic function. This decline may play some role in the decline in immune function during aging in humans and mice. The thymus reaches its maximal size at puberty and then atrophies, with a significant decrease in both cortical and medullary cells and an increase in the total fat content of the organ.Whereas the average weight of the thymus is 70 g in infants, its age-dependent involution leaves an organ with an average weight of only 3 g in the elderly.And a number of experiments have been designed to look at the effect of age on the immune function of the thymus. In one experiment, the thymus from a 1-day-old or 33-monthold mouse was grafted into thymectomized adults. (For most laboratory mice, 33 months is very old.) Mice receiving the newborn thymus graft showed a significantly larger improvement in immune function than mice receiving the 33-month-old thymus.

BONE MARROW

In humans and mice, bone marrow is the site of B-cell origin and development. Arising from lymphoid progenitors, immature B cells proliferate and differentiate within the bone marrow, and stromal cells within the bone marrow interact directly with the B cells and secrete various cytokines that are required for development. Like thymic selection during Tcell maturation, a selection process within the bone marrow eliminates B cells with self-reactive antibody receptors. This process is explained in detail in Chapter 11. Bone marrow is not the site of B-cell development in all species. In birds, a lymphoid organ called the bursa of Fabricius, alymphoid tissue associated with the gut, is the primary site of B-cell maturation. In mammals such as primates and rodents, there is no bursa and no single counterpart to it as a primary lymphoid organ. In cattle and sheep, the primary lymphoid tissue hosting the maturation, proliferation, and diversification of B cells early in gestation is the fetal spleen. Later in gestation, this function is assumed by a patch of tissue embedded in the wall of the intestine called the ileal Peyer's patch,which contains a large number B cells. The rabbit, too, uses gut-associated tissues such as the appendix as primary lymphoid tissue for important steps in the proliferation and diversification of B cells.

Lymphatic System

As blood circulates under pressure, its fluid component **(plasma)** seeps through the thin wall of the capillaries into the surrounding tissue.Much of this fluid, called **interstitial fluid,** returns to the blood through the capillary membranes. The remainder of the interstitial fluid, now called **lymph,** flows from the spaces in connective tissue into a network of tiny open lymphatic capillaries and then into a series of progressively larger collecting vessels called **lymphatic vessels**

The largest lymphatic vessel, the **thoracic duct,** empties into the left subclavian vein near the heart (see Figure 2-13). 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.

Secondary Lymphoid Organs

Various types of organized lymphoid tissues are located along the vessels of the lymphatic system. Some lymphoid tissue in the lung and lamina propria of the intestinal wall consists of diffuse collections of lymphocytes and macrophages. Other lymphoid tissue is organized into structures called lymphoid follicles, which consist of aggregates of lymphoid and nonlymphoid cells surrounded by a network of draining lymphatic capillaries. Until it is activated by antigen, a lymphoid follicle—called a **primary follicle**—comprises a network of follicular dendritic cells and small resting B cells. After an antigenic challenge, a primary follicle becomes a larger **secondary follicle**—a ring of concentrically packed B lymphocytes surrounding a center (the **germinal center**) in which one finds a focus of proliferating B lymphocytes and an area that contains nondividing B cells, and some helper T cells interspersed with macrophages and follicular dendritic cells (Figure 2-17).

Most antigen-activated B cells divide and differentiate into antibody-producing plasma cells in lymphoid follicles, but only a few B cells in the antigen-activated population find their way into germinal centers. Those that do undergo one or more rounds of cell division, during which the genes that encode their antibodies mutate at an unusually high rate. Following the period of division and mutation, there is a rigorous selection process in which more than 90% of these B cells die by apoptosis. In general, those B cells producing antibodies that bind antigen more strongly have a much better chance of surviving than do their weaker companions. The small number of B cells that survive the germinal center's rigorous selection differentiate into plasma cells or memory. cells and emerge. The process of B-cell proliferation, mutation, and selection in germinal centers is described more fully in Chapter 11

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..

LYMPH NODES

Lymph nodes are the sites where immune responses are mounted to antigens in lymph. They are encapsulated beanshaped structures containing a reticular network packed with lymphocytes, macrophages, and dendritic cells. Clustered at junctions of the lymphatic vessels, lymph nodes are the first organized lymphoid structure to encounter antigens that enter the tissue spaces. As lymph percolates through a node, any particulate antigen that is brought in with the lymph will be trapped by the cellular network of phagocytic cells and dendritic cells (follicular and interdigitating). The overall architecture of a lymph node supports an ideal microenvironment for lymphocytes to effectively encounter and respond

to trapped antigens.

Morphologically, a lymph node can be divided into three roughly concentric regions: the cortex, the paracortex, and the medulla, each of which supports a distinct microenvironment (Figure 2-18). The outermost layer, the **cortex,** contains lymphocytes (mostly B cells), macro-phages, and follicular dendritic cells arranged in primary follicles. After antigenic challenge, the primary follicles enlarge into secondary follicles, each containing a germinal center. In children with B-cell deficiencies, the cortex lacks primary follicles and germinal centers. Beneath the cortex is the **paracortex,** which is populated largely by T lymphocytes and also contains interdigitating dendritic cells thought to have migrated from tissues to the node. These interdigitating dendritic cells express high levels of class II MHC molecules,which are necessary for presenting antigen to TH cells. Lymph nodes taken from neonatally thymectomized mice have unusually few cells in the paracortical region; the paracortex is therefore sometimes referred to as a **thymus-dependent area** in contrast to the cortex, which is a **thymus-independent area.** The innermost layer of a lymph node, the **medulla,** is more sparsely populated with lymphoid-lineage cells; of those present, many are plasma cells actively secreting antibody molecules.

As antigen is carried into a regional node by the lymph, it is trapped, processed, and presented together with class II MHC molecules by interdigitating dendritic cells in the paracortex, resulting in the activation of TH cells. The initial activation of B cells is also thought to take place within the T-cell-rich paracortex. Once activated, TH and B cells form small foci consisting largely of proliferating B cells at the edges of the paracortex. Some B cells within the foci differentiate into plasma cells secreting IgM and IgG. These foci reach maximum size within 4–6 days of antigen challenge. Within 4–7 days of antigen challenge, a few B cells and TH cells migrate to the primary follicles of the cortex. It is not known what causes this migration.Within a primary follicle, cellular interactions between follicular dendritic cells, B cells, and TH cells take place, leading to development of a secondary follicle with a central germinal center. Some of the plasma cells generated in the germinal center move to the medullary areas of the lymph node, and many migrate to bone marrow Afferent lymphatic vessels pierce the capsule of a lymph node at numerous sites and empty lymph into the subcapsular sinus (see Figure 2-18b). Lymph coming from the tissues percolates slowly inward through the cortex, paracortex, and medulla, allowing phagocytic cells and dendritic cells to trap any bacteria or particulate material (e.g., antigen-antibody complexes) carried by the lymph. After infection or the introduction of other antigens into the body, the lymph leaving a node through its single efferent lymphatic vessel is enriched with antibodies newly secreted by medullary plasma cells and also has a fiftyfold higher concentration of lymphocytes than the afferent lymph. The increase in lymphocytes in lymph leaving a node is due in part to lymphocyte proliferation within the node in response to antigen. Most of the increase, however, represents blood-borne lymphocytes that migrate into the node by passing between specialized endothelial cells that line the **postcapillary venules** of the node. Estimates are that 25% of the lymphocytes leaving a lymph node have migrated across this endothelial layer and entered the node from the blood. Because antigenic stimulation within a node can increase this migration tenfold, the concentration of lymphocytes in a node that is actively responding can increase greatly, and the node swells visibly. Factors released in lymph nodes during antigen stimulation are thought to facilitate this increased migration

SPLEEN

The spleen plays a major role in mounting immune responses to antigens in the blood stream. It is a large, ovoid secondary lymphoid organ situated high in the left abdominal cavity. While lymph nodes are specialized for trapping antigen from local tissues, the spleen specializes in filtering blood and

trapping blood-borne antigens; thus, it can respond to systemic infections. Unlike the lymph nodes, the spleen is not supplied by lymphatic vessels. Instead, bloodborne antigens and lymphocytes are carried into the spleen through the splenic artery. Experiments with radioactively labeled lymphocytes show that more recirculating lymphocytes pass daily through the spleen than through all the lymph nodes combined.

The spleen is surrounded by a capsule that extends a number of projections (trabeculae) into the interior to form a compartmentalized structure. The compartments are of two types, the red pulp and white pulp, which are separated by a diffuse marginal zone (Figure 2-19). The splenic **red pulp** consists of a network of sinusoids populated by macrophages and numerous red blood cells (erythrocytes) and few lymphocytes; it is the site where old and defective red blood cells are destroyed and removed.Many of the macrophages within the red pulp contain engulfed red blood cells or iron pigments from degraded hemoglobin. The splenic **white pulp** surrounds the branches of the splenic artery, forming a **periarteriolar lymphoid sheath (PALS)** populated mainly by T lymphocytes. Primary lymphoid follicles are attached to the PALS. These follicles are rich in B cells and some of them contain germinal centers. The **marginal zone,** located peripheral to the PALS, is populated by lymphocytes and macrophages.

Blood-borne antigens and lymphocytes enter the spleen through the splenic artery, which empties into the marginal zone. In the marginal zone, antigen is trapped by interdigitating dendritic cells, which carry it to the PALS. Lymphocytes in the blood also enter sinuses in the marginal zone and migrate to the PALS

The initial activation of B and T cells takes place in the Tcell- rich PALS. Here interdigitating dendritic cells capture antigen and present it combined with class II MHC molecules to TH cells. Once activated, these TH cells can then activate B cells. The activated B cells, together with some TH cells, then migrate to primary follicles in the marginal zone. Upon antigenic challenge, these primary follicles develop into characteristic secondary follicles containing germinal centers (like those in the lymph nodes), where rapidly dividing B cells (centroblasts) and plasma cells are surrounded by dense clusters of concentrically arranged lymphocytes.

The effects of splenectomy on the immune response depends on the age at which the spleen is removed. In children, splenectomy often leads to an increased incidence of bacterial sepsis caused primarily by *Streptococcus pneumoniae, Neisseria meningitidis,* and *Haemophilus influenzae.* Splenectomy in adults has less adverse effects, although it leads to some increase in blood-borne bacterial infections **(bacteremia).**

MUCOSAL-ASSOCIATED LYMPHOID TISSUE

The mucous membranes lining the digestive, respiratory, and urogenital systems have a combined surface area of about 400 m2 (nearly the size of a basketball court) and are the major sites of entry for most pathogens. These vulnerable membrane surfaces are defended by a group of organized lymphoid tissues mentioned earlier and known collectively as **mucosal-associated lymphoid tissue (MALT).** Structurally, these tissues range from loose, barely organized clusters of lymphoid cells in the lamina propria of intestinal villi to well-organized structures such as the familiar tonsils and appendix, as well as Peyer's patches, which are found within the submucosal layer of the intestinal lining. The functional importance of MALT in the body's defense is attested to by its large population of antibody-producing plasma cells, whosenumber far exceeds that of plasma cells in the spleen, lymph nodes, and bone marrow combined.

The **tonsils** are found in three locations: lingual at the base of the tongue; palatine at the sides of the back of the mouth; and pharyngeal (adenoids) in the roof of the nasopharynx (Figure 2-20). All three tonsil groups are nodular structures consisting of a meshwork of reticular cells and fibers interspersed with lymphocytes, macrophages, granulocytes, and mast cells. The B cells are organized into follicles and germinal centers; the latter are surrounded by regions showing T-cell activity. The tonsils defend against antigens entering through the nasal and oral epithelial routes.

The best studied of the mucous membranes is the one that lines the gastrointestinal tract. This tissue, like that of the respiratory and urogenital tracts, has the capacity to endocytose antigen from the lumen. Immune reactions are initiated against pathogens and antibody can be generated and exported to the lumen to combat the invading organisms. As shown in Figures 2-21 and 2-22, lymphoid cells are found in various regions within this tissue. The outer mucosal epithe- lial layer contains so-called **intraepithelial lymphocytes (IELs).** Many of these lymphocytes are T cells that express unusual receptors (_T-cell receptors, or _ TCRs), which exhibit limited diversity for antigen. Although this population of T cells is well situated to encounter antigens that enter through the intestinal mucous epithelium, their actual function remains largely unknown The lamina propria, which lies under the epithelial layer, contains large numbers of B cells, plasma cells, activated TH cells, and macrophages in loose clusters.Histologic sections have revealed more than 15,000 lymphoid follicles within the intestinal lamina propria of a healthy child. The submucosal layer beneath the lamina propria contains Peyer's patches, nodules of 30–40 lymphoid follicles. Like lymphoid follicles in other sites, those that compose Peyer's patches can develop into secondary follicles with germinal centers. The epithelial cells of mucous membranes play an important role in promoting the immune response by delivering small samples of foreign antigen from the lumina of the respiratory, digestive, and urogenital tracts to the underlying mucosal-associated lymphoid tissue. This antigen transport is carried out by specialized **M cells.** The structure of the Mcell is striking: these are flattened epithelial cells lacking the microvilli that characterize the rest of the mucous epithelium. In addition, M cells have a deep invagination, or pocket, in the basolateral plasma membrane; this pocket is filled with a cluster of B cells, T cells, and macrophages (Figure 2-22a). Luminal antigens are endocytosed into vesicles that are transported from the luminal membrane to the underlying pocket membrane. The vesicles then fuse with the pocket membrane, delivering the potentially response-activating antigens to the clusters of lymphocytes contained within the pocket. M cells are located in so-called **inductive sites**—small regions of a mucous membrane that lie over organized lymphoid follicles (Figure 2-22b). Antigens transported across the mucous membrane by M cells can activate B cells within these lymphoid follicles. The activated B cells differentiate into plasma cells, which leave the follicles and secrete the IgA class of antibodies. These antibodies then are transported across the epithelial cells and released as **secretory IgA** into the lumen, where they can interact with antigens.

As described in Chapter 1,mucous membranes are an effective barrier to the entrance of most pathogens, which thereby contributes to nonspecific immunity. One reason for this is that the mucosal epithelial cells are cemented to one another by tight junctions that make it difficult for pathogens to penetrate. Interestingly, some enteric pathogens, including both bacteria and viruses, have exploited the M cell as an entry route through the mucous-membrane barrier. In some cases, the pathogen is internalized by the M cell and transported into the pocket. In other cases, the pathogen binds to the M cell and disrupts the cell, thus allowing entry of the pathogen. Among the pathogens that use M cells in these ways are several invasive *Salmonella* species, *Vibrio cholerae,* and the polio virus.

Cutaneous-Associated Lymphoid Tissue

The skin is an important anatomic barrier to the external environment, and its large surface area makes this tissue important in nonspecific (innate) defenses. The epidermal (outer) layer of the skin is composed largely of specialized epithelial cells called keratinocytes. These cells secrete a number of cytokines that may function to induce a local inflammatory reaction. In addition, keratinocytes can be induced to express class II MHC molecules and may function as antigen-presenting cells. Scattered among the epithelial-cell matrix of the epidermis are Langerhans cells, a type of dendritic cell, which internalize antigen by phagocytosis or endocytosis. The Langerhans cells then migrate from the epidermis to regional lymph nodes, where they differentiate into interdigitating dendritic cells. These cells express high levels of class II MHC molecules and function as potent activators of naive TH cells.

The epidermis also contains so-called *intraepidermal lymphocytes*. These are similar to the intraepithelial lymphocytes of MALT in that most of them are CD8_ T cells, many of which express _ T-cell receptors, which have limited diversity for antigen. These intraepidermal T cells are well situated to encounter antigens that enter through the skin and some immunologists believe that they may play a role in combating antigens that enter through the skin. The underlying dermal layer of the skin contains scattered CD4_ and CD8_ T cells and macrophages.Most of these dermal T cells were either previously activated cells or are memory cells.

T-Cell Maturation

The attribute that distinguishes antigen recogenation by mostT cells from recognition byB cells inis MHC restriction. In most cases both the maturation of progenitor T cells in the thymus and the acti- vation of mature T cells in the periphery are influenced by the involvement of MHC molecules. The potential antigenic diversity of the T-cell population is reduced during matura- tion by a selection process that allows only MHC-restricted and nonself-reactive T cells to mature. The final stages in the maturation of most T cells proceed along two different de- velopmental pathways, which generate functionally distinct $CD4^+$ and $CD8^+$ subpopulations that exhibit class II and class I MHC restriction, respectively.

T-Cell Maturation and the Thymus Progenitor T cells from the early sites of hematopoiesis begin to migrate to the thymus at about day 11 of gestation in mice and in the eighth or ninth week of gestation in humans. In a manner similar to B-cell maturation in the bone marrow, T- cell maturation involves rearrangements of the germ-line TCR genes and the expression of various membrane mark- ers. In the thymus, developing T cells, known as thymocytes, proliferate and differentiate along developmental pathways that generate functionally distinct subpopulations of mature T cells. the thymus occupies a central role in T-cell biology. Aside from being the main source of all T cells, it is where T cells diversify and then are shaped into an effective primary T-cell repertoire by an extraordinary pair of selection processes. One of these, positive selection, permits the survival of only those T cells whose TCRs are capable of recognizing self-MHC molecules. It is thus responsible for the creation of a self-MHC-restricted repertoire of T cells. The other, negative selection, eliminates T cells that reacttoo strongly with self-MHC or with self-MHC plus self- peptides. It is an extremely important factor in generating primary T-cell repertoire that is self-tolerant

when T-cell precursors arrive at the thymus, they do not express such signaturesurface mark-ers of T cells as the T-cell receptor, the CD3 complex, or the

coreceptors CD4 and CD8. In fact, these progenitor cells hav not yet rearranged their TCR genes and do not express pro- teins, such as RAG-1 and RAG-2, that are required for re- arrangement. After arriving at the thymus, these T-cell precursors enter the outer cortex and slowly proliferate. Dur- ing approximately three weeks of development in the thy- mus, the differentiating T cells progress through a series of stages that are marked by characteristic changes in their cell- surface phenotype. For example, as mentioned previously, thymocytes early in development lack detectable CD4 and CD8. Because these cells are $CD4^+CD8^+$, they are referred to as double-negative (DN) cells. Even though these coreceptors are not expressed during the DN early stages, the differentiation program is progress- ing and is marked by changes in the expression of such cell surface molecules as c-Kit, CD44, and CD25. The initial thy- mocyte population displays c-Kit, the receptor for stem-cell growth factor, and CD44, an adhesion molecule involved in homing; CD25, the β-chain of the IL-2 receptor, also appears 222 PART II Generation of B-Cell and T-Cell Responses on early-stage DN cells. During this period, the cells are pro- liferating but the TCR genes remain unrearranged. Then the cells stop expressing c-Kit, markedly reduce CD44 expres- sion, turn on expression of the recombinase genes RAG-1 and RAG-2 and begin to rearrange their TCR genes. Al- though it is not shown in Figure 10-1, a small percentage (5%) of thymocytes productively rearrange the β- and βchain genes and develop into double- negative CD3 T cells. In mice, this thymocyte subpopulation can be detected by day 14 of gestation, reaches maximal numbers between days 17 and 18, and then declines until birth.

Most double-negative thymocytes progress down the developmental pathway. They stop proliferating and begin to rearrange the TCR -chain genes, then express the β chain. Those cells of the lineage that fail to productively re- arrange and express βchains die. Newly synthesized chains combine with a 33-kDa glycoprotein known as the pre-Tβchain and associate with the CD3 group to form a novel com plex called the pre-T-cell receptor or pre-TCR Some researchers have suggested that the pre-TCR recog- nizes some intra-thymic ligand and transmits a signal through the CD3 complex that activates signal-transduction pathways that have several effects I Indicates that a cell has made a productive TCR β-chain rearrangement and signals its further proliferation and maturation. I Suppresses further rearrangement of TCR β-chain genes, resulting in allelic exclusion. I Renders the cell permissive for rearrangement of theTCR β chain.I Induces developmental progression to the CD4+8+ double-positive state. After advancing to the double-positive (DP) stage, whereboth CD4 and CD8 coreceptors are expressed, the thymo- cytes begin to proliferate. However, during this proliferative phase, TCR β-chain gene rearrangement does not occur; both the RAG-1 and RAG-2 genes are transcriptionally ac- tive, but the RAG-2 protein is rapidly degraded in proliferat- ing cells, so rearrangement of theβchain genes cannot take place. The rearrangement of β-chain genes does not begin until the double-positive thymocytes stop proliferating and RAG-2 protein levels increase. The proliferative phase prior to the rearrangement of the β-chain increases the diversity of the T-cell repertoire by generating a clone of cells with a sin- gle TCR β-chain rearrangement. Each of the cells within this clone can then rearrange a different β-chain gene, thereby generating a much more diverse population than if the original cell had first undergone rearrangement at both the β- and β-chain loci before it proliferated. In mice, the TCR β chain genes are not expressed until day 16 or 17 of gestation; double-positive cells expressing both CD3 and the β T-cell receptor begin to appear at day 17 and reach maximal levels about the time of birth (see Figure 10-2). The possession of a complete TCR enables DP thymocytes to undergo the rigors of positive and negative selection. T-cell development is an expensive process for the host. An estimated 98% of all thymocytes do not mature—they die by apoptosis within the thymus either because they fail to make a productive

TCR-gene rearrangement or because they fail to survive thymic selection. Double-positive thymocytes that express the β TCR-CD3 complex and survive thymic selection develop into immature single-positive $CD4^+$ thymocytes or single-positive $CD8^+$ thymocytes. These single-positive cells undergo additional negative selection and migrate from the cortex to the medula, where they pass from the thymus into the circulatory system.

TH-Cell Activation

The central event in the generation of both humoral and cell-mediated immune responses is the activation and clonal ex-pansion of TH cells. Activation of TC cells, which is generally similar to TH-cell activation, is described in Chapter 14. TH- cell activation is initiated by interaction of the TCR-CD3 complex with a processed antigenic peptide bound to a class II MHC molecule on the surface of an antigen-presenting cell. This interaction and the resulting activating signals also involve various accessory membrane molecules on the TH cell and the antigen-presenting cell. Interaction of a TH cell with antigen initiates a cascade of biochemical events that in-duces the resting TH cell to enter the cell cycle, proliferating and differentiating into memory cells or effectors cells. Manyof the gene products that appear upon interaction with anti- gen can be grouped into one of three categories depending on how early they can be detected after antigen recognition (I Immediate genes, expressed within half an hour ofantigen recognition, encode a number of transcription factors, including c-Fos, c-Myc, c-Jun, NFAT, and NF-B I Early genes, expressed within 1–2 h of antigen recognition, encode IL-2, IL-2R (IL-2 receptor), IL-3,IL-6, IFN-¥, and numerous other proteinsI Late genes, expressed more than 2 days after antigen recognition, encode various adhesion molecules These profound changes are the result of signal-transduction pathways that are activated by the encounter between the TCR and MHC-peptide complexes. An overview of some of the basic strategies of cellular signaling will be useful back- ground for appreciating the specific signaling pathways used by T cells. T-Cell Differentiation CD4+ and CD8+ T cells leave the thymus and enter the cir-culation as resting cells in the G0 stage of the cell cycle. There are about twice as many CD4+ T cells as CD8+ T cells in the periphery. T cells that have not yet encountered antigen(naive T cells) are characterized by condensed chromatin, very little cytoplasm, and little transcriptional activity. NaiveT cells continually recirculate between the blood and lymph systems. During recirculation, naive T cells reside in sec- ondary lymphoid tissues such as lymph nodes. If a naive cell does not encounter antigen in a lymph node, it exits through the efferent lymphatic, ultimately draining into the thoracic duct and rejoining the blood. It is estimated that each naive T cell recirculates from the blood to the lymph nodes and back again every 12–24 hours. Because only about 1 in 105 naive T cells is specific for any given antigen, this large-scale recircu- lation increases the chances that a naive T cell will encounter appropriate antigen.

B-Cell Maturation

The generation of mature B cells first occurs in the embryoand continues throughout life. Before birth, the yolk sac, fetal liver, and fetal bone marrow are the major sites of B-cell maturation; after birth, generation of mature B cells occurs in the bone marrow. Progenitor B Cells Proliferate in Bone Marrow B-cell development begins as lymphoid stem cells differenti- ate into the earliest distinctive B-lineage cell—the progeni-tor B cell (pro-B cell)—which expresses a transmembran tyrosine phosphatase called CD45R (sometimes called B220 in mice). Pro-B cells proliferate within the bone marrow, fill- ing the extravascular spaces between large sinusoids in theshaft of a bone. Proliferation and differentiation of pro-B
cells into precursor B cells (pre-B cells) requires the micro-environment provided by the bone-marrow stromal cells. If pro-B cells are removed from the bone marrow and cultured in vitro, they will not

progress to more mature B-cell stages unless stromal cells are present. The stromal cells play two important roles: they interact directly with pro-B and pre-B cells, and they secrete various cytokines, notably IL-7, thatsupport the developmental process.

At the earliest developmental stage, pro-B cells require direct contact with stromal cells in the bone marrow. This in- teraction is mediated by several cell-adhesion molecules, including VLA-4 on the pro-B cell and its ligand, VCAM-1, on the stromal cell (Figure 11-2). After initial contact is made, a receptor on the pro-B cell called c-Kit interacts with a stromal-cell surface molecule known as stem-cell factor (SCF). This interaction activates c-Kit, which is a tyrosine kinase, and the pro-B cell begins to divide and differentiate into a pre-B cell and begins expressing a receptor for IL-7. The IL-7 secreted by the stromal cells drives the maturation process, eventually inducing down-regulation of the adhesion molecules on the pre-B cells, so that the proliferating cells can detach from the stromal cells. At this stage, pre-B cells no longer require direct contact with stromal cells but continue to require IL-7 for growth and maturation.

Ig-Gene Rearrangment Produces Immature B Cells

B-cell maturation depends on rearrangement of the immuno-globulin DNA in the lymphoid stem cells. The mechanisms of Ig-gene rearrangement were described in Chapter 5. First to occur in the pro-B cell stage is a heavy-chain DH-to-JH gene rearrangement; this is followed by a VH-to-DHJH rearrangement. If the first heavy-chain re- arrangement is not productive, then VH-DH-JH rearrange- ment continues on the other chromosome. Upon completion of heavy-chain rearrangement, the cell is classified as a pre-B cell. Continued development of a pre-B cell into an imma- ture B cell requires a productive light-chain gene rearrange- ment. Because of allelic exclusion, only one light-chain isotype is expressed on the membrane of a B cell. Completion of a productive light-chain rearrangement commits the now im- mature B cell to a particular antigenic specificity determined by the cell's heavy-chain VDJ sequence and light-chain VJ sequence. Immature B cells express mIgM (membrane IgM) on the cell surface.

As would be expected, the recombinase enzymes RAG-1 and RAG-2, which are required for both heavy-chain and light-chain gene rearrangements, are expressed during the pro-B and pre-B cell stages. The enzyme terminal deoxyribonucleotidyl transferase (TdT), which cat- alyzes insertion of N-nucleotides at the DH-JH and VH-DH- JH coding joints, is active during the pro-B cell stage and ceases to be active early in the pre–B-cell stage. Because TdT expression is turned off during the part of the pre–B-cell stage when light-chain rearrangement occurs, N-nucleotides are not usually found in the VL-JL coding joints. The bone-marrow phase of B-cell development culmi- nates in the production of an IgM-bearing immature B cell.At this stage of development the B cell is not fully functional, and antigen induces death or unresponsiveness (anergy) rather than division and differentiation. Full maturation is signaled

The Pre–B-Cell Receptor Is Essential for B-Cell Development

During one stage in T-cell develop-ment, the chain of the T-cell receptor associates with pre-T to form the pre–T-cell receptor. Aparallel situation occurs during B-cell development. In thepre-B cell, the membrane chain is associated with the sur- rogate light chain, a complex consisting of two proteins: a V-like sequence called Vpre-B and a C-like sequence called 5, which associate noncovalently to form a light-chain–like structure.

The membrane-bound complex of βheavy chain and sur-rogate light chain appears on the pre-B cell associated with the Ig-/Ig-heterodimer to form the pre–B-cell receptor. Only pre-B cells that are able to express membrane bound heavy chains in association with surrogate light chains are able to proceed

along the maturation pathway. There is speculation that the pre–B-cell receptor recog- nizes a not-yet-identified ligand on the stromal-cell mem- brane, thereby transmitting a signal to the pre-B cell that prevents VH to DHJH rearrangement of the other heavy-chain allele, thus leading to allelic exclusion. Following the estab- lishment of an effective pre–B-cell receptor, each pre-B cell undergoes multiple cell divisions, producing 32 to 64 descen- dants. Each of these progeny pre-B cells may then rearrange different light-chain gene segments, thereby increasing the overall diversity of the antibody repertoire. The critical role of the pre–B-cell receptor was demon- strated with knockout mice in which the gene encoding the 5 protein of the receptor was disrupted. B-cell development in these mice was shown to be blocked at the pre-B stage, which suggests that a signal generated through the receptor is neces- sary for pre-B cells to proceed to the immature B-cell stage. Knockout Experiments Identified Essential Transcription Factors

Many different transcription fac- tors act in the development of hematopoietic cells. Nearly a dozen of them have so far been shown to play roles in B-cell development. Experiments in which particular transcription factors are knocked out by gene disruption have shown that four such factors, E2A, early B-cell factor (EBF), B-cell– specific activator protein (BSAP), and Sox-4 are particularly important for B-cell development. Micethat lack E2A do not express RAG-1, are unable to makeDHJH rearrangements, and fail to express 5, a critical com- ponent of the surrogate light chain. A similar pattern is seen in EBF-deficient mice. These findings point to important roles for both of these transcription factors early in B-cell development, and they may play essential roles in the earlystages of commitment to the B-cell lineage. Knocking out the Pax-5 gene, whose product is the transcription factor BSAP, also results in the arrest of B-cell development at an early stage. Binding sites for BSAP are found in the promoter re- gions of a number of B-cell–specific genes, including Vpre-B and 5, in a number of Ig switch regions, and in the Ig heavy- chain enhancer. This indicates that BSAP plays a role beyond the early stages of B-cell development. This factor is also ex- pressed in the central nervous system, and its absence results in severe defects in mid-brain development.Although the ex- act site of action of Sox-4 is not known, it affects early stages of B-cell activation. While Figure 11-3 shows that all of these transcription factors affect development at an early stage, some of them are active at later stages also.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: II M.Sc MB COURSE NAME: ADVANCED IMMUNOLOGY

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Sl. No	Question	Option A	Option B	Option C	Option D	Correct Ans
1	Antibody affinity is _____in the case of secondary response	Higher	Lower	Moderate	Significant	Higher
2	Humoral mediated immunity coming under the ___	Innate immunity	Acquired Immunity	Local Immunity	Herd immunity	Acquired Immunity
3	Cell mediated immunity is mediated by ___	T cells	B cells	Native immunity	Adaptive immunity	T cells
4	Delayed type hypersensitivity is an example of __	CMI	HMI	Native immunity	Adaptive immunity	CMI
5	Digeorge syndrome is the lacking of ____	HMI	CMI	Native immunity	Adaptive immunity	CMI
6	An example of non-specific cell involved in CMI __	CD4TH cells	Macrophages	Cytotoxic T cells	T cells	Macrophages
7	ABO haemolytic disease is largely seen in _____ blood	AB group	o group	grouping	Rh blood	o group
8	Cells which develop into blood cells is called ____	Pluripotent	T cells	Null cells	T cells	Pluripotent
9	The stem cells is develop into blood cells through a process called ____	Cytokines	Stomal cells	Hematopoiesis	Macrophages	Null cells
10	Microglial cells are located in the _____	Liver	Kidney	Brain	Lungs	Hematopoiesis
11	The bursa of fabricins arises from _____of birds	Spleen	Cloace	Bursa	Thymus	Cloace
12	Lobe of thymus is organized into lobules which are separated from one another by septa collect ___	Medulla	Trabeculae	Trabule	Cortex	Trabeculae
13	The major part of the lymphoid tissue is arranged around a central arteriole and it is known as ____	Malt	Galt	Pals	Spleen	Pals
14	Immune responses are secreted by activated macrophages, it includes _____	IL- 2 and 3	IL 1 and 6	HNO_2	O_2	IL- 2 and 3
15	The ingested material is digested by phagolysosome is excreated by a process called ____	Hematopoiesis	Exocytosis	Chemotaxis	Phagocytosis	Exocytosis
16	Particulate Ag are rendered more susceptible to phagocytosis is called __	Endocytsis	Opsonization	Exocytosis	Cytolysis	Opsonization

17	The ability to induce a response and the ability to react with the products of that response is called as _	Immunogenecity	Antigenicity	Immunocompetest	Antigenic drift	Antigenicity
18	Substances capable of inducing a specific immune response are called__	Antibody	Hapten	Antigen	Paratope	Antigen
19	The best immunogens tend to have a molecular mass	400 kDa	100kDa	20 kDa	5kda	100kDa
20	The sites either on or within the antigen with which antibodies and T cell receptors react.	Epitope	Haptens	Paratope	Autoantigens	Epitope
21	The A and B blood group antigens are examples of___	syngenic antigens	Autologous antigen	Isoantigens	Xenogenic antigen	Isoantigens
22	____ are organ specific antigen that are not normally exposed to the immune system	Sequestered antigen	Heterophile antigen	Allogenic antigens	Iso antigen	Sequestered antigen
23	A transplant of a monkey's kidneys to a human is an example of ___	Isograft	Xenograft	Autograft	Allograft	Xenograft
24	The area of the antibody molecule that interacts with the epitopes is called ___	Epitope	Paratope	Hapten	FaB	Paratope
25	Small molucules or chemical groups that can be function as epitopes when they bound to other molecules is this way are called as ___	Antibody	Haptens	Antigen	Paratope	Haptens
26	Substaneces when mixed with an antigen and injected enhances the immunogenecity of an antigen are reffered to as __	Carrier Molecule	Tissue specific antigen	Adjuvants	Immunogens	Adjuvants
27	The antibody elicited by one antigen can cross react with an unrelated antigen, Such reactions are termed as ___	Precipitin	Diffusion	Cross reactivity	Agglutinin	Cross reactivity
28	Cross reactions occur due to__	Avidity of an epitope	Affinity of an epitope	Two different antigens share an	Affinity of antigen	Two different antigens share an

				individual epitope		individual epitope
29	Eosinophils stains with ___	acid dye	Basic dye	Acid dye eosin	Both acid and basic dye	Acid dye eosin
30	Freund's complete adjuvant had bacterial compound drawn from	*E. coli*	*Staphylococcus*	*Pseudomonas*	*Mycobacterium*	*Mycobacterium*
31	A group of structurally hetrogenous compounds which increase an immune response to an antigen is called ___	Hormones	Adjuvants	Vaccines	Antibiotics	Adjuvants
32	____ Adjuvants contains heat killed Mycobacteria in the water in oil emulsion	Freund's incomplete adjuvant	Freund's complete adjuvant	Ribi's adjuvants	Hanter's Titer max	Freund's complete adjuvant
33	____component of the Mycobacterial cell wall present in freund's complete adjuvant	Muramyl dipetide	Mannide monooleate	Monophosphoryl lipid A	Lipopolysaccharide	Muramyl dipetide
34	Major component of the surfactant in freund's Adjuvant is ___	Mannide oleate	Muramyl dipeptide	Monophosphoryl lipid A	Muramyl dipeptide	Mannide oleate
35	One mycobacterial component present in Ribi's Adjuvansts is ___	Mannide oleate	Trehalose dimycolate	Monophosphoryl lipid A	Muramyl dipeptide	Trehalose dimycolate
36	___ is a new aqueous phase adjuvant that does not have a depot effect	Freund's complete adjuvant	Gerbce Adjuvant	Rib's adjuvant	Aluminium salt adjuvant	Gerbce Adjuvant
37	T lymphocytes are derived from the site of maturation	liver	spleen	Thymus	Bone marrow cells	Thymus
38	T cells that express in __	CD_4	CD_8	CD_4 and CD_8	CD_{28}	CD_4 and CD_8
39	_____ the discover of complement, won the prize in 1919	Jules Bordet	Donnall Thomas	Neils Jerne	Rosalyn	Jules Bordet
40	Example for cytokines__	Chemicals	lymphokines	Immunoglobulin	histamine	lymphokines
41	Opsonization is promoted by	Antigens	serum proteins	Macrophages and neutrophills	T cells and B cells	Macrophages and neutrophills

42	Which antibody can have the ability to transport across the placental tissue that separates mother and fetus?	IgM	IgD	IgG	IgA	IgG
43	Primary humoral response characterized by the production of	Macrophages	lymphocytes	Monocytes	AB secreting plasma cell and memory B cell	AB secreting plasma cell and memory B cell
44	Secondary humoral response depends on the existence of a population of	Macrophages and T cell	Macrophages and B cell	Macrophages and lymphochyes	Memory B cells and memory T cells	Memory B cells and memory T cells
45	The predominant antibody mainly secreted during secondary response is	Macrophages and T cell	Macrophages and B cell	Macrophages and lymphochyes	Memory B cells and memory T cells	Memory B cells and memory T cells
46	Primary lymphoid organ are ___	Increase in size with age	Atropy with age	Becomes in Maximum size	Decerease in size with age	Atropy with age
47	___happens due to cross reactivity	Autoimmune reactions	Precipitation reaction	Agglutination reactions	hypersensitive reaction	Autoimmune reactions
48	ABO blood group antigens namely antigen A,B and H are completely absent in ___ blood group	O group	Bombay group	MN group	AB group	Bombay group
49	Rhesus factor discovered from Rhesns monkey in 1940 by __	Landsteiner	Fisher	Landsteiner and Wiener	VonDe castallo and starle	Landsteiner and Wiener
50	In 1972 two protein chemists ____and ____ received the prize for demonstrating the chemical structure of antibody molecule	Rosalyn yalow and Berson	George kobler and Cesar Milstein	Rodney porter and Gerald Edelman	Donnall Thomas and Joseph Murray	Rodney porter and Gerald Edelman
51	Cross reactivity between S pyogens and myosin present in cardiae muscles is throught to be responsible for the ___damage observed in acute rheumatic fever that follows a streptococcal infection	Cardiac damage	Muscular damage	Neuronal damage	Respiratory damage	Cardiac damage

52	_____is a disease due to cross reactivity	Addison's disease	Diarrbborea	AIDS	Nephritis	Addison's disease
53	Lymphocytes consists _____ % of body's WBC	30-50	20- 30 %	20-40%	10-20 %	30-50 %
54	After cell division, the lymphoblasts generates __ and ___	T and B cells	Effector and memory	Marcrophages and monocytes	Peripheral blood mononuclear cell	Effector and memory
55	In lymphoid cells the native cells are __	Small lymphocytes	large lymphocytes	Blast cells	Plasma cells	Small lymphocytes
56	A small group of peripheral blood lymphocyte called ___	MAST cells	NK cells	Null cells	Macrophages	Null cells
57	T lymphocytes are derived from the site of maturation	liver	spleen	Thymus	Bone marrow cells	Thymus
58	T cells that express in __	CD_4	CD_8	CD_4 and CD_8	CD_{28}	CD_4 and CD_8
59	Antibody affinity is _____in the case of secondary response	Higher	Lower	Moderate	Significant	Higher
60	Humoral mediated immunity coming under the ___	Innate immunity	Acquired Immunity	Local Immunity	Herd immunity	Acquired Immunity

UNIT – II

ANTIGEN

Substance that can be recognized by the immunoglobulin receptor of B cells, or by the Tcell receptor when complexed with MHC, are called **antigens**. The molecular properties of antigens and the way in which these properties ultimately contribute to immune activation are central to our understanding of theimmune system. This chapter describes some of the molecular features of antigens recognized by B or T cells. The chapter also explores the contribution made to immunogenicity by the biological system of the host; ultimately the biological system determines whether a molecule that combines with a B or T cell's antigen-binding receptor can then induce an immune response. Fundamental differences in the way B and T lymphocytes recognize antigen determine which molecular features of an antigen are recognized by each branch of the immune system.

FACTORS THAT INFLUNCE CELL IMMUNOGENECITY

To protect against infectious disease, the immune system must be able to recognize bacteria, bacterial products, fungi, parasites, and viruses as immunogens. In fact, the immune system actually recognizes particular macromolecules of an infectious agent, generally either proteins or polysaccharides. Proteins are the most potent immunogens, with polysaccharides ranking second. In contrast, lipids and nucleic acids of an infectious agent generally do not serve as immunogens unless they are complexed with proteins or polysaccharides. Immunologists tend to use proteins or polysaccharides as immunogens in most experimental studies of humoral immunity (Table 3-1). For cell-mediated immunity, only proteins and some lipids and glycolipids serve as immunogens. These molecules are not recognized directly. Proteins must first be processed into small peptides and then presented together with MHC molecules on the membrane of a cell before they can be recognized as immunogens. Recent work shows that those lipids and glycolipids that can elicit cellmediated immunity must also be combined with MHC-like membrane molecules called CD1.

The Nature of the Immunogen Contributes to Immunogenicity

Immunogenicity is determined, in part, by four properties of the immunogen: its foreignness, molecular size, chemical composition and complexity, and ability to be processed and presented with an MHC molecule on the surface of an antigen- presenting cell or altered self-cell.

FOREIGNNESS

In order to elicit an immune response, a molecule must be recognized as nonself by the biological system. The capacity to recognize nonself is accompanied by tolerance of self, a specific unresponsiveness to self antigens. Much of the ability to tolerate self antigens arises during lymphocyte development, during which immature lymphocytes are exposed to self-components. Antigens that have not been exposed to immature lymphocytes during this critical period may be later recognized as nonself, or foreign, by the immune system. When an antigen is introduced into an organism, the degree of its immunogenicity depends on the degree of its foreignness. Generally, the greater the phylogenetic distance between two species, the greater the

structural (and therefore the antigenic) disparity between them. For example, the common experimental antigen bovine serum albumin (BSA) is not immunogenic when injected into a cow but is strongly immunogenic when injected into a rabbit. Moreover, BSA would be expectedto exhibit greater immunogenicity in a chicken than in a goat, which is more closely related to bovines. There are some exceptions to this rule. Some macromolecules (e.g., collagen and cytochrome *c*) have been highly conserved throughout evolution and therefore display very little immunogenicity across diverse species lines. Conversely, some self-components (e.g., corneal tissue and sperm) are effectively sequestered from the immune system, so that if these tissues are injected even into the animal from which they originated, they will function as immunogens.

MOLECULAR SIZE

There is a correlation between the size of a macromolecule and its immunogenicity. The most active immunogens tend to have a molecular mass of 100,000 daltons (Da). Generally, substances with a molecular mass less than 5000–10,000 Da are poor immunogens, although a few substances with a molecular mass less than 1000 Da have proven to be immunogenic.

CHEMICAL COMPOSITION AND HETEROGENEITY

Size and foreignness are not, by themselves, sufficient to make a molecule immunogenic; other properties are needed as well. For example, synthetic homopolymers (polymer composed of a single amino acid or sugar) tend to lack immunogenicity regardless of their size. Studies have shown that copolymers composed of different amino acids or sugars are usually more immunogenic than homopolymers of their constituents. These studies show that chemical complexity contributes to immunogenicity. In this regard it is notable that all four levels of protein organization—primary, secondary, tertiary, and quaternary—contribute to the structural complexity of a protein and hence affect its immunogenicity

LIPIDS AS ANTIGENS

Appropriately presented lipoidal antigens can induce B- and T-cell responses. For the stimulation of B-cell responses, lipids are used as haptens and attached to suitable carrier molecules such as the proteins keyhole limpet hemocyanin (KLH) or bovine serum albumin (BSA).By immunizing with these lipid-protein conjugates it is possible to obtain antibodies that are highly specific for the target lipids. Using this approach, antibodies have been raised against a wide variety of lipid molecules including steroids, complex fatty-acid derivatives, and fat-soluble vitamins such as vitamin E. Such antibodies are of considerable practical importance since many clinical assays for the presence and amounts of medically important lipids are antibody-based. For example, a determination of the levels of a complex group of lipids known as leukotrienes can be useful in evaluating asthma patients. Prednisone, an immunosuppressive steroid, is often given as part of the effort to prevent the rejection of a trans- planted organ. The achievement and maintenance of adequateblood levels of this and other immunosuppressive drugs is important to a successful outcome of transplantation, and antibody-based immunoassays

are routinely used to make these evaluations. The extraordinary sensitivity and specificity of assays based on the use of anti-lipid antibodies, which shows the specificity of an antibody raised against leukotriene C4. This antibody allows the detection of as little as 16–32 picograms per ml of leukotriene C4. Because it has little or no reactivity with similar compounds, such as leukotriene D4 or leukotriene E4, it can be used to assay leukotriene C4 in samples that contain this compound and a variety of other structurally related lipids.T cells recognize peptides derived from protein antigens when they are presented as peptide-MHC complexes. However, some lipids can also be recognized by T cells. Lipoidal compounds such as glycolipids and some phospholipids can be recognized by T-cell receptors when presented as complexes with molecules that are very much like MHC molecules. These lipid-presenting molecules are members of the CD1 family and are close structural relatives of class I MHC molecules. The lipid molecules recognized by the CD1–T-cell receptor system all appear to share the common feature of a hydrophobic portion and a hydrophilic head group. The hydrophobic portion is a long-chain fatty acid oralcohol and the hydrophilic head group is composed of highly polar groups that often contain carbohydrates.Recognition of lipids is a part of the immune response to some pathogens, and T cells that recognize lipids arising from *Mycobacterium tuberculosis* and *Mycobacterium leprae,* which respectively cause tuberculosis and leprosy, have been isolated from humans infected by these mycobacteria. Epitopes immune cells do not interact with, or recognize, an entire immunogen molecule; instead, lymphocytes recognize discrete sites on the macromolecule called **epitopes,** or **antigenic determinants.** Epitopes are the immunologically active regions of an immunogen that bind to antigen-specific membrane receptors on lymphocytes or to secreted antibodies. Studies with small antigens have revealed that B and T cells recognize different epitopes on the same antigenic molecule. For example, when mice were immunized with glucagon, a small human hormone of 29 amino acids, antibody was elicited to epitopes in the aminoterminalc aminoterminal portion, whereas the T cells responded only to epitopes in the carboxyl-terminal portion.

Lymphocytes may interact with a complex antigen on several levels of antigen structure. An epitope on a protein antigen may involve elements of the primary, secondary, tertiary, and even quaternary structure of the protein (see Figure 3-1). In polysaccharides, branched chains are commonly present, and multiple branches may contribute to the conformation of epitopes. The recognition of antigens by T cells and B cells is fundamentally different (Table 3-4). B cells recognize soluble antigen when it binds to their membrane-bound antibody. Because B cells bind antigen that is free in solution, the epitopes they recognize tend to be highly accessible sites on the exposed surface of the immunogen. As noted previously, most T cells recognize only peptides combined with MHC molecules on the surface of antigen-presenting cells and altered self-cells; T-cell epitopes, as a rule, cannot be considered apart from their associated MHC molecules.

Properties of B-Cell Epitopes Are Determined by the Nature of the Antigen-Binding Site

Several generalizations have emerged from studies in which the molecular features of the epitope recognized by B cells have been established. The ability to function as a B-cell epitope is

determined by the nature of the antigen- binding site on the antibody molecules displayed by B cells. Antibody binds to an epitope by weak noncovalent interactions, which operate only over short distances. For a strong bond, the antibody's binding site and the epitope must have complementary shapes that place the interacting groups near each other. This requirement poses some restriction on the properties of the epitope. The size of the epitope recognized by a B cell can be no larger than the size of the antibody's binding site. For any given antigen-antibody reaction, the shape of the epitope that can be recognizedby the antibody is determined by the shape assumed by the sequences of amino acids in the binding site and the chemical environment that they produce.Smaller ligands such as carbohydrates, small oligonucleotides, peptides, and haptens often bind within a deep pocket of an antibody. For example, angiotensin II, a small octapeptide hormone, binds within a deep and narrow groove (725 Å2) of a monoclonal antibody specific for the hormone. Within this groove, the bound peptide hormone folds into a compact structure with two turns, which brings its amino (N-terminal) and carboxyl (C-terminal) termini close together. All eight amino acid residues of the octapeptide are in van der Waals contact with 14 residues of the antibody's groove. A quite different picture of epitope structure emerges from x-ray crystallographic analyses of monoclonal antibodies bound to globular protein antigens such as hen egg-white lysozyme (HEL) and neuraminidase (an envelope glycoprotein of influenza virus). These antibodies make contact with the antigen across a large flat face. The interacting face between antibody and epitope is a flat or undulating surface in which protrusions on the epitope or antibody are matched by corresponding depressions on the antibody or epitope. These studies have revealed that 15–22 amino acids on the surface of the antigen make contact with a similar number of residues in the antibody's binding site; the surface area of this large complementary interface is between 650 Å2 and 900 Å2. For these globular protein antigens, then, the shape of the epitope is entirely determined by the tertiary conformation of the native protein. Thus, globular protein antigens and small peptide antigens interact with antibody in different ways. Typically, larger areas of protein antigens are engaged by the antibody binding site. In contrast, a small peptide such as angiotensin II can fold into a compact structure that occupies less space and fits into a pocket or cleft of the binding site. This pattern is not unique to small peptides; it extends to the binding of low-molecular-weight antigens of various chemical types. However, these differences between the binding of small and large antigenic determinants do not reflect fundamental differences in the regions of the antibody molecule that make up the binding site. Despite differences in the binding patterns of small haptens and large antigens.

The B-cell epitopes on native proteins generally are composed of hydrophilic amino acids on the protein surface that are topographically accessible to membrane-bound or free antibody. A B-cell epitope must be accessible in order to be able to bind to an antibody; in general, protruding regions on the surface of the protein are the most likely to be recognized as epitopes, and these regions are usually composed of predominantly hydrophilic amino acids. Amino acid sequences that are hidden within the interior of a protein often consist of predominantly hydrophobic amino acids, and cannot function as B-cell epitopes unless the protein is first denatured. In the crystallized antigen-antibody complexes analyzed to date, the interface between antibody and antigen shows numerous complementary protrusions and depressions. Between 15

and 22 amino acids on the antigen contact the antibody by 75–120 hydrogen bonds as well as by ionic and hydrophobic interactions. *B-cell epitopes can contain sequential or nonsequential amino acids.* Epitopes may be composed of sequential contiguous residues along the polypeptide chain or nonsequential residues from segments of the chain brought together by the folded conformation of an antigen. Most antibodies elicited by globular proteins bind to the protein only when it is in its native conformation. Because denaturation of such antigens usually changes the structure of their epitopes, antibodies to the native protein do not bind to the denatured protein.Five distinct sequential epitopes, each containing six to eight contiguous amino acids, have been found in sperm whale myoglobin. Each of these epitopes is on the surface of the molecule at bends between the α-helical regions. Sperm whale myoglobin also contains several nonsequential epitopes, or conformational determinants. The residues that constitute these epitopes are far apart in the primary amino acid sequence but close together in the tertiary structure of the molecule. The B-cell epitopes on native proteins generally are composed of hydrophilic amino acids on the protein surface that are topographically accessible to membrane-bound or free antibody. A B-cell epitope must be accessible in order to be able to bind to an antibody; in general, protruding regions on the surface of the protein are the most likely to be recognized as epitopes, and these regions are usually composed of predominantly hydrophilic amino acids. Amino acid sequences that native protein conformation for their topographical structure. One well-characterized nonsequential epitope in hen egg-white lysozyme (HEL) is shown in Figure 3-6b.Although the amino acid residues that compose this epitope of HEL are far apart in the primary amino acid sequence, they are brought together by the tertiary folding of the protein.

Sequential and nonsequential epitopes generally behave differently when a protein is denatured, fragmented, or reduced. For example, appropriate fragmentation of sperm whale myoglobin can yield five fragments, each retaining one sequential epitope, as demonstrated by the observation that antibody can bind to each fragment. On the other hand, fragmentation of a protein or reduction of its disulfide bonds often destroys nonsequential epitopes. For example, HEL has four intrachain disulfide bonds, which determine the final protein conformation (Figure 3-7a). Many antibodies to HEL recognize several epitopes, and each of eight different epitopes have been recognized by a distinct antibody.Most of these epitopes are conformational determinants dependent on the overall structure of the protein. If the intrachain disulfide bonds of HEL are reduced with mercaptoethanol, the nonsequential epitopes are lost; for this reason, antibody to native HEL does not bind to reduced HEL

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The inhibition experiment shown in Figure 3-7 nicely demonstrates this point. An antibody to a conformational determinant, in this example a peptide loop present in native HEL, was able to bind the epitope only if the disulfide bond that maintains the structure of the loop was intact. Information about the structural requirements of the antibody combining site was obtained by examining the ability of structural relatives of the natural antigen to bind to that antibody. If a structural relative has the critical epitopes present in the natural antigen, it will bind to the antibody combining site, thereby blocking its occupation by the natural antigen. In

this inhibition assay, the ability of the closed loop to inhibit binding showed that the closed loop was sufficiently similar to HEL to be recognized by antibody to native HEL. Even though the open loop had the same sequence of aminoacids as the closed loop, it lacked the epitopes recognized by the antibody and therefore was unable to block binding of HEL. *B-cell epitopes tend to be located in flexible regions of an immunogen and display site mobility*. John A. Tainer and his colleagues analyzed the epitopes on a number of protein antigens (myohemerytherin, insulin, cytochrome *c,* myoglobin, and hemoglobin) by comparing the positions of the known B-cell epitopes with the mobility of the same residues. Their analysis revealed that the major antigenic determinants in these proteins generally were located in the most mobile regions. These investigators proposed that site mobility of epitopes maximizes complementarity with the antibody's binding site, permitting an antibody to bind with an epitope that it might bind ineffectively if it were rigid. However, because of the loss of entropy due to binding to a flexible site, the binding of antibody to a flexible epitope is generally of lower affinity than the binding of antibody to a rigid epitope. *Complex proteins contain multiple overlapping B-cell epitopes, some of which are immunodominant.* For many years, it was dogma in immunology that each globular protein had a small number of epitopes, each confined to a highly accessible region and determined by the overall conformation of the protein.However, it has been shown more recently that most of the surface of a globular protein is potentially antigenic. This has been demonstrated by comparing the antigen-binding profiles of different monoclonal antibodies to various globular proteins. For example, when 64 different monoclonal antibodies to BSA were compared for their ability to bind to a panel of 10 different mammalian albumins, 25 different overlapping antigen-binding profiles emerged, suggesting that these 64 different antibodies recognized a minimum of 25 different epitopes on BSA. Similar findings have emerged for other globular proteins, such as myoglobin and HEL. The surface of a protein, then, presents a large number of potential antigenic sites. The subset of antigenic sites on a given protein that is recognized by the immune system of an animal is much smaller than the potential antigenic repertoire, and it varies from species to species and even among individual members of a given species.Within an animal, certain epitopes of an antigen are recognized as immunogenic, but others are not. Furthermore, some epitopes, called immunodominant**,** induce a more pronounced immune response than other epitopes in a particular animal. It is highly likely that the intrinsic topographical properties of the epitope as well as the animal's regulatory mechanisms influence the immunodominance of epitopes.

Antigen-Derived Peptides Are the Key Elements of T-Cell Epitopes

Studies by P. G. H. Gell and Baruj Benacerraf in 1959 suggested that there was a qualitative difference between the Tcell and the B-cell response to protein antigens. Gell and Benacerraf compared the humoral (B-cell) and cell-mediated (T-cell) responses to a series of native and denatured protein antigens. They found that when primary immunization was with a native protein, only native protein, not denatured protein, could elicit a secondary antibody (humoral) response. In contrast, both native and denatured protein could elicit a secondary cell-mediated response. The finding that a secondary response mediated by T cells was induced by denatured protein, even when the primary immunization had been with native protein, initially

puzzled immunologists. In the 1980s, however, it became clear that T cells do not recognize soluble native antigen but rather recognize antigen that has been processed into antigenic peptides, which are presented in combination with MHC molecules. For this reason, destruction of the conformation of a protein by denaturation does not affect its T-cell epitopes. Because the T-cell receptor does not bind free peptides, experimental systems for studying T-cell epitopes must include antigen-presenting cells or target cells that can display the peptides bound to an MHC molecule. *Antigenic peptides recognized by T cells form trimolecular complexes with a T-cell receptor and an MHC molecule*. The structures of TCR-peptide-MHC trimolecular complexes have been determined by x-ray crystallography and are described in Chapter 9. These structural studies of class I or class II MHC molecules crystallized with known Tcell antigenic peptides has shown that the peptide binds to a cleft in the MHC molecule .Unlike B-cell epitopes,which can be viewed strictly in terms of their ability to interact with antibody, T-cell epitopes must be viewed in terms of their ability to interact with both a T-cell receptorand an MHC molecule.

The binding of an MHC molecule to an antigenic peptide does not have the fine specificity of the interaction between an antibody and its epitope. Instead, a given MHC molecule can selectively bind a variety of different peptides. For example, the class II MHC molecule designated IA*d* can bind peptides from ovalbumin (residues 323–339), hemagglutinin (residues 130– 142), and lambda repressor (residues 12–26). Studies revealing structural features, or motifs, common to different peptides that bind to a single MHC molecule are described in Chapter 7.

Antigen processing is required to generate peptides that interact specifically with MHC molecules., endogenous and exogenous antigens are usually processed by different intracellular pathways . Endogenous antigens are processed into peptides within the cytoplasm, while exogenous antigens are processed by the endocytic pathway. The details of antigen processing and presentation are described in Chapter 8.

Epitopes recognized by T cells are often internal. T cells tend to recognize internal peptides that are exposed by processing within antigen-presenting cells or altered self-cells. J. Rothbard analyzed the tertiary conformation of hen egg-white lysozyme and sperm whale myoglobin to determine which amino acids protruded from the natural molecule. He then mapped the major T-cell epitopes for both proteins and found that, in each case, the T-cell epitopes tended to be on the “inside” of the protein molecule.

Haptens

The pioneering work of Karl Landsteiner in the 1920s and 1930s created a simple, chemically defined system for studying the binding of an individual antibody to a unique epitope on a complex protein antigen. Landsteiner employed various haptens, small organic molecules that are antigenic but not immunogenic. Chemical coupling of a hapten to a large protein, called a carrier, yields an immunogenic hapten-carrier conjugate. Animals immunized with such a conjugate produce antibodies specific for (1) the hapten determinant, (2) unaltered epitopes on

the carrier protein, and (3) new epitopes formed by combined parts of both the hapten and carrier. By itself, a hapten cannot function as an immunogenic epitope.But when multiple molecules of a single hapten are coupled to a carrier protein (or nonimmunogenic homopolymer), the hapten becomes accessible to the immune system and can function as an immunogen. The beauty of the hapten-carrier system is that it provides immunologists with a chemically defined determinant that can be subtly modified by chemical means to determine the effect of various chemical structures on immune specificity. In his studies, Landsteiner immunized rabbits with a haptencarrier conjugate and then tested the reactivity of the rabbit's immune sera with that hapten and with closely related haptens coupled to a different carrier protein.He was thus able to measure, specifically, the reaction of the antihapten antibodies in the immune serum and not that of antibodies to the original carrier epitopes. Landsteiner tested whether an antihapten antibody could bind to other haptens having a slightly different chemical structure. If a reaction occurred, it was called a cross-reaction. By observing which hapten modifications prevented or permitted cross-reactions, Landsteiner was able to gain insight into the specificity of antigenantibody interactions. Using various derivatives of aminobenzene as haptens, Landsteiner found that the overall configuration of a hapten plays a major role in determining whether it can react with a given antibody. For example, antiserum from rabbits immunized with aminobenzene or one of its carboxyl derivatives (*o*-aminobenzoic acid, *m*-aminobenzoic acid, or *p*aminobenzoic acid) coupled to a carrier protein reacted only with the original immunizing hapten and did not cross- react with any of the other haptens (Table 3-6). In contrast, if theoverall configuration of the hapten was kept the same and the hapten was modified in the para position with various nonionic derivatives, then the antisera showed various degrees of cross-reactivity. Landsteiner's work not only demonstrated the specificity of the immune system, but also demonstrated the enormous diversity of epitopes that the immunesystem is capable of recognizing.

Many biologically important substances, including drugs, peptide hormones, and steroid hormones, can function as haptens. Conjugates of these haptens with large protein carriers can be used to produce hapten-specific antibodies. These antibodies are useful for measuring the presence of various substances in the body. For instance, the original home pregnancy test kit employed antihapten antibodies to determine whether a woman's urine contained human chorionic gonadotropin (HCG), which is a sign of pregnancy. However, as shown in the Clinical Focus, the formation of drug-protein conjugates in the body can produce drug allergies that may be life-threatening.

Pattern-Recognition Receptors

The receptors of adaptive and innate immunity differ. Antibodies and T-cell receptors, the receptors of adaptive immunity, recognize details of molecular structure and can discriminate with exquisite specificity between antigens featuring only slight structural differences. The receptors of innate immunity recognize broad structural motifs that are highly conserved within microbial species but are generally absent from the host. Because they recognize particular overall molecular patterns, such receptors are called pattern recognition receptors (PRRs). Patterns recognized by this type of receptor include combinations of sugars, certain proteins,

particular lipid-bearing molecules, and some nucleic acid motifs. Typically, the ability of pattern-recognition receptors to distinguish between self and nonself is perfect because the molecular pattern targeted by the receptor is produced only by the pathogen and never by the host. This contrasts sharply with the occasional recognition of self antigens by receptors of adaptive immunity, which can lead to autoimmune disorders. Like antibodies and T-cell receptors, pattern-recognition receptors are proteins. However, the genes that encode PRRs are present in the germline of the organism. In contrast, the genes that encode the enormous diversity of antibodies and TCRs are not present in the germline. They are generated by an extraordinary process of genetic recombination. Many different pattern-recognition receptors have been identified. Some are present in the bloodstream and tissue fluids as soluble circulating proteins and others are on the membrane of cells such as macrophages, neutrophils, and dendritic cells. Mannosebinding lectin (MBL) and C-reactive protein (CRP) are soluble pattern receptors that bind to microbial surfaces and promote their opsonization. Both of these receptors also have the ability to activate the complement system when they are bound to the surface of microbes, thereby making the invader a likely target of complement-mediated lysis. Yet another soluble receptor of the innate immune system, lipopolysaccharide-binding protein, is an important part of the system that recognizes and signals a response to lipopolysaccharide, a component of the outer cell wall of gram-negative bacteria.

Pattern-recognition receptors found on the cell membrane include scavenger receptors and the toll-like receptors. Scavenger receptors (SRs) are present on macrophages and many types of dendritic cells, and are involved in the binding and internalization of gram-positive and gram-negative bacteria, as well as the phagocytosis of apoptotic host cells. The exact roles and mechanisms of action of the many types of scavenger receptors known to date are under active investigation. The toll-like receptors (TLRs) are important in recognizing many microbial patterns. This family of proteins is ancient—toll-like receptors mediate the recognition and generation of defensive responses to pathogens in organisms as widely separated in evolutionary history as humans and flies. Typically, signals transduced through the TLRs cause transcriptional activation and the synthesis and secretion of cytokines, which promote inflammatory responses that bring macrophages and neutrophils to sites of inflammation. TLR signaling can also result in the recruitment and activation of macrophages, NK cells, and dendritic cells, key agents in the presentation of antigen to T cells. The links to T cells and cytokine release shows the intimate relationship between innate and adaptive responses.

A search of the human genome has uncovered 10 TLRs,and the functions of six members of this PRR family have been determined. TLR2, often with the collaboration of TLR6, binds a wide variety of molecular classes found in microbes, including peptidoglycans, zymosans, and bacterial lipopeptides. TLR4 is the key receptor for most bacterial lipopolysaccharides, although TLR2 also binds some varieties of LPS. The binding of LPS by either of these TLRs is complex and involves the participation of three additional proteins, one of which is the lipopolysaccharide-binding protein mentioned above, abbreviated LBP. The first step in the process is the binding of LPS by circulating LBP, which then delivers it to a complex of TLR4

(or TLR2) with two additional proteins, CD14 and MD2. The engagement of LPS by this complex causes its TLR component to initiate a signal- transduction process that can produce a cellular response. Another family member, TLR5, recognizes flagellin, the major structural component of bacterial flagella. TLR3 recognizes the double-stranded RNA (dsRNA) that appears after infection by RNA viruses. dsRNA is also recognized by dsRNA-activated kinase. Finally, TLR9 recognizes and initiates a response to CpG (unmethylated cytosine linked to guanine) sequences. These sequences are represented in abundance in microbial sequences but are much less common in mammalian sequences.

Antibody

Antibodies (Immuno globulins) are the antigen binding protein present on the B-cell membrane and secreted by plasma cells. Membrane-bound antibody confers antigenic specificity on B cells; antigen-specific proliferation of B-cell clones is elicted by the interaction of membrane antibody with antigen. Secreted antibodies circulate in the blood, where they serve as the effectors of humoral immunity by searching out and neutralizing antigens or marking them for elimination.All antibodies share structural features, bind to antigen, and participate in a limited number of effector functions. The antibodies produced in response to a particular antigen are heterogeneous.Most antigens are complex and contain many different antigenic determinants, and the immune system usually responds by producing antibodies to several epitopes on the antigen. This response requires the recruitment of several clones of B cells. Their outputs are monoclonal antibodies, each of which specifically binds a single antigenic determinant. Together, these monoclonal antibodies make up the polyclonal and heterogeneous serum antibody response to an immunizing antigen.

Basic Structure of Antibodies

Blood can be separated in a centrifuge into a fluid and a cellular fraction. The fluid fraction is the **plasma** and the cellular fraction contains red blood cells, leukocytes, and platelets. Plasma contains all of the soluble small molecules and macromolecules of blood, including fibrin and other proteins required for the formation of blood clots. If the blood or plasma is allowed to clot, the fluid phase that remains is called serum. It has been known since the turn of the century that antibodies reside in the serum. The first evidence that antibodies were contained in particular serum protein fractions came from a classic experiment by A. Tiselius and E. A.Kabat, in 1939. They immunized rabbits with the protein ovalbumin (the albumin of egg whites) and then divided the immunized rabbits' serum into two aliquots. Electrophoresis of one serum aliquot revealed four peaks corresponding to albumin and the alpha (α), beta (β), and gamma (γ) globulins. The other serum aliquot was reacted with ovalbumin, and the precipitate that formed was removed; the remaining serum proteins, which did not react with the antigen, were then electrophoresed. A comparison of the electrophoretic profiles of these two serum aliquots revealed that there was a significant drop in the γ-globulin. peak in the aliquot that had been reacted with antigen. Thus, the γ-globulin fraction was identified as containing serum antibodies, which were called immunoglobulins, to distinguish them from any other proteins that might be contained in the γ-globulin fraction. The early experiments of Kabat and Tiselius resolved serum

proteins into three major nonalbumin peaks. We now know that although immunoglobulin G (IgG), the main class of antibody molecules, is indeed mostly found in the γ-globulin fraction, significant amounts of it and other important classes of antibody molecules are found in the fractions of serum.

Antibodies Are Heterodimers

Antibody molecules have a common structure of four peptide chains. This structure consists of two identical light (L) chains, polypeptides of about 25,000 molecular weight, and two identical heavy (H) chains, larger polypeptides of molecular weight 50,000 ormore.Likethe antibody molecules they constitute, H and L chains are also called immunoglobulins. Each light chain is bound to a heavy chain by a disulfide bond, and by such noncovalent interactions as salt linkages, hydrogen bonds, and hydrophobic bonds, to form a heterodimer (H-L). Similar noncovalent interactions and disulfide bridges link the two identical heavy and light (H-L) chain combinations to each other to form the basic four-chain (H-L)2 antibody structure, a dimer of dimers. As we shall see, the exact number and precise positions of these interchain disulfide bonds differs among antibody classes and subclasses.

The first 110 or so amino acids of the amino-terminal region of a light or heavy chain varies greatly among antibodies of different specificity. These segments of highly variable sequence are called V regions:VL in light chains and VH in heavy. All of the differences in specificity displayed by different antibodies can be traced to differences in the amino acid sequences of V regions. In fact, most of the differences among antibodies fall within areas of the V regions called complementarity- determining regions (CDRs), and it is these CDRs, on both light and heavy chains, that constitute the antigenbinding site of the antibody molecule. By contrast, within the same antibody class, far fewer differences are seen when one compares sequences throughout the rest of the molecule. The regions of relatively constant sequence beyond the variable regions have been dubbed C regions, CL on the light chain and CH on the heavy chain.Antibodies are glycoproteins; with few exceptions, the sites of attachment for carbohydrates are restricted to the constant region.We do not completely understand the role played by glycosylation of antibodies, but it probably increases the solubility of the molecules. Inappropriate glycosylation, or its absence, affects the rate at which antibodies are cleared from the serum, and decreases the efficiency of interaction between antibody and the complement system and between antibodies and Fc receptors.

Chemical and Enzymatic Methods Revealed Basic Antibody Structure

Our knowledge of basic antibody structure was derived from a variety of experimental observations.When the -globulin fraction of serum is separated into high- and low-molecularweight fractions, antibodies of around 150,000-MW, designated as immunoglobulin G (IgG) are found in the lowmolecular- weight fraction. In a key experiment, brief digestion of IgG with the enzyme papain produced three fragments, two of which were identical fragments and a third that was quite different. The two identical fragments (each with a MW of 45,000), had

antigen-binding activity and were called Fab fragments ("fragment, antigen binding"). The other fragment (MW of 50,000) had no antigenbinding activity at all. Because it was found to crystallize during cold storage, it was called the Fc fragment ("fragment, crystallizable").Digestion with pepsin, a different proteolytic enzyme, also demonstrated that the antigen-binding properties of an antibody can be separated from the rest of the molecule. Pepsin digestion generated a single 100,000- MW fragment composed of two Fab-like fragments designated the F(ab)2 fragment, which binds antigen. The Fc fragment was not recovered from pepsin digestion because it had been digested into multiple fragments.

A key observation in deducing the multichain structure of IgG was made when the molecule was subjected to mercaptoethanol reduction and alkylation, a chemical treatment that irreversibly cleaves disulfide bonds. If the sample is chromatographed on a column that separates molecules by size following cleavage of disulfide bonds, it is clear that the intact 150,000-MW IgG molecule is, in fact, composed of subunits. Each IgG molecule contains two 50,000-MW polypeptide chains, designated as heavy (H) chains, and two 25,000-MW chains, designated as light (L) chains. Antibodies themselves were used to determine how the enzyme digestion products—Fab, F(ab)2, and Fc—were related to the heavy-chain and light-chain reduction products. The antibody to the Fab fragment could react with both the H and the L chains, whereas antibody to the Fc fragment reacted only with the H chain. These observations led to the conclusion that the Fab fragment consists of portions of a heavy and a light chain and that Fc contains only heavy-chain components. the IgG molecule consists of two identical H chains and two identical L chains, which are linked by disulfide bridges. The enzyme papain cleaves just above the interchain disulfide bonds linking the heavy chains, whereas the enzyme pepsin cleaves just below these bonds, so that the two proteolytic enzymes generate different digestion products. Mercaptoethanol reduction and alkylation allow separation of the individual heavy and light chains.

Isotypes of Immmunoglobulin

The various immunoglobulin isotypes and classes have been mentioned briefly already. Each class is distinguished by unique amino acid sequences in the heavy-chain constant region that confer class-specific structural and functional properties.

Immunoglobulin G (IgG)

IgG, the most abundant class in serum, constitutes about 80% of the total serum immunoglobulin. The IgG molecule consists of two _ heavy chains and two or two light chains. There are four human IgG subclasses, distinguished by differences in _-chain sequence and numbered according to their decreasing average serum concentrations: IgG1, IgG2, IgG3, and IgG4. The amino acid sequences that distinguish the four IgG subclasses are encoded by different germ-line CH genes, whose DNA sequences are 90%–95% homologous. The structural characteristics that distinguish these subclasses from one another are the size of the hinge region and the number and position of the interchain disulfide bonds between the heavy chains. The subtleamino acid differences between subclasses of IgG affect the biological activity of the molecule: IgG1, IgG3, and IgG4 readily cross the placenta and play an important role in

protecting the developing fetus IgG3 is the most effective complement activator, followed by IgG1; IgG2 is less efficient, and IgG4 is not able to activate complement at all. IgG1 and IgG3 bind with high affinity to Fc receptors on phagocytic cells and thus mediate opsonization. IgG4 has an intermediate affinity for Fc receptors, and IgG2 has an extremely low affinity.

Immunoglobulin M (IgM)

IgM accounts for 5%–10% of the total serum immunoglobulin, with an average serum concentration of 1.5 mg/ml. Monomeric IgM, with a molecular weight of 180,000, is expressed as membrane-bound antibody on B cells. IgM is secreted by plasma cells as a pentamer in which five monomer units are held together by disulfide bonds that link their carboxyl- terminal heavychain domains (C_4/C_4) and their C_3/C_3 domains.The five monomer subunits are arranged with their Fc regions in the center of the pentamer and the ten antigen-binding sites on the periphery of the molecule. Each pentamer contains an additional Fc-linked polypeptide called the **J (joining) chain,** which is disulfide-bonded to the carboxyl-terminal cysteine residue of two of the ten _ chains. The J chain appears to be required for polymerization of the monomers to form pentameric IgM; it is added just before secretion of the pentamer. IgM is the first immunoglobulin class produced in a primary response to an antigen, and it is also the first immunoglobulin to be synthesized by the neonate. Because of its pentameric structure with 10 antigen-binding sites, serum IgM has a higher valency than the other isotypes. An IgM molecule can bind 10 small hapten molecules; however, because of steric hindrance, only 5 or fewer molecules of larger antigens can be bound simultaneously.Because of its high valency, pentameric IgM is more efficient than other isotypes in binding antigens with many repeating epitopes such as viral particles and red blood cells (RBCs). For example, when RBCs are incubated with specific antibody, they clump together into large aggregates in a process called agglutination. It takes 100 to 1000 times more molecules of IgG than of IgM to achieve the same level of agglutination. A similar phenomenon occurs with viral particles: less IgM than IgG is required to neutralize viral infectivity. IgM is also more efficient than IgG at activating complement. Complement activation requires two Fc regions in close proximity, and the pentameric structure of a single molecule of IgM fulfills this requirement Because of its large size, IgM does not diffuse well and therefore is found in very low concentrations in the intercellular tissue fluids. The presence of the J chain allows IgM to bind to receptors on secretory cells,which transport it across epithelial linings to enter the external secretions that bathe mucosal surfaces. Although IgA is the major isotype found in these secretions, IgM plays an important accessory role as a secretory immunoglobulin.

Immunoglobulin A (IgA)

Although IgA constitutes only 10%–15% of the total immunoglobulin in serum, it is the predominant immunoglobulin class in external secretions such as breast milk, saliva, tears, and mucus of the bronchial, genitourinary, and digestive tracts. In serum, IgA exists primarily as a monomer, but polymeric forms (dimers, trimers, and some tetramers) are sometimes seen, all containing a J-chain polypeptide The IgA of external secretions, called **secretory IgA,** consists of a dimer or tetramer, a J-chain polypeptide, and a polypeptide chain called **secretory component**. As is explained below, secretory component is derived from the receptor that is

responsible for transporting polymeric IgA across cell membranes. The J-chain polypeptide in IgA is identical to that found in pentameric IgM and serves a similar function in facilitating the polymerization of both serum IgA and secretory IgA. The secretory component is a 70,000-MW polypeptide produced by epithelial cells of mucous membranes. It consists of five immunoglobulin-like domains that bind to the Fc region domains of the IgA dimer. This interaction is stabilized by a disulfide bond between the fifth domain of the secretory component and one of the chains of the dimeric IgA. The daily production of secretory IgA is greater than that of any other immunoglobulin class. IgA-secreting plasma cells are concentrated along mucous membrane surfaces.

Secretory IgA serves an important effector function at mucous membrane surfaces, which are the main entry sites. for most pathogenic organisms. Because it is polymeric, secretory IgA can cross-link large antigens with multiple epitopes. Binding of secretory IgA to bacterial and viral surface antigens prevents attachment of the pathogens to the mucosal cells, thus inhibiting viral infection and bacterial colonization. Complexes of secretory IgA and antigen are easily entrapped in mucus and then eliminated by the ciliated epithelial cells of the respiratory tract or by peristalsis of the gut. Secretory IgA has been shown to provide an important line of defense against bacteria such as *Salmonella, Vibrio cholerae,* and *Neisseria gonorrhoeae* and viruses such as polio, influenza, and reovirus. Breast milk contains secretory IgA and many other moleculesthat help protect the newborn against infection during the first month of life.

Immunoglobulin E (IgE)

The potent biological activity of IgE allowed it to be identified in serum despite its extremely low average serum concentration (0.3 g/ml). IgE antibodies mediate the immediate hypersensitivity reactions that are responsible for the symptoms of hay fever, asthma, hives, and anaphylactic shock. The identification of IgE was accomplished by K. and T. Ishizaka in 1966. They obtained serum from an allergic individual and immunized rabbits with it to prepare antiisotype antiserum. The rabbit antiserum was then allowed to react with each class of human antibody known at that time (i.e., IgG, IgA, IgM, and IgD). IgE binds to Fc receptors on the membranes of blood basophils and tissue mast cells. Cross-linkage of receptor bound IgE molecules by antigen (allergen) induces basophils and mast cells to translocate their granules to the plasma membrane and release their contents to the extracellular environment, a process known as degranulation.

Immunoglobulin D (IgD)

IgD was first discovered when a patient developed a multiple myeloma whose myeloma protein failed to react with antiisotype antisera against the then-known isotypes: IgA, IgM, and IgG.When rabbits were immunized with this myeloma protein, the resulting antisera were used to identify the same class of antibody at low levels in normal human serum. The new class, called IgD, has a serum concentration of 30 _g/ml and constitutes about 0.2% of the total immunoglobulin in serum. IgD, together with IgM, is the major membranebound immunoglobulin expressed by mature B cells, and its role in the physiology of B cells is under investigation. No biological effectors function has been identified for IgD.

ANTIGENIC DETERMINANTS ON IMMUNOGLOBULINS

Since antibodies are glycoproteins, they can themselves function as potent immunogens to induce an antibody response. Such anti-Ig antibodies are powerful tools for the study ofB-cell development and humoral immune responses. The antigenic determinants, or epitopes, on immunoglobulin molecules fall into three major categories: isotypic, allotypic, and idiotypic determinants,which are located in characteristic portions of the molecule.

Isotype

Isotypic determinants are constant-region determinants that collectively define each heavy-chain class and subclass and each light-chain type and subtype within a species. Each isotype is encoded by a separate constantregion gene, and all members of a species carry the same constant-region genes (which may include multiple alleles). Within a species, each normalindividual will express all isotypes in the serum. Different species inherit different constant- region genes and therefore express different isotypes. Therefore, when an antibody from one species is injected into another species, the isotypic determinants will be recognized as foreign, inducing an antibody response to the isotypic determinants on the foreign antibody. Anti-isotype antibody is routinely used for research purposes to determinethe class or subclass of serum antibody produced during an immune response or to characterize the class of membrane-bound antibody present on B cells.

Allotype

Although all members of a species inherit the same set of isotype genes, multiple alleles exist for some of the genes. These alleles encode subtle amino acid differences, called allotypic determinants, that occur in some, but not all, members of a species. The sum of the individual allotypic determinants displayed by an antibody determines its **allotype.** In humans, allotypes have been characterized forall four IgG subclasses, for one IgA subclass, and for the light chain. The _-chain allotypes are referred to as Gm markers. At least 25 different Gm allotypes have been identified; they are designated by the class and subclass followed by the allele number, for example, G1m(1), G2m(23), G3m(11), G4m(4a). Of the two IgA subclasses, only the IgA2 subclass has allotypes, as A2m(1) and A2m(2). The light chain has three allotypes, designated m(1), m(2), and m(3). Each of these allotypic determinants represents differences in one to four amino acids that are encoded by different alleles. Antibody to allotypic determinants can be produced by injecting antibodies from one member of a species into another member of the same species who carries different allotypic determinants. Antibody to allotypic determinants sometimes is produced by a mother during pregnancy in response to paternal allotypic determinants on the fetal immunoglobulins. Antibodies to allotypic determinants can also arise from a blood transfusion.

Idiotype

The unique amino acid sequence of the VH and VL domains of a given antibody can function not only as an antigen-binding site but also as a set of antigenic determinants. The idiotypic determinants arise from the sequence of the heavy- and light-chain variable regions. Each individual antigenic determinant of the variable region is referred to as an **idiotope.** In some cases an idiotope may be the actual antigen-binding site, and in some cases an idiotope

may comprise variable-region sequences outside of the antigenbinding site. Each antibody will present multiple idiotopes; the sum of the individual idiotopes is called the **idiotype** of the antibody. Because the antibodies produced by individual B cells derived from the same clone have identical variable-region sequences, they all have the same idiotype. Anti-idiotype antibody is produced by injecting antibodies that have minimal variation in their isotypes and allotypes, so that the idiotypic difference can be recognized.

THE IMMUNOGLOBULIN SUPERFAMILY

The structures of the various immunoglobulin heavy and light chains described earlier share several features, suggesting that they have a common evolutionary ancestry. In particular, all heavy- and light-chain classes have the immunoglobulin-fold domain structure. The presence of this characteristic structure in all immunoglobulin heavy and light chains suggests that the genes encoding them arose from a common primordial gene encoding a polypeptide of about 110amino acids. Gene duplication and later divergence could then have generated the various heavy- and light-chain genes. Large numbers of membrane proteins have been shown to possess one or more regions homologous to an immunoglobulin domain. Each of these membrane proteins isclassified as a member of the **immunoglobulin superfamily.** The term *superfamily* is used to denote proteins whose corresponding genes derived from a common primordial gene encoding the basic domain structure. These genes have evolved independently and do not share genetic linkage or function. The following proteins, in addition to the immunoglobulins themselves, are representative members of the immunoglobulin superfamily.

- Ig-α/Ig-β heterodimer, part of the B-cell receptor
- Poly-Ig receptor, which contributes the secretory component to secretory IgA and IgM
- T-cell receptor
- T-cell accessory proteins, including CD2, CD4, CD8, and CD28, and the γ, δ and ε chains of CD3
- Class I and class II MHC molecules 2-microglobulin, an invariant protein associated with class I MHC molecules
- Various cell-adhesion molecules, including VCAM-1, ICAM-1, ICAM-2, and LFA-3 _
- Platelet-derived growth factor

Numerous other proteins, some of them discussed in other chapters, also belong to the immunoglobulin superfamily. X-ray crystallographic analysis has not been accomplished for all members of the immunoglobulin superfamily. Nevertheless, the primary amino acid sequence of these proteins suggests that they all contain the typical immunoglobulin- fold domain. Specifically, all members of the immunoglobulin superfamily contain at least one or more stretches of about 110 amino acids, capable of arrangement into pleated sheets of antiparallel strands, usually with aninvariant intrachain disulfide bond that closes a loop spanning 50–70 residues. Most members of the immunoglobulin superfamily cannot bind antigen. Thus, the characteristic Ig-fold structure found in so many membrane proteins musthave some function other than antigen binding. One possibility is that the immunoglobulin fold may facilitate interactions between membrane proteins. As described earlier, interactions can occur between the faces of _ pleated sheets both of homologous immunoglobulin domains (e.g., CH2/CH2 interaction) and of nonhomologous domains (e.g., VH/VL and CH1/CL interactions).

Organization and Expression of Immunoglobulin Genes

As immunoglobulin (Ig) sequence data accumulated virtually every antibody molecule studied was found to contain a unique amino acid sequence in its variable region but only one of a limited number of invariant sequences in its constant region. The genetic basis for this combination of constancy and tremendous variation in a single protein molecule lies in the organization of the immunoglobulin genes. In germ-line DNA, multiple gene segments encode portions of a single immunoglobulin heavy or light chain. These gene segments are carried in the germ cells but cannot be transcribed and translated into complete chains until they are rearranged into functional genes. During B-cell maturation in the bone marrow, certain of these gene segments are randomly shuffled by a dynamic genetic system capable of generating more than 106 combinations. Subsequent processes increase the diversity of the repertoire of antibodybinding sites to a very large number that exceeds 106 by at least two or three orders of magnitude. The processes of Bcell development are carefully regulated: the maturation of a progenitor B cell progresses through an ordered sequence of Ig-gene rearrangements, coupled with modifications to the gene that contribute to the diversity of the final product. By the end of this process, a mature, immunocompetent B cell will contain coding sequences for one functional heavychain variable-region and one light-chain variable-region. The individual B cell is thus antigenically committed to aspecific epitope. After antigenic stimulation of a mature Bcell in peripheral lymphoid organs, further rearrangement of constant-region gene segments can generate changes in the isotype expressed, which produce changes in the biological effector functions of the immunoglobulin molecule without changing its specificity. Thus, mature B cells contain chromosomal DNA that is no longer identical to germ-line DNA. While we think of genomic DNA as a stable genetic blueprint, the lymphocyte cell lineage does not retain an intact copy of this blueprint. Genomic rearrangement is an essential feature of lymphocyte differentiation, and no other vertebrate cell type has been shown to undergo this process. This chapter first describes the detailed organization of the immunoglobulin genes, the process of Ig-gene rearrangement, and the mechanisms by which the dynamic immunoglobulin genetic system generates more than 108 different antigenic specificities. Then it describes the mechanism of class switching, the role of differential RNA processing in the expression of immunoglobulin genes, and the regulation of Ig-gene transcription. The chapter concludes with the application of our knowledge of the molecular biology of immunoglobulin genes to the engineering of antibody molecules for therapeutic and research applications. Chapter 11 covers in detail the entire process of B-cell development from the first gene rearrangements in progenitor B cells to final differentiation into memory B cells and antibody- secreting plasma cells. Figure 5-1 outlines the sequential stages in B-cell development, many of which result from critical rearrangements.

Germ-Line and Somatic-Variation Models Contended To Explain Antibody Diversity

For several decades, immunologists sought to imagine a genetic mechanism that could explain

the tremendous diversity of antibody structure. Two different sets of theories emerged. The germ-line theories maintained that the genome contributed by the germ cells, egg and sperm, contains a large repertoire of immunoglobulin genes; thus, these theories invoked no special genetic mechanisms to account for antibody diversity. They argued that the immense survival valueof the immune system justified the dedication of a significant fraction of the genome to the coding of antibodies. In contrast, the somatic-variation theories maintained that the genome contains a relatively small number of immunoglobulin genes, from which a large number of antibody specificities are generated in the somatic cells by mutation ore combination. As the amino acid sequences of more and more immunoglobulin's were determined, it became clear that there must be mechanisms not only for generating antibody diversity but also for maintaining constancy. Whether diversity was generated by germ-line or by somatic mechanisms, a paradox remained: How could stability be maintained in the constant (C) region while some kind of diversifying mechanism generated the variable (V) region? Neither the germ-line nor the somatic-variation proponents could offer a reasonable explanation for this central feature of immunoglobulin structure. Germ-line proponents found it difficult to account for an evolutionarymechanism that could generate diversity in the variable part of the many heavy- and light-chain genes while preserving the constant region of each unchanged. Somatic-variation proponents found it difficult to conceive of a mechanism that could diversify the variable region of a single heavy- or light-chain gene in the somatic cells without allowing alteration in the amino acid sequence encoded by the constant region. A third structural feature requiring an explanation emerged when amino acid sequencing of the human myeloma protein called Ti1 revealed that identical variable region sequences were associated heavychain constant regions. A similar phenomenon was observed in rabbits by C. Considerable additional evidence has confirmed that a single variable-region sequence, defining a particular antigenic specificity, can be associated with multiple heavy-chain constant-region sequences; in other words, different classes, or isotypes, of antibody (e.g., IgG, IgM) can be expressed with identical variable-region sequences.

Variable-Region Gene Rearrangements

The preceding sections have shown that functional genes that encode immunoglobulin light and heavy chains are assembled by recombinational events at the DNA level. These events and the parallel events involving T-receptor genes are the only known site-specific DNA rearrangements in vertebrates. Variable-region gene rearrangements occur in an ordered sequence during B-cell maturation in the bone marrow. The heavy-chain variable-region genes rearrange first, then the light-chain variable-region genes. At the end of this process, each B cell contains a single functional variable region DNA sequence for its heavy chain and another for itslight chain. The process of variable-region gene rearrangement produces mature, immunocompetent B cells; each such cell is committed to produce antibody with a binding site encoded by the particular sequence of its rearranged V genes. As described later in this chapter, rearrangements of the heavychain constant-region genes will generate further changes in the immunoglobulin class (isotype) expressed by a B cell, but those changes will not affect the cell's antigenic specificity.The steps in variable-region gene rearrangement occur in an ordered sequence, but they are random events that result in the random determination of B-cell specificity. The order,

mechanism, and consequences of these rearrangements are described in this section.

Light-Chain DNA Undergoes-J Rearrangements

Expression of both _ and _ light chains requires rearrangement of the variable-region V and J gene segments. In humans, any of the functional V_ genes can combine with any of the four functional J_-C_ combinations. In the mouse, things are slightly more complicated. DNA rearrangement can join the V_1 gene segment with either the J_1 or the J_3 gene segment, or the V_2 gene segment can be joined with the J_2 gene segment. In human or mouse _ light-chain DNA, any one of the V_ gene segments can be joined withany one of the functional J_ gene segments Rearranged _ and _ genes contain the following regions in order from the 5_ to 3_ end: a short leader (L) exon, a noncoding sequence (intron), a joined VJ gene segment, a second intron, and the constant region. Upstream from each leader gene segment is a promoter sequence. The rearranged lightchain sequence is transcribed by RNA polymerase from the exon throughthe C segment to the stop signal, generating a light-chain primary RNA transcript (Figure 5-4). The introns in the primary transcript are removed by RNA processing enzymes, and the resulting light-chain messenger RNA then exits from the nucleus. The light chain mRNA binds to ribosomes and is translated into the light-chain protein. The leader sequence at the amino terminus pulls the growing polypeptide chain into the lumen of the rough endoplasmic reticulum and is then cleaved, so it is not present in the finished light-chain protein product.

Heavy-Chain DNA Undergoes V-D-J Rearrangement

Generation of a functional immunoglobulin heavy-chai gene requires two separate rearrangement events within thevariable region. As illustrated in Figure 5-5, a DH gene segmen first joins to a JH segment; the resulting DHJH segment then moves next to and joins a VH segment to generate a VHDHJH unit that encodes the entire variable region. Inheavy-chain DNA, variable-region rearrangement produces a rearranged gene consisting of the following sequences,starting from the 5_ end: a short L exon, an intron, a joinedVDJ segment, another intron, and a series of C gene segments. As with the light-chain genes, a promoter sequence islocated a short distance upstream from each heavy-chain leader sequence.Once heavy-chain gene rearrangement is accomplished, RNA polymerase can bind to the promoter sequence andtranscribe the entire heavy-chain gene, including the introns. Initially, both C_ and C_ gene segments are transcribed. Differential polyadenylation and RNA splicing remove the intronsand process the primary transcript to generate mRNA including either the C_ or the C_ transcript. These tw mRNAs are then translated, and the leader peptide of the resultingnascent polypeptide is cleaved, generating finished. The production of two different heavy-chain mRNAs allows a mature, immunocompetent B cell to express both IgM and IgD with identical antigenic specificity on its surface.

Recombination Signal Sequences Direct Recombination

The discovery of two closely related conserved sequences in variable-region germ-line DNA

paved the way to fuller understanding of the mechanism of gene rearrangements.DN sequencing studies revealed the presence of unique recombination signal sequences (RSSs) flanking each germ-line V, D, and J gene segment. One RSS is located 3_ to each V gene segment, 5_ to each J gene segment, and on both sides of eachD gene segment. These sequences function as signals for the recombination process that rearranges the genes. Each RSS contains a conserved palindromic heptamer and a conserved AT-rich nonamer sequence separated by an intervening sequence of 12 or 23 base pairs. The intervening12- and 23-bp sequences correspond, respectively, to one and two turns of the DNA helix; for this reason the sequences arecalled one-turn recombination signal sequences and twoturn signal sequences. The V_ signal sequence has a one-turn spacer, and the J_signal sequence has a two-turn spacer. In _ light-chain DNA, this order is reversed; that is, the V_ signal sequence has atwo-turn spacer, and the J_ signal sequence has a one-turnspacer. In heavy-chain DNA, the signal sequences of the VH and JH gene segments have two-turn spacers, the signals oneither side of the DH gene segment have one-turn spacers. Signal sequences having a one-turn spacer canjoin only with sequences having a two-turn spacer (the socalled one-turn/two-turn joining rule). This joining rule ensures, for example, that a VLsegment joins only to a JL segment and not to another VL segment; the rule likewise ensuresthat VH, DH, and JH segments join in proper order and that segments of the same type do not join each other.

Gene Segments Are Joined by Recombinases

V-(D)-J recombination, which takes place at the junctionsbetween RSSs and coding sequences, is catalyzed by enzymes collectively called **V(D)J recombinase.** Identification of the enzymes that catalyze recombinationof V,D, and J gene segments began in the late 1980s and is stillongoing. In 1990 David Schatz, Marjorie Oettinger, and David Baltimore first reported the identification of two recombination-activating genes, designated *RAG-1* and*RAG-2,* whose encoded proteins act synergistically and are required to mediate V-(D)-J joining.The RAG-1 and RAG-2 proteins and the enzyme terminal deoxynucleotidyl transferase (TdT) are the only lymphoid-specific gene products that have been shown to be involved in V-(D)-J rearrangement.The recombination of variable-region gene segments consists of the following steps, catalyzed by a system of recombinase enzymes.Recognition of recombination signal sequences (RSSs) by recombinase enzymes, followed by synapsis in which two signal sequences and the adjacent coding sequences (gene segments) are brought into proximity _ Cleavage of one strand of DNA by RAG-1 and RAG-2 at the junctures of the signal sequences and coding sequences _ A reaction catalyzed by RAG-1 and RAG-2 in which the free 3_-OH group on the cut DNA strand attacks the phosphodiester bond linking the opposite strand to the signal sequence, simultaneously producing a hairpin structure at the cut end of the coding sequence and a flush, 5_-phosphorylated, double-strand break at thesignal sequence _ Cutting of the hairpin to generate sites for the addition of P-region nucleotides, followed by the trimming of afew nucleotides from the coding sequence by a single strand endonuclease_ Addition of up to 15 nucleotides, called N-region nucleotides, at the cut ends of the V, D, and J coding sequences of the heavy chain by the enzyme terminal deoxynucleotidyl transferase_ Repair and ligation to join the coding sequences and to join the signal sequences, catalyzed by normal doublestrand break

repair (DSBR) enzymes Recombination results in the formation of a coding joint, falling between the coding sequences, and a signal joint, between the RSSs. The transcriptional orientation of the gene segments to be joined determines the fate of the signal joint and intervening DNA. When the two gene segments are inthe same transcriptional orientation, joining results in deletion of the signal joint and intervening DNA as a circular excision product (Figure 5-8). Less frequently, the two gene segments have opposite orientations. In this case joining occurs by inversion of the DNA, resulting in the retention of both the coding joint and the signal joint (and intervening DNA) on the chromosome. In the human _ locus, about half of the V_ gene segments are inverted with respect to J_ and their joining is thus by inversion.

Generation of Antibody Diversity

As the organization of the immunoglobulin genes was deciphered,the sources of the vast diversity in the variable region began to emerge. The germ-line theory, mentioned earlier, argued that the entire variable-region repertoire is encoded in the germ line of the organism and is transmitted from parent to offspring through the germ cells (egg and sperm). The somatic-ariation theory held that the germ line contains alimited number of variable genes,which are diversified in the somatic cells by mutational or recombinational events during development of the immune system. With the cloning and sequencing of the immunoglobulin genes, both models were partly vindicated. To date, seven means of antibody diversification have been identified in mice and humans: _ Multiple germ-line gene segments _ Combinatorial V-(D)-J joining _ Junctional flexibility_ P-region nucleotide addition (P-addition) _ N-region nucleotide addition (N-addition) _ Somatic hypermutation _ Combinatorial association of light and heavy chains Although the exact contribution of each of these avenues of diversification to total antibody diversity is not known, they each contribute significantly to the immense number of distinct antibodies that the mammalian immune system is capable of generating.

There Are Numerous Germ-Line V, D, and J Gene Segments

An inventory of functional V, D, and J gene segments in the germ-line DNA of one human reveals 51 VH, 25 D, 6 JH,40 V_, 5 J_, 31 V_, and 4 J_ gene segments. In addition to these functional segments, there are many pseudogenes. It should be borne in mind that these numbers were largely derived from a landmark study that sequenced the DNA of the immunoglobulin loci of a single individual. The immunoglobulinloci of other individuals might contain slightly different numbers of particular types of gene segments. In the mouse, although the numbers are known with less precision than in the human, there appear to be about 85 V_gene segments and 134 VH gene segments, 4 functional JH, 4 functional J_, 3 functional J_, and an estimated 13 DH gene segments, but only three V_ gene segments. Although the number of germ-line genes found in either humans or mice is far fewer than predicted by early proponents of the germline model, multiple germ-line V, D, and J gene segments clearly do contribute to the diversity of the antigen-binding sites in antibodies.

Combinatorial V-J and V-D-J Joining Generates Diversity

The contribution of multiple germ-line gene segments to antibody diversity is magnified by the random rearrangement of these segments in somatic cells. It is possible to calculate how much diversity can be achieved by gene rearrangements). In humans, the ability of any of the 51 VH gene segments to combine with any of the 27 DH segments and any of the 6 JH segments allows a considerable amount of heavy-chain gene diversity to be generated (51 _ 27 _6 _ 8262 possible combinations). Similarly, 40 V_ gene segments randomly combining with 5 J_ segments has the potential of generating 200 possible combinations at the _ locus,while 30 V_ and 4 J_ gene segments allow up to 120 possible combinations at the human _ locus. It is important to realize that these are minimal calculations of potential diversity. Junctional flexibility and P- and N-nucleotide addition, as mentioned above, and, especially, somatic hypermutation, which will be described shortly, together make an enormous contribution to antibody diversity. Although it is not possible to make an exact calculation of their contribution, most workers in this field agree that they raise the potential for antibody combining-site diversity in humans to well over 1010. This does not mean that, at any given time, a single individual hasa repertoire of 1010 different antibody combining sites. These very large numbers describe the set of possible variations, of which any individual carries a subset that is smaller by several orders of magnitude.

Junctional Flexibility Adds Diversity

The enormous diversity generated by means of V, D, and J combinations is further augmented by a phenomenon called junctional flexibility. As described above, recombination involves both the joining of recombination signal sequences to form a signal joint and the joining of coding sequences to form a coding joint. Although the signal sequences are always joined precisely, joining of the coding sequences is often imprecise. In one study, for example, joining of the V_21 and J_1 coding sequences was analyzed in several pre-B cell lines. Sequence analysis of the signal and coding joints revealed the contrast in junctional precision. As illustrated previously, junctional flexibility leads to many nonproductive rearrangements, but it also generates productive combinations that encode alternative amino acids at each coding joint, thereby increasing antibody diversity. The amino acid sequence variation gener-ated by junctional flexibility in the coding joints has been shown to fall within the third hypervariable region (CDR3) in immunoglobulin heavy-chain and light-chain DNA. Since CDR3 often makes a major contribution to antigen binding by the antibody molecule, amino acid changes generated by junctional flexibility are important in the generation of antibody diversity.

P-Addition Adds Diversity at Palindromic Sequences

As described earlier, after the initial single-strand DNA cleavage at the junction of a variable-region gene segment and attached signal sequence, the nucleotides at the end of the coding sequence turn back to form a hairpin structure. This hairpin is later cleaved by an endonuclease. This second cleavage sometimes occurs at a position that leaves a short single strand at the end

of the coding sequence. The subsequent addition of complementary nucleotides to this strand (**P-addition**) by repair enzymes generates a palindromic sequence in the coding joint, and so these nucleotides are called **P-nucleotides**. Variation in the position at which the hairpin is cut thus leads to variation in the sequence of the coding joint.

N-Addition Adds Considerable Diversity by Addition of Nucleotides

Variable-region coding joints in rearranged heavy-chain genes have been shown to contain short amino acid sequences that are not encoded by the germ-line V,D, or J gene segments. These amino acids are encoded by nucleotides added during the D-J and V to D-J joining process by a terminal deoxynucleotidyl transferase (TdT) catalyzed reaction Evidence that TdT is responsible for the addition of these **N-nucleotides** has come from transfection studies in fibroblasts.When fibroblasts were transfected withthe *RAG-1* and *RAG-2* genes,V-D-J rearrangement occurred but no N-nucleotides were present in the coding joints.However,when the fibroblasts were also transfected with the gene encoding TdT, then V-D-J rearrangement was accompanied by addition of N-nucleotides at the coding joints. Up to 15 N-nucleotides can be added to both the DH-JH and VH-DHJH joints. Thus, a complete heavy-chain variable region is encoded by a VHNDHNJH unit. The additional heavychain diversity generated by N-region nucleotide addition is quite large because N regions appear to consist of wholly random sequences. Since this diversity occurs at V-D-J coding joints, it is localized in CDR3 of the heavy-chain genes.

Somatic Hypermutation Adds Diversity in Already-Rearranged Gene Segments

All the antibody diversity described so far stems from mechanisms that operate during formation of specific variable regions by gene rearrangement. Additional antibody diversity is generated in rearranged variable-region gene units by a process called **somatic hypermutation.** As a result of somatic hypermutation, individual nucleotides in VJ or VDJ units are replaced with alternatives, thus potentially altering the specificity of the encoded immunoglobulins. Normally, somatic hypermutation occurs only within germinal centers (see Chapter 11), structures that form in secondary lymphoid organs within a week or so of immunization with an antigen that activates a T-cell-dependent B-cell response. Somatic hypermutation is targeted to rearranged Vregions located within a DNA sequence containing about 1500 nucleotides, which includes the whole of the VJ or VDJ segment. Somatic hypermutation occurs at a frequency approaching 10_3 per base pair per generation. This rate is at least a hundred thousand-fold higher (hence the name *hypermutation*) than the spontaneous mutation rate, about 10_8/bp/generation, in other genes. Since the combined length of the H-chain and L-chain variable-region genes is about 600 bp, one expects that somatic hypermutation willintroduce at least one mutation per every two cell divisions in the pair of VH and VL genes that encode an antibody. The mechanism of somatic hypermutation has not yet been determined.Most of the mutations are nucleotide substitutions rather than deletions or insertions. Somatic hypermutation introduces these substitutions in a largely, but not completely, random fashion. Recent evidence suggests that certain nucleotide motifs and palindromic sequences within VH and VL may be especially susceptible to somatic hypermutation. Somatic hypermutations occur throughout the VJ or VDJsegment, but in mature

B cells they are clustered within theCDRs of the VH and VL sequences,where they are most likelyto influence the overall affinity for antigen. Following exposure to antigen, those B cells with higher-affinity receptors will be preferentially selected for survival. Since CDR3 often makes a major contribution to antigen binding by the antibody molecule, amino acid changes generated by junctional flexibility are important in the generation of antibody diversity.

P-Addition Adds Diversity at Palindromic Sequences

As described earlier, after the initial single-strand DNA cleavage at the junction of a variable-region gene segment and attached signal sequence, the nucleotides at the end of the coding sequence turn back to form a hairpin structure. This hairpin is later cleaved by an endonuclease. This second cleavage sometimes occurs at a position that leaves a short single strand at the end of the coding sequence. The subsequent addition of complementary nucleotides to this strand (P-addition) by repair enzymes generates a palindromic sequence in the coding joint, and so these nucleotides are called P-nucleotides (Figure 5-13a). Variation in the position at which the hairpin is cut thus leads to variation in the sequence of the coding joint.

N-Addition Adds Considerable Diversity by Addition of Nucleotides

Variable-region coding joints in rearranged heavy-chain genes have been shown to contain short amino acid sequences that are not encoded by the germ-line V,D, or J gene segments. Theseamino acids are encoded by nucleotides added during the D-J and V to D-J joining process by a terminal deoxynucleotidyl transferase (TdT) catalyzed reaction differential selection is an increase in the antigen affinity of a population of B cells. The overall process, called affinity maturation, takes place within germinal centers, and is described more fully in Chapter 11. Claudia Berek and Cesar Milstein obtained experimental evidence demonstrating somatic hypermutation during the course of an immune response to a hapten-carrier conjugate. These researchers were able to sequence mRNA that encoded antibodies raised against a hapten in response to primary, secondary, or tertiary immunization (first, second, or third exposure) with a hapten-carrier conjugate. The hapten they chose was 2-phenyl-5-oxazolone (phOx), coupled to a protein carrier. They chose this hapten because it had previously been shown that the majority of antibodies it induced were encoded by a single germ-line VH and V_ gene segment. Berek and Milstein immunized mice with the phOx-carrier conjugate and then used the mouse spleen cells to prepare hybridomas secreting monoclonal antibodies specific for the phOx hapten. The mRNA sequence for the H chain and _ light chain of each hybridoma was then determined to identify deviations from the germ-line sequences.The results of this experiment are depicted. Of the 12 hybridomas obtained from mice seven days after a primary immunization, all used a particular VH, the VH Ox-1 gene segment, and all but one used the same VL gene segment, V_ Ox-1. Moreover, only a few mutations from the germ-line sequence were present in these hybridomas. By day 14 after primary immunization, analysis of eight hybridomas revealed that six continued to use the germ-line VH Ox-1 gene segment and all continued to use the V_ Ox-1 gene segment. Now, however, all of these hybridomas included one or more mutations from the

germ-linesequence. Hybridomas analyzed from the secondary and tertiary responses showed a larger percentage utilizing germ-line VH gene segments other than the VH Ox-1 gene. In those hybridoma clones that utilized the VH Ox-1 and V_Ox-1 gene segments, most of the mutations were clustered in the CDR1 and CDR2 hypervariable regions. The numberof mutations in the anti-phOx hybridomas progressively increased following primary, secondary, and tertiary immunizations, as did the overall affinity of the antibodies for phOx.

Synthesis, Assembly, and Secretion of Immunoglobulins

Immunoglobulin heavy- and light-chain mRNAs are translated on separate polyribosomes of the rough endoplasmic reticulum (RER). Newly synthesized chains contain an amino-terminal leader sequence, which serves to guide the chains into the lumen of the RER, where the signal sequence is then cleaved. The assembly of light (L) and heavy (H) chains into the disulfide-linked and glycosylated immunoglobulin molecule occurs as the chains passthrough the cisternae of the RER. The complete molecules are transported to the Golgi apparatus and then into secretory vesicles, which fuse with the plasma membrane. The order of chain assembly varies among the immunoglobulin classes. In the case of IgM, the H and L chains assemble within the RER to form half-molecules, and then two half-molecules assemble to form the complete molecule. In the case of IgG, two H chains assemble, then an H2L intermediate is assembled, and finally the complete H2L2 moleculeis formed. Interchain disulfide bonds are formed, and the polypeptides are glycosylated as they move through theGolgi apparatus. If the molecule contains the transmembrane sequence ofthe membrane form, it becomes anchored in the membraneof asecretory vesicle and is inserted into the plasma membrane as the vesicle fuses with the plasma membrane. If the molecule contains the hydrophilic sequence of secreted immunoglobulins, it is transported as a free molecule in a secretory vesicle and is released from thev cell when the vesicle fuses with the plasma membrane.

Regulation of Ig-Gene Transcription

The immunoglobulin genes are expressed only in B-lineage cells, and even within this lineage, the genes are expressed at different rates during different developmental stages. As withother eukaryotic genes, three major classes of cis regulatory sequences in DNA regulate transcription of immunoglobulin genes:_ Promoters: relatively short nucleotide sequences, extending about 200 bp upstream from the transcription initiation site, that promote initiation of RNA transcription in a specific direction_ Enhancers: nucleotide sequences situated some distance upstream or downstream from a gene that activat etranscription from the promoter sequence in an orientation-independent manner _ Silencers: nucleotide sequences that down-regulate transcription, operating in both directions over a distance. The locations of the three types of regulatory elements ingerm-line immunoglobulin DNA are shown in Figure 5-19.All of these regulatory elements have clusters of sequencemotifs that can bind specifically to one or more nuclear proteins. Each VH and VL gene segment has a promoter located just upstream from the leader sequence. In addition, the J_cluster and each of the DH genes of the heavy-chain locus are

preceded by promoters. Like other promoters, the immunoglobulin promoters contain a highly conserved ATrich sequence called the TATA box, which serves as a site for the binding of a number of proteins that are necessary for the initiation of RNA transcription. The actual process of transcription is performed by RNA polymerase II, which starts transcribing DNA from the initiation site, located about 25 bp downstream of the TATA box. Ig promoters also contain an essential and conserved octamer that confers B-cell specificity on the promoter. The octamer binds two transcription factors, oct-1, found in many cell types, and oct-2, found only in B cells.

Antibody Genes and Antibody Engineering

There are many clinical applications in which the exquisite specificity of a mouse monoclonal antibody would be useful. However, when mouse monoclonal antibodies are introducedinto humans they are recognized as foreign and evoke an antibody response that quickly clears the mouse monoclonal antibody from the bloodstream. In addition, circulating complexes of mouse and human antibodies can cause allergic reactions. In some cases, the buildup of these complexes in organs such as the kidney can cause serious andeven life-threatening reactions. Clearly, one way to avoid these undesirable reactions is to use human monoclonal antibodies for clinical applications .However, the preparation of human monoclonal antibodies has been hampered by numerous technical problems. In response to the difficulty of producing human monoclonal antibodies and the complications resulting from the use of mouse monoclonal antibodies in humans, there is now a major effort to engineer monoclonal antibodies and antibody binding sites with recombinant DNA technology. The growing knowledge of antibody gene structure and regulation has made possible what Cesar Milstein, one of the inventors of monoclonal antibody technology, has called “man-made antibodies.” It is now possible to design and construct genes that encode immunoglobulin molecules in which the variable regions come from one species and the constant regions come from another. New genes have been created that link nucleotide sequences coding non antibody proteins with sequences that encode antibody variable regions specific for particular antigens. These molecular hybrids or **chimeras** may be able to deliver powerful toxins to particular antigenic targets, such as tumor cells. Finally, by replacement of the immunoglobulin loci of one species with that of another, animals of one species have been endowed with the capacity to respond to immunization by producing antibodies encoded by the donor’s genetically transplanted Iggenes. By capturing a significant sample of all of the immunoglobulin heavy- and light-chain variable-region genes via incorporation into libraries of bacteriophage, it has been possible to achieve significant an useful reconstructions of the entire antibody repertoires of individuals. The next few sections describe each of these types of antibody genetic engineering.

Chimeric and Hybrid Monoclonal Antibodies Have Potent Clinical Potential

One approach to engineering an antibody is to clone recombinant DNA containing the promoter, leader, and variable region sequences from a mouse antibody gene and the constant-region exons from a human antibody gene. The antibody encoded by such a recombinant gene is a mouse-

human chimera, commonly known as a humanized antibody. Its antigenic specificity ,which is determined by the variable region, is derived from the mouse DNA; its isotype, which is determined by the constant region, is derived from the human DNA. Because the constant regions of these chimeric antibodies are encoded by human genes, the anti- bodies have fewer mouse antigenic determinants and are far less immunogenic when administered to humans tha mouse monoclonal antibodies. The ability ofthe mouse variable regions remaining in these humanized antibodies to provide the appropriate binding site to allow specific recognition of the target antigen has encouraged further rexploration of this approach. It is possible to produce chimeric human-mouse antibodies in which only the sequences of the CDRs are of mouse origin. Another advantage of humanized chimeric antibodies is that they retain the biological effector functions of human antibody and are more likely to trigger human complement activation or Fc receptor binding. One such chimeric human mouse antibody has been used to treat patients with B-cell varieties of non-Hodgkin's lymphoma. Chimeric monoclonal antibodies that function as immunotoxins can also be prepared. In thiscase, the terminal constant-region domain in a tumor specific monoclonal antibody is replaced with toxin chains. Because these immunotoxins lack the terminal Fc domain, they are not able to bind to cells bearingFc receptors. These immunotoxins can bind only to tumo cells, making them highly specific as therapeutic reagents. Hetero conjugates, or bispecific antibodies, are hybrids of two different antibody molecules. They can be constructed by chemically cross linking two different antibodies or by synthesizing them in hybridomas consisting of two different monoclonal-antibody-producing cell lines that have been fused. Both of these methods generate mixtures of monospecific and bispecific antibodies from which the desired bi specific molecule must be purified. Using genetic engineering to construct genes that will encode molecules only with the two desired specificitiesis a much simpler and more elegant approach. Several bi specific molecules have been designed in which one half of the antibody has specificity for a tumor and the other half has specificity for a surface molecule on an immune effector cell, such as an NK cell, an activated macrophage, or a cytotoxic T lymphocyte(CTL). Such hetero conjugates have been designed to activate the immune effector cell when it is cross linked to the tumor cell so that it begins to mediate destruction of the tumor cell.

Monoclonal Antibodies Can Be Constructed from Ig-Gene Libraries

A quite different approach for generating monoclonal antibodies employs the polymerase chain reaction (PCR) to amplify the DNA that encodes antibody heavy-chain and light-chain Fab fragments from hybridoma cells or plasma cells. A promoter region and *Eco*RI restriction site (see Chapter 23) are added to the amplified sequences, and the resulting constructs are inserted into bacteriophage, yielding separate heavy- and light-chain libraries. Cleavage with *Eco*RI and random joining of the heavy- and light-chain genes yield numerous novel heavy-light construct. This procedure generates an enormous diversity of antibody specificities—libraries with _1010 unique members have been obtained—and clones containing these random combinations of H-L chains can be rapidly screened for those secreting antibody to a particular antigen. The level of diversity is comparable to the human in vivo repertoire, and it is possible to demonstrate that

specificities against a wide variety of antigens can be obtained from these libraries. Such a combinatorial library approach opens the possibility of obtaining specific antibodies without any need whatsoever for immunization. However, the real challenge to bypassing in vivo immunization in the derivation of useful antibodies of high affinity lies in finding ways to mimic the biology of the humoral libraries will allow the routine and widespread production of useful antibodies from any desired species without the limitations of immunization and hybridoma technology that currently complicate the production of monoclonal antibodies.

MONOCLONAL ANTIBODIES

As noted in Chapter 3, most antigens offer multiple epitopes and therefore induce proliferation and differentiation of a variety of B-cell clones, each derived from a B cell that recognizes a particular epitope. The resulting serum antibodies areheterogeneous, comprising a mixture of antibodies, each specific for one epitope . Such a **polyclonal antibody** response facilitates the localization, phagocytosis, and complement-mediated lysis of antigen; it thus has clear advantagesfor the organism in vivo. Unfortunately, the antibody heterogeneity that increases immune protection in vivooften reduces the efficacy of an antiserum for various in vitro uses. For most research, diagnostic, and therapeutic purposes,**monoclonal antibodies,** derived from a single clone and thus specific for a single epitope, are preferable. Direct biochemical purification of a monoclonal antibody from a polyclonal antibody preparation is not feasible. In 1975, Georges Köhler and Cesar Milstein devised a method for preparing monoclonal antibody, which quickly became one of immunology's key technologies. By fusing a normal activated, antibody-producing B cell with a myeloma cell (a cancerous plasma cell), they were able to generate a hybrid cell, called a **hybridoma,** that possessed the immortalgrowth properties of the myeloma cell and secreted the antibody produced by the B cell The resulting clones of hybridoma cells, which secrete large quantities of monoclonal antibody, can be cultured indefinitely.

Clinical Uses of Monoclonal Antibodies

Monoclonal antibodies are proving to be very useful as diagnostic, imaging, and therapeutic reagents in clinical medicine. Initially, monoclonal antibodies were used primarily as in vitro diagnostic reagents. Among the many monoclonal antibody diagnostic reagents now available are products for detecting pregnancy, diagnosing numerous pathogenic microorganisms, measuring the blood levels of various drugs, matching histocompatibility antigens, and detecting antigens shed by certain tumors. Radiolabeled monoclonal antibodies can also be used in vivo for detecting or locating tumor antigens, permitting earlier diagnosis of some primary or metastatic tumors in patients. For example, monoclonal antibody to breast-cancer cells is labeled with iodine-131 and introduced into the blood to detect the spread of a tumor to regional lymph nodes. This monoclonal imaging technique can reveal breast-cancer metastases that would be undetected by other, less sensitive scanning techniques.

Immunotoxins composed of tumor-specific monoclonal antibodies coupled to lethal toxins are potentially valuable therapeutic reagents. The toxins used in preparing immunotoxins include ricin, *Shigella* toxin, and diphtheria toxin, all of which inhibit protein synthesis. These toxins are so potent that a single molecule has been shown to kill a cell. Each of these toxins

consists of two types of functionally distinct polypeptide components, an inhibitory (toxin) chain and one or more binding chains,which interact with receptors on cell surfaces; without the binding polypeptide(s) the toxin cannot get into cells and therefore is harmless. An immunotoxin is prepared by replacing the binding polypeptide(s) with a monoclonal antibody that is specific for a particular tumor cell. In theory, the attached monoclonal antibody will deliver the toxin chain specifically to tumor cells, where it will cause death by inhibiting protein synthesis . The initial clinical responses to such immunotoxins in patients with leukemia, lymphoma,and some other types of cancer have shown promise, and research to develop and demonstrate their safety and effectiveness is underwa

Abzymes Are Monoclonal Antibodies That Catalyze Reactions

The binding of an antibody to its antigen is similar in many ways to the binding of an enzyme to its substrate. In both cases the binding involves weak, noncovalent interactions and exhibits high specificity and often high affinity. What distinguishes an antibody-antigen interaction from an enzyme substrate interaction is that the antibody does not alter the antigen, whereas the enzyme catalyzes a chemical changein its substrate. However, like enzymes, antibodies of appropriate specificity can stabilize the transition state of a bound substrate, thus reducing the activation energy for chemical modification of the substrate. The similarities between antigen-antibody interactions and enzyme-substrate interactions raised the question of whether some antibodies could behave like enzymes and catalyze chemical reactions. To investigate this possibility, a hapten-carrier complex was synthesized in which the hapten structurally resembled the transition state of an ester undergoing hydrolysis. Spleen cells from mice immunized with this transition state analogue were fused with myeloma cells togenerate monoclonal antihapten monoclonal antibodies. When these monoclonal antibodies were incubated with an ester substrate, some of them accelerated hydrolysis by about 1000-fold; that is, they acted like the enzyme that normally catalyzes the substrate's hydrolysis. The catalytic activity of these antibodies was highly specific; that is, they hydrolyzed only esters whose transition-state structure closely resembled the transition state analogue used as a hapten in the immunizing conjugate. These catalytic antibodies have been called **abzymes** in reference to their dual role as antibody and enzyme.

A central goal of catalytic antibody research is the derivation of a battery of abzymes that cut peptide bonds at specific amino acid residues, much as restriction enzymes cut DNA at specific sites. Such abzymes would be invaluable tools in the structural and functional analysis of proteins.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: II M.Sc MB COURSE NAME: ADVANCED IMMUNOLOGY

COURSE CODE: 18MBP301 UNIT: II BATCH-2018-2020

Sl. No	Question	Option A	Option B	Option C	Option D	Correct Ans
1	The mononuclear cells consists of circulating _____ in the blood and _____ in the tissues	Macrophage and mast tissue	Null cells and NK cells	monocytes and macrophages	T and B cells	Macrophage and mast tissue
2	Basophils release the contents of their granule plays a vital role in ____	Certain allergic responses	Against parasitic organisms	Tumour cells	blood pathogens	Certain allergic responses
3	IL-10 acts to	Activate macrophage	Supress Ab production	Enhance IgE production	suppress cytokine production	suppress cytokine production
4	Transforming growth factor b ____	Activates fibroblast	Enhances T cells function	Activates macrophages	Enhance B cell function	Activates fibroblast
5	Lymphotoxin is a	Transforming growth factor b	Tumour necrosis factorb	Tumour necrosis factor Z	Transforming growth factor a	Tumour necrosis factorb
6	Il- 10 is produced by	Th_1 and Th_2 cells	B cells	T_c cells	T_H cells	Th_1 and Th_2 cells
7	B cell growth factors produced by T cell is known as	IL-4	IL- 14	IL-3	IL-10	IL- 14
8	In an antibody, an antigen can bind at ___ region	V_H and V_L domain	V_H domain	V_L domain	C domine	V_H and V_L domain
9	Complex formation between neuraminidase and antineuraminidase is due to	V_H and V_L domain	V_H domain	V_L domain	C domine	V_H and V_L domain
10	The concept that the rejection of foreign tissue is the result of an immune response to the cell surface called ____	Change in orientation of epitopes	change in orientation of epitope and Ag binding site	Change in orientation of antigen binding site	Change in conformation of antigen	change in orientation of epitope and Ag binding site
11	Major histocompaticibility complex was discovered in the year ___	Histocompactibility	Histocomapatibility Ag	pagocytosis	All the above	Histocomapatibility Ag
12	Major histocompatibility complex was discovered by	1948	1980	1984	1955	1980
13	The presence of a serum component responsible for allergic reaction was first demonstrated in 1921 by	K. Prausnitz	Donnall Thomas	Rosalyn	Jules Bordet	K. Prausnitz

14	The basic structure of the immunoglobulin was elucidated by___	Charly Richet	Rodney and Gerald Edelman	Joseph Murray and Donnal Thomas	George Kohler	Rodney and Gerald Edelman
15	The first immunoglobulin class produced in a primary response to an antigen is ___	IgG	IgA	IgM	IgD	IgM
16	Which of the following immunoglobulin forms bas a dimeric structure ___	IgD	IgE	IgG	IgA	IgA
17	Five monomer unit in an IgM molecule are held together by ___	vander waals force	Ionic bond	Covalent bond	Disulphide bond	Disulphide bond
18	The diversity of the molecules results in ___	Polymorphism	Totipotency	Pluripotency	Unipotency	Polymorphism
19	Process stimulating the B cells to produce antibodies by humoral immune response is called ___	B cell activation	Proliferation	Maturation	Regenaration	B cell activation
20	The activation antigen entering through the tissues reaches the _____and that enter through the blood reaches spleen	B cell activation	Proliferation	Maturation	Regenaration	B cell activation
21	B cell on its surface contains antigen-binding sites called ____	Epitope	paratope	Receptors	Haptens	Receptors
22	Transfer of processed antigen from the macrophages to B lymphocyte is called__	antigen presentation	Proliferation	Differentiation	Activation	Antigen presentation
23	In addition the macrophage secretes a soluble factor (Monokine) called ___	Histamin	Fibrin	Interleukin	Cytokinin	Interleukin
24	Lymphotoxin produced by activation of _____	CD_{18+} T cells	CD_4^+ T cells	CD_{24}^+ T cells	CD_{26}^+ T cells	CD_4^+ T cells
25	Single chain glycoprotein secreted by activated T_{h2} cells are	IL -9	IL-10	TNFa	TNF b	IL -9
26	The lethal characteristics syndrome developed by the infection of gram negative bacteria is _	Immunodeletion	Weight loss	Inflammation	Septic shock	Septic shock
27	Interaction - a enhances	ADCC	Macrophage	N-K cells activity	TCR	ADCC

			activation			
28	Which of is not a lymphokine	Interferon	Histamine	Lymphotoxin	TNF	Histamine
29	A typical ¡ shaped four chain immunoglobulin is ____	IgE	IgG	IgD	IgM	IgM
30	Immunoglobulin IgM has a ___structure	monomeric	Dimeric	Hexameric	Pentameric	4
31	Which immunoglobulin induces mast cell degranulation	IgG	IgG_3	IgE	IgD	IgE
32	IgM and IgD molecules are present on the membrane of mature _____	Null cells	B-cells	T-cells	Macrophages	B-cells
33	How many antigen-binding sites are present on an antibody?	3	6	2	4	2
34	Lymphokines may aggregate to form____	Monomer	dimmer	Trimer	Oligomer	Oligomer
35	Disulphide linked homodimeric glycoprotein is ____	IL –3	IL-4	IL-5	IL-6	IL-5
36	Il-5 is mainly produced by	T_{H1} cells	T_{H2} cells	T_c cells	Peripheral blood mononuclear cell	Peripheral blood mononuclear cell
37	TNF have the ability to kill	Nk Cells	T_H cells	Tumor cells	B cell	Tumor cells
38	a & b chain of IL- 2 receptor is required for	High affinity	Low affinity	Signal transduction	binding	Signal transduction
39	Antigen binding components of TCR s are _____	Glycogen beterodimers	Glycoprotein heterodimers	Glucose heterodimers	Carboxybydratase	Glycoprotein heterodimers
40	The receptor that help the T cells bind to APC include	CD_4	CD_8	CD4 and CD8	CD_3	CD4 and CD8
41	The ¡ chain found in TCRs has a molecular weight of_____	55 kDa	40-55 KDa	49KDa	40 KDa	40-55 KDa
42	An elongated peptide that can extend from the T cell surface to bind to a site on the MHC class II	CD_3	CD_2	CD_4	CD_8	CD_4

	molecules is					
43	After the activation of a T_H cell, it secretes growth factors known as	Cytokines	Gibberllins	Lymphokines	Auxin	Cytokines
44	The two N - terminal domains of the CD_8 a and b chains bind to	MHC class I Molecules	MHC class II molecules	MHC class III molecules	HLA	MHC class I Molecules
45	Immunoglobulins and glycoproteins are molecules that are produced by _	Antibodies	Immunoglobulins	Plasma cells	Tcells	Plasma cells
46	Multiple myeloma also occurs in _____	Animals	Plants	Humans	Insects	Animals
47	An antibody is a___ molecule	Glycogen	Glycoprotien	Polysaccharide	Lipid	Glycoprotien
48	Two heavy chains are linked together by	Covalent bond	Ionic bond	Disulphide bond	vander walls force	Disulphide bond
49	An antigen – binding site is also called as	Paratope	Epitope	Haptens	Adjuvants	Paratope
50	Activated T_M cell secretes _____ called as B cell stimulatory factor (BSF) which activates the B cell	lymphokine	Monokine	cytokine	Histamin	lymphokine
51	B cell is triggered by the bound antigen or _____ secreted by the T_H cell	Lymphokine	Monokine	cytokine	Histamine	Lymphokine
52	Proliferating B cells finally produce 2 types of cells namely ____and ____	null cell & naive cell	NK cell & T cell	Memory & plasma cell	Basophil	Memory & plasma cell
53	T_H cell attaches itself to the processed antigen present on the surface of _____	Macrophage	monocyte	Mast cell	Basophil	Macrophage
54	The mononuclear cells consists of circulating _____ in the blood and _____ in the tissues	Macrophage and mast tissue	Null cells and NK cells	monocytes and macrophages	T and B cells	Macrophage and mast tissue
55	Basophils release the contents of their granule plays a vital role in ____	Certain allergic responses	Against parasitic organisms	Tumour cells	blood pathogens	Certain allergic responses
56	IL-10 acts to	Activate macrophage	Supress Ab production	Enhance IgE production	suppress cytokine production	suppress cytokine production
57	Transforming growth factor b ____	Activates fibroblast	Enhances T cells	Activates	Enhance B cell	Activates fibroblast

			function	macrophages	function	
58	Lymphotoxin is a	Transforming growth factor b	Tumour necrosis factorb	Tumour necrosis factor Z	Transforming growth factor a	Tumour necrosis factorb
59	Il- 10 is produced by	Th_1 and Th_2 cells	B cells	T_c cells	T_H cells	Th_1 and Th_2 cells
60	B cell growth factors produced by T cell is known as	IL-4	IL- 14	IL-3	IL-10	IL- 14

UNIT III
Antigen Processing and Presentation

Recognition of foreign proteins antigen as T cell that peptides derived from the antigen be displayed within the cleft of an MHC molecule on the membrane of a cell. The formation of these peptide-MHC complexes requires that a protein antigen be degraded into peptides by a sequence of events called antigen processing. The degraded peptides then associate with MHC molecules within the cell interior, and the peptide- MHC complexes are transported to the membrane, where they are displayed (antigen presentation). Class I and class II MHC molecules associate with peptides that have been processed in different intracellular compartments. Class I MHC molecules bind peptides derived from endogenous antigens that have been processed within the cytoplasm of the cell (e.g., normal cellular proteins, tumor proteins, or viral and bacterial proteins produced within infected cells). Class II MHC molecules bind peptides derived from exogenous antigens that are internalized by phagocytosis or endocytosis and processed within the endocytic pathway. This chapter examines in more detail the mechanism of antigen processing and the means by which processed antigen and MHC molecules are combined. In addition, a third pathway for the presentation of nonapeptide antigens derived from bacterial pathogens is described.

Self-MHC Restriction of T Cells
Both CD4_and CD8_T cells can recognize antigen only when it is presented by a self-MHC molecule, an attribute called *self- MHC restriction.* Beginning in the mid-1970s, experiments conducted by a number of researchers demonstrated self- MHC restriction in T-cell recognition.A. Rosenthal and E. Shevach, for example, showed that antigen-specific proliferation of TH cells occurred only in response to antigen presented by macrophages of the same MHC haplotype as the T cells. In their experimental system, guinea pig macrophages from strain 2 were initially incubated with an antigen. After the "antigen-pulsed" macrophages had processed the antigen and presented it on their surface, they were mixed with T cells from the same strain (strain 2), a different strain (strain 13), or (2 13) F1 animals, and the magnitude of T-cell proliferation in response to the antigen-pulsed macrophages was measured. That strain-2 antigen-pulsed macrophages activated strain-2 and F1 T cells but not strain-13 T cells. Similarly, strain-13 antigen-pulsed macrophages activated strain-13 and F1 T cells but not strain-2 T cells. Subsequently, congenic and recombinant congenic strains of mice, which differed from each other only in selected regions of the H-2 complex, were used as the source of macrophages and T cells. These experiments confirmed that the CD4_ TH cell is activated and proliferates only in the presence of antigen-pulsed macrophages that share class II MHC alleles. Thus, antigen recognition by the CD4- TH cell is *class II MHC restricted.* In 1974 R. Zinkernagel and P. Doherty demonstrated the self-MHC restriction of CD8-T cells. In their experiments, mice were immunized with lymphocytic choriomeningitis (LCM) virus; several days later, the animals' spleen cells, which included TC cells specific for the virus, were isolated and incubated with LCM-infected target cells of the same or different haplotype. They found that the TC cells killed

only syngeneic virus-infected target cells. Later studies with congenic and recombinant congenic strains showed that the TC cell and the virus-infected target cell must share class I moleculesencoded by the K or D regions of the MHC. Thus, antigen recognition by CD8_ TC cells is *class I MHC restricted*. In 1996, Doherty and Zinkernagel were awarded the Nobel prize for their major contribution to the understanding of cell-mediated immunity.

Role of Antigen-Presenting Cells

As early as 1959, immunologists were confronted with data suggesting that T cells and B cells recognized antigen by different mechanisms. The dogma of the time, which persisted until the 1980s, was that cells of the immune system recognize the entire protein in its native conformation. However, experiments by P. G. H. Gell and B. Benacerraf demonstrated that, while a primary antibody response and cell-mediated response were induced by a protein in its native conformation, a secondary antibody response (mediated by B cells) could be induced only by native antigen, whereas a secondary cell-mediated response could be induced by either the native or the denatured antigen. These findings were viewed as an interesting enigma, but implications for antigen presentation were completely over looked until the early 1980s.

Processing of Antigen Is Required for Recognition by T Cells

The results obtained by K. Ziegler and E. R. Un an ue were among those that contradicted the prevailing dogma that antigen recognition by B and T cells was basically similar. These researchers observed that TH-cell activation by bacterial protein antigens was prevented by treating the antigen presenting cells with paraformaldehyde prior to antigen exposure. However, if the antigen-presenting cells were first allowed to ingest the antigen and were fixed with paraformaldehyde1–3 h later, TH-cell activation still occurred. During that interval of 1–3 h, the antigen presenting cells had processed the antigen and had displayed it on the membrane in a form able to activate T cells .Subsequent experiments by R. P. Shimonkevitz showed that internalization and processing could be bypassed if antigen-presenting cells were exposed to peptide digests of an antigen instead of the native antigen. In these experiments, antigen-presenting cells were treated with glutaraldehyde (this chemical, like paraformaldehyde, fixes the cell, making the membrane impermeable) and then incubated with native ovalbumin or with ovalbumin that had been subjected to partial enzymatic digestion. The digested ovalbumin was able to interact with the glutaraldehyde-fixed antigen-presenting cells, thereby activating ovalbumin specificTH cells, whereas the native ovalbumin failed to dose. These results suggest that antigen processing involves the digestion of the protein into peptides that are recognized bythe ovalbumin-specific TH cells. At about the same time, A. Townsend and his colleagues began to identify the proteins of influenza virus that were recognized by TC cells. Contrary to their expectations, they found that internal proteins of the virus, such as matrix and nucleocapsid proteins, were often recognized by TC cells better than the more exposed envelope proteins. Moreover, Townsend's work revealed that TC cells recognized short linear peptide sequences of the influenza protein. In fact, when non infected target cells were incubated in vitro with synthetic peptides corresponding to sequences of internal influenza proteins, these cells could be recognized by TC cells and subsequently lysed just as well as target cells that had been infected

with live influenza virus. These findings along with those presented in suggest that antigenprocessing is a metabolic process that digests proteins into peptides, which can then be displayed on the cell membrane together with a class I or class II MHC molecule.

Most Cells Can Present Antigen with Class IMHC; Presentation with Class II MHCIs Restricted to APCs

Since all cells expressing either class I or class II MHC molecules can present peptides to T cells, strictly speaking they all could be designated as antigen-presenting cells. However, by convention, cells that display peptides associated with class IMHC molecules to CD8_ TC cells are referred to as *target cells;* cells that display peptides associated with class II MHC molecules to CD4- TH cells are called antigen-presenting cells (APCs). This convention is followed throughout this text. A variety of cells can function as antigen-presenting cells. Their distinguishing feature is their ability to express class IIMHC molecules and to deliver a co-stimulatory signal. Three cell types are classified as *professional* antigen-presenting cells: dendritic cells, macrophages, and B lymphocytes. These cells differ from each other in their mechanisms of antigen uptake, in whether they constitutively express class II MHC molecules, and in their co-stimulatory activity: Dendritic cells are the most effective of the antigen presenting cells. Because these cells constitutively expressed high level of class II MHC molecules and costimulatory activity, they can activate naive TH cells Macrophages must be activated by phagocytosis of particulate antigens before they express class II MHC molecules or the co-stimulatory B7 membrane molecule._ B cells constitutively express class II MHC molecules but must be activated before they express the co-stimulatoryB7 molecule. Several other cell types, classified as *nonprofessional* antigen-presenting cells, can be induced to express class II MHC molecules or a co-stimulatory signal (Table 8-1).Many of these cells function in antigen presentation only for short periods of time during a sustained inflammatory response. Because nearly all nucleated cells express class I MHC molecules, virtually any nucleated cell is able to function as a target cell presenting endogenous antigens to TC cells. Most often, target cells are cells that have been infected by a virus or some other intracellular microorganism. However, altered self-cells such as cancer cells, aging body cells, or allogeneic cells from a graft can also serve as targets

Evidence for Two Processing and Presentation Pathways

The immune system uses two different pathways to eliminate intracellular and extracellular antigens. Endogenous antigens (those generated within the cell) are processed in the *cytosolic pathway* and presented on the membrane with class IMHC molecules; exogenous antigens (those taken up by endocytosis)are processed in the *endocytic pathway* and presented on the membrane with class II MHC molecules Experiments carried out by L. A. Morrison and T. J.Braciale provided early evidence that the antigenic peptides presented by class I and class II MHC molecules are derived from different processing pathways. These researchers based their experimental protocol on the properties of two clone of TC cells, one that recognized influenza

hemagglutinin(HA) associated with a class I MHC molecule, and antitypical TC line that recognized the same antigen associated with a class II MHC molecule. (In this case, and others as well, the association of T cell function with MHC restriction is not absolute). In one set of experiments, target cells that expressed both class I and class II MHC molecules were incubated with infectious influenza virus or with UV inactivated influenza virus. (The inactivated virus retainedits antigenic properties but was no longer capable of replicating within the target cells.) The target cells were then incubated with the class I–restricted or the atypical class II–restricted TC cells and subsequent lysis of the target cells was determined. The class I–restricted TC cells responded only to target cells treated with infectious virions. Similarly, target cells that had been treated with infectious influenza virions in the presence of emetine, which inhibits viral protein synthesis, stimulated the class II–restricted TC cells but not th classI restricted TC cells. Conversely, target cells that had been treated with infectious in the presence of chloroquine, a drug that blocks the endocytic processing pathway, stimulated class I– but not class II–restricted TC cells. These results support the distinction between the processing of exogenous and endogenous antigens, including the preferential association of exogenous antigens with class IIMHC molecules and of endogenous antigens with class IMHC molecules. Association of viral antigen with class IMHC molecules required replication of the influenza virus and viral protein synthesis within the target cells; association with class II did not. These findings suggested that the peptides presented by class I and class II MHC molecules are trafficked through separate intracellular compartments; class I MHC molecules interact with peptides derived from cytosolic degradation of endogenously synthesized proteins ,class II molecules with peptides derived from endocytic degradation of exogenous antigens

The Cytosolic Pathway

In eukaryotic cells, protein levels are carefully regulated. Every protein is subject to continuous turnover and is degraded at a rate that is generally expressed in terms of its half-life. Some proteins (e.g., transcription factors, cyclins, and key metabolic enzymes) have very short half-lives; denatured, misfolded, or otherwise abnormal proteins also are degrading rapidly. The pathway by which endogenous antigens are degraded for presentation with class I MHC molecules utilizes the same pathways involved in the normal turnover of intracellular proteins.

Peptides for Presentation Are Generated by Protease Complexes Called Proteasomes

Intracellular proteins are degraded into short peptides by a cytosolic proteolytic system present in all cells. Those proteins targeted for proteolysis often have a small protein, called *ubiquitin,* attached to them. Ubiquitin-protein conjugates can be degraded by a multifunctional protease complex called a proteasome. Each proteasome is a large (26S), cylindrical particle consisting of four rings of protein subunits with a central channel of diameter 10–50 Å.A proteasome can cleave peptide bonds between 2 or 3different amino acid combinations in an ATP-dependent process. Degradation of ubiquitin-protein complexes is thought to occur within the central

hollow of the proteasome. Experimental evidence indicates that the immune system utilizes this general pathway of protein degradation toproduce small peptides for presentation with class I MHC molecules. The proteasomes involved in antigen processing include two subunits encoded within the MHC gene cluster, LMP2 and LMP7, and a third non-MHC protein, LMP10(also called MECL-1). All three are induced by increased levelsof the T-cell cytokine IFN-_. The peptidase activities ofproteasomes containing LMP2, LMP7, and LMP10 preferentially generate peptides that bind to MHC class I molecules.

Peptides Are Transported from the Cytosol to the Rough Endoplasmic Reticulum

Insight into the role that peptide transport, the delivery of peptides to the MHC molecule, plays in the cytosolic processing pathway came from studies of cell lines with defects in peptide presentation by class I MHC molecules. One such mutant cell line, called RMA-S, expresses about 5% of thenormal levels of class I MHC molecules on its membrane. Although RMA-S cells synthesize normal levels of class I chains and 2-microglobulin, neither molecule appears onthe membrane. A clue to the mutation in the RMA-S cell line was the discovery by A. Townsend and his colleagues that "feeding" these cells peptides restored their level of membrane-associated class I MHC molecules to normal. These investigators suggested that peptides might be required to stabilize the interaction between the class I chain and 2-microglobulin. The ability to restore expression of class I MHC molecules on the membrane by feeding the cells predigested peptides suggested that the RMA-S cell line might have a defect in peptide transport. Subsequent experiments showed that the defect in the RMA-S cell line occurs in the protein that transports peptides from the cytoplasm to the RER, where class I molecules are synthesized. When RMA-S cells were transected with a functional gene encoding the transporter protein, the cells began to express class I molecules on the membrane. The transporter protein, designated TAP (for transporter associated with antigen processing) is a membrane-spanning heterodimer consisting of two proteins: TAP1 and TAP2. In addition to their multiple transmembrane segments, the TAP1 and TAP2 proteins each have a domain projecting into the lumen of the RER, and an ATP-binding domain that project into the cytosol. Both TAP1 and TAP2belong to the family of ATP-binding cassette proteins found in the membranes of many cells, including bacteria; these proteins mediate ATP-dependent transport of amino acids, sugars, ions, and peptides. Peptides generated in the cytosol by the proteasome are translocated by TAP into the RER by a process that requires the hydrolysis of ATP.TAP has the highest affinity for peptides containing 8–10 amino acids, which is the optimal peptide length for class I MHC binding. In addition, TAP appears to favor peptides with hydrophobic or basic carboxyl-terminal amino acids, the preferred anchor residues for class I MHC molecules. Thus, TAP is optimized to transport peptides that will interact with class I MHC molecules. The *TAP1* and *TAP2* genes map within the class II MHC region, adjacent to the *LMP2* and *LMP7* genes. Both the transporter genes and these *LMP* genes are polymorphic; that is, different allelic forms of these genes exist within the population. Allelic differences in LMP-mediated proteolytic cleavage of protein antigens or in the transport of different peptides from the cytosol into the

RER may contribute to the observed variation among individuals in their response to different endogenous antigens. TAP deficiencies can lead to a disease syndrome that has aspects of both immunodeficiency an autoimmunity

Peptides Assemble with Class I MHC Aided by Chaperone Molecules

Like other proteins, the chain and 2-microglobulin components of the class I MHC molecule are synthesized on polysomes along the rough endoplasmic reticulum. Assembly of these components into a stable class I MHC molecular complex that can exit the RER requires the presence of a peptide in the binding groove of the class I molecule. The assembly process involves several steps and includes the participation of *molecular chaperones,* which facilitate the folding of polypeptides. The first molecular chaperone involved in class I MHC assembly is *calnexin,* a resident membrane protein of the endoplasmic reticulum. Calnexin associates with the free class I _ chain and promotes its folding. When 2-microglobulin binds to the _ chain, calnexin is released and the class I molecule associates with the chaperone *calreticulin* and with *tapasin*. Tapasin (TAP-associated protein) brings the TAP transporter into proximity with the class I molecule and allows it to acquire an antigenic peptide. The physical association of the _ chain–_2-microglobulinheterodimer with the TAP protein promotes peptide capture by the class I molecule before the peptides are exposed to the luminal environment of the RER. Peptides not bound by class I molecules are rapidly degraded. As a consequence of peptide binding, the class I molecule displays increased stability and can dissociate from calreticulin and tapasin, exit from the RER, and proceed to the cell surface via the Golgi. An additional chaperone protein, ERp57, has been observed in association with calnexin and calreticulin complexes. The precise role of this resident endoplasmic reticulum protein in the class I peptide assembly and loading process has not yet been defined, but it is thought to contribute to the formation of disulfide bonds during the maturation of class I chains. Because its role is not clearly defined,

Exogenous Antigens: The Endocytic Pathway

Recapitulates the endogenous pathway discussed previously (left side), and compares it with the separate exogenous pathway (right), which we shall now consider.Whether an antigenic peptide associates with class I or with class II molecules is dictated by the mode of entry into the cell, either exogenous or endogenous, and by the site of processing. Antigen-presenting cells can internalize antigen by phagocytosis, endocytosis, or both.Macrophages internalize antigen by both processes, whereas most other APCs are not phagocytic or are poorly phagocytic and therefore internalize exogenous antigen only by endocytosis (either receptor-mediated endocytosis or pinocytosis). B cells, for example, internalize antigen very effectively by receptor-mediated endocytosis using antigen-specific membrane antibody as the receptor.

Peptides Are Generated from Internalized Molecules in Endocytic Vesicles

Once an antigen is internalized, it is degraded into peptides within compartments of the endocytic processing pathway. The endocytic pathway appears to involve three increasingly acidic compartments: early endosomes (Ph 6.0–6.5); late endosomes, or endolysosomes (pH 5.0–6.0); and lysosomes (pH 4.5–5.0). Internalized antigen moves from early to late endosomes and finally to lysosomes, encountering hydrolytic enzymes and a lower pH in each compartment 9). Lysosomes, for example, contain a unique collection of more than 40 acid-dependent hydrolases, including proteases, nucleases, glycosidases, lipases, phospholipases, and phosphatases. Within the compartments of the endocytic pathway, antigen is degraded into oligopeptides of about 13–18 residues,which bind to class II MHC molecules.Because the hydrolytic enzymes are optimally active under acidic conditions (low pH), antigen processing can be inhibited by chemical agents that increase the pH of the compartments (e.g., chloroquine) as well as by protease inhibitors (e.g., leupeptin). The mechanism by which internalized antigen moves from one endocytic compartment to the next has not been conclusively demonstrated. It has been suggested that early endosomes from the periphery move inward to become late endosomes and finally lysosomes. Alternatively, small transport vesicles may carry antigens from one compartment to the next. Eventually the endocytic compartments, or portions of them, return to the cell periphery, where they fuse with the plasma membrane. In this way, the surface receptors are recycled.

The Invariant Chain Guides Transport of Class II MHC Molecules to Endocytic Vesicles

Since antigen-presenting cells express both class I and class II MHC molecules, some mechanism must exist to prevent class II MHC molecules from binding to the same set of antigenic peptides as the class I molecules.When class II MHC molecule are synthesized within the RER, three pairs of class II___chains associate with a preassembled trimer of a Assembly and stabilization of class I MHC molecules. Newly formed class I _ chains associate with calnexin, a molecular chaperone, in the RER membrane. Subsequent binding to _2-microglobulin releases calnexin and allows binding to the chaperonin calreticulin and to tapasin, which is associated with the peptide transporter TAP. This association promotes binding of an antigenic peptide, which stabilizes the class I molecule–peptide complex, allowing its release from the RER. + Protein called invariant chain (Ii, CD74**).** This trimeric protein interacts with the peptide-binding cleft of the class II molecules, preventing any endogenously derived peptides from binding to the cleft while the class II molecule is within the RER. The invariant chain also appears to be involved in the folding of the class II _ and_ chains, their exit from the RER,and the subsequent routing of class II molecules to the endocytic processing pathway from the trans-Golgi network. The role of the invariant chain in the routing of class II molecules has been demonstrated in transfection experiments with cells that lack the genes encoding class II MHC molecules and the invariant chain. Immunofluorescent labeling of such cells transfected only with class II MHC genes revealed class II molecules localized within the Golgi complex. However, in cells transfected with both the class II MHC genes and invariantchain gene, the class II molecules were localized in the cytoplasmic vesicular structures of the endocytic pathway. The invariant chain contains sorting signals in its cytoplasmic tail that directs the transport of the class II MHC complex from the trans-Golgi network to the endocytic compartments.

Peptides Assemble with Class II MHC Molecules by Displacing CLIP

Recent experiments indicate that most class II MHC–invariant chain complexes are transported from the RER, where they are formed, through the Golgi complex and trans-Golgi network, and then through the endocytic pathway, moving from early endosomes to late endosomes, and finally to lysosomes. As the proteolytic activity increases in each successive compartment, the invariant chain is gradually degraded. However, a short fragment of the invariant chain termed *CLIP* (for *class II–associated invariant chain peptide*) remains bound to the class II molecule after the invariant chain has been cleaved within the endosomal compartment. CLIP physically occupies the peptide-binding groove of the class II MHC molecule, presumably preventing any premature binding of antigenic peptide. A nonclassical class II MHC molecule called *HLA-DM* is required to catalyze the exchange of CLIP with antigenic peptide. MHC class II genes encoding HLADM have been identified in the mouse and rabbit, indicating that HLA-DM is widely conserved among mammalian species. Like other class II MHC molecules, HLA-DM is a heterodimer of and chains. However, unlike other class II molecules, HLA-DM is not polymorphic and is not expressed at the cell membrane but is found predominantly within the endosomal compartment. The *DM_* and *DM_* genes are located near the *TAP* and *LMP* genes in the MHC complex of humans and DM is expressed in cells that express classical class II molecules. The reaction between HLA-DM and the class II CLIP complex facilitating exchange of CLIP for another peptide is impaired in the presence of HLA-DO, which binds to HLADM and lessens the efficiency of the exchange reaction. HLADO, like HLA-DM, is a non classical and non polymorphic class II molecule that is also found in the MHC of other species. HLA-DO differs from HLA-DM in that it is expressed only by B cells and the thymus, and unlike other class II molecules, its expression is not induced by IFN-_. An additional difference is that the genes encoding the _ and the _ chains of HLA-DO are not adjacent in the MHC as are all other class II _ and _ pairs (see Fig 7-15). An HLA-DR3 molecule associated with CLIP was isolated from a cell line that did not express HLA-DM and was therefore defective in antigen processing. Superimposing the structure of HLA-DR3–CLIP on another DR molecule bound to antigenic peptide reveals that CLIP binds to class II in the same stable manner as the antigenic peptide. The discovery of this stable complex in a cell with defective HLA-DM supports the argument that HLA-DM is required for the replacement of CLIP. Although it certainly modulates the activity of HLA-DM, the precise role of HLA-DO remains obscure. One possibility is that it acts in the selection of peptides bound to class II MHC molecules in B cells. DO occurs in complex with DM in these cells and this association continues in the endosomal compartments. Conditions of higher acidity weaken the association of DM/DO and increase the possibility of antigenic peptide binding despite the presence of DO. Such a pH-dependent interaction could lead to preferential selection of class II-bound peptides from lysosomal compartments in B cells as compared with other APCs. As with class I MHC molecules, peptide binding is required to maintain the structure and stability of class II MHC molecules. Once a peptide has bound, the peptide–class II complex is transported to the plasma membrane, where the neutral pH appears to

enable the complex to assume a compact, stable form. Peptide is bound so strongly in this compact form that it is difficult to replace a class II–bound peptide on the membrane with another peptide at physiologic conditions.

Presentation of Nonpeptide Antigens

To this point the discussion has been limited to peptide antigens and their presentation by classical class I and II MHC molecules. It is well known that nonprotein antigens also are recognized by the immune system, and there are reports dating back to the 1980s of T cell proliferation in the presence of non protein antigens derived from infectious agents. More recent reports indicate that T cells that express the TCR (Tcell receptors are dimers of either or chains) that react with glycolipid antigens derived from bacteria such as *Mycobacterium tuberculosis*. These nonprotein antigens are presented by members of the CD1 family of nonclassical class I molecules. The CD1 family of molecules associates with _2-microglobulin and has general structural similarity to class I MHC molecules. There are five genes encodinghuman CD1 molecules (*CD1A-E,* encoding the gene products CD1a-d, with no product yet identified for *E*). These genes are located not within the MHC but on chromosome 1 . The genes are classified into two groups based on sequence homology. Group 1 includes *CD1A, B, C,* and *E*; *CD1D* is in group 2. All mammalian species studied have CD1 genes, although the number varies. Rodents have only group 2 *CD1* genes, the counterpart of human *CD1D,* whereas rabbits, like humans, have five genes, including both group 1 and 2 types. Sequence identity of CD1 with classical class I molecules is considerably lower than the identity of the class I molecules with each other. Comparison of the three-dimensional structure of the mouse CD1d1 with the class I MHC molecule H- 2*kb* shows that the antigen-binding groove of the CD1d1 molecules is deeper and more voluminous than that of the classical class I molecule (Fig 8-11b). Expression of CD1 molecules varies according to subset; *CD1D1* genes are expressed mainly in nonprofessional APCs and on certain B-cell subsets. The mouse CD1d1 is more widely distributed and found on T cells, B cells, dendritic cells, hepatocytes, and some epithelial cells. The *CD1A, B,* and *C* genes are expressed on immature thymocytes and professional APCs, mainly those of the dendritic type. *CD1C* gene expression is seen on B cells, whereas the *CD1A* and *B* products are not. *CD1* genes can be induced by exposure to certain cytokines such as GM-CSF or IL-3. The intracellular trafficking patterns of the CD1 molecules differ; for example, CD1a is found mostly in early endosomes or on the cell surface; CD1b and CD1d localize to late endosomes; and CD1c is found throughout the endocytic system. Certain CD1 molecules are recognized by T cells in the absence of foreign antigens, and self restriction can be demonstratedin these reactions. Examination of antigens presented by CD1 molecules revealed them to be lipid components (mycolic acid) of the *M. tuberculosis* cell wall. Further studies of CD1 presentation indicated that a glycolipid (lipoarabinomannan) from *Mycobacterium leprae* could also be presented by these molecules. The data concerning CD1 antigen presentation point out the existence of a third pathway for the processing of antigens, a pathway with distinct intracellular steps that do not involve the molecules found to facilitate class I antigen processing. For example, CD1 molecules are able to process antigen in TAP-deficient cells. Recent data

indicate that the CD1a and 1b molecules traffic differently, with CD1a at the surface or in the recycling endocytic compartments and CD1b and CD1d in the lysomal compartments. Exactly how the CD1 pathway complements or intersects the better understood class I and class II pathways remains an open question. The T-cell types reactive to CD1 were first thought to be limited to T cells expressing the_TCR and lacking both CD4 and CD8, or T cells with a single TCR _ chain, but recent reports indicate that a wider range of T-cell types will recognize CD1-presenting cells. Recent evidence indicates that natural killer T cells recognize CD1d molecules presenting autologous antigen. This may represent a mechanism for eliminating cells that are altered by stress, senescence, or neoplasia

Transplantation Immunology

immunology, refers to the act of transferring cells, tissues, or organs from one site to another. The desire to accomplish transplants stems from the realization that many diseases can be cured by implantation of a healthy organ, tissue, or cells (a graft) from one individual (the donor) to another in need of the transplant (the recipient or host). The development of surgical techniques that allow the facile reimplantation of organs has removed one barrier to successful transplantation, but others remain. One is the lack of organs for transplantation. Although a supply of organs is provided by accident victims and, in some cases, living donors, there are more patients in need of transplants than there are organs available. The seriousness of the donor organ shortage is reflected in the fact that, as of November 2000, an estimated 73,000 patients in the United States were on the waiting list for an organ transplantation. The majority of those on the list (~70%) require a kidney; at present, the waiting period for this organ averages over 800 days. While the lack of organs for transplantation is a serious issue, the most formidable barrier to making transplantation a routine medical treatment is the immune system. The immune system has evolved elaborate and effective mechanisms to protect the organism from attack by foreign agents, and these same mechanisms cause rejection of grafts from anyone who is not genetically identical to the recipient. Alexis Carrel reported the first systematic study of transplantation in 1908; he interchanged both kidneys in a series of nine cats. Some of those receiving kidneys from other cats maintained urinary output for up to 25 days. Although all the cats eventually died, the experiment established that a transplanted organ could carry out its normal function in the recipient. The first human kidney transplant, attempted in 1935 by a Russian surgeon, failed because there was a mismatch of blood types between donor and recipient. This incompatibility caused almost immediate rejection of the kidney, and the patient died without establishing renal function. The rapid immune response experienced here, termed hyperacute rejection, is mediated by antibodies and will be described in this chapter. The first successful human kidney transplant, which was between identical twins, was accomplished in Boston in 1954. Today, kidney, pancreas, heart, lung, liver, bone-marrow, and cornea transplantations are performed among non identical individuals with ever increasing frequency and success. A variety of immunosuppressive agents can aid in the survival of the transplants, including drugs and specific antibodies developed to diminish the immunologic attack on grafts, but the majority of these

agents have an overall immunosuppressive effect, and their long-term use is deleterious. New methods of inducing specific tolerance to the graft without suppressing other immune responses are being developed and promise longer survival of transplants without compromise of host immunity. This chapter describes the mechanisms underlying graft rejection, various procedures that are used to prolong graft survival, and a summary of the current status of transplantation as a clinical tool. A Clinical Focus section examines the use of organs from nonhuman species (xenotransplants) to circumvent the shortage of organs available for patients in need of them.

Immunologic Basis of Graft Rejection

The degree of immune response to a graft varies with the type of graft. The following terms are used to denote different types of transplants:

Autograft is self-tissue transferred from one body site to another in the same individual. Transferring healthy skin to a burned area in burn patients and use of healthy blood vessels to replace blocked coronary arteries are examples of frequently used autografts.

Isograft is tissue transferred between genetically identical individuals. In inbred strains of mice, an isograft can be performed from one mouse to another syngeneic mouse. In humans, an isograft can be performed between genetically identical (monozygotic) twins.

Allograft is tissue transferred between genetically different members of the same species. In mice, an allograft is performed by transferring tissue or an organ from one strain to another. In humans, organ grafts from one individual to another are allografts unless the donor and recipient are identical twins.

Xenograft is tissue transferred between different species (e.g., the graft of a baboon heart into a human). Because of significant shortages in donated organs, raising animals for the specific purpose of serving as organ donors for humans is under serious consideration. Autografts and isografts are usually accepted, owing to the genetic identity between graft and host (Figure 21-1a). Because an allograft is genetically dissimilar to the host, it is often recognized as foreign by the immune system and is rejected. Obviously, xenografts exhibit the greatest genetic disparity and therefore engender a vigorous graft rejection.

Allograft Rejection Displays Specificity and Memory

The rate of allograft rejection varies according to the tissue involved. In general, skin grafts are rejected faster than other tissues such as kidney or heart. Despite these time differences, the immune response culminating in graft rejection always displays the attributes of specificity and memory. If an inbred mouse of strain A is grafted with skin from strain B, primary graft rejection, known as first-set rejection, occurs. The skin first becomes revascularized between

days 3 and 7; as the reaction develops, the vascularized transplant becomes infiltrated with lymphocytes, monocytes, neutrophils, and other inflammatory cells. There is decreased vascularization of the transplanted tissue by 7–10 days, visible necrosis by 10 days, and complete rejection by 12–14 days. Immunologic memory is demonstrated when a second strain-B graft is transferred to a previously grafted strain-A mouse. In this case, a graft-rejection reaction develops more quickly, with complete rejection occurring within 5–6 days; this secondary response is designated second-set rejection. The specificity of second-set rejection can be demonstrated by grafting an unrelated strain-C graft at the same time as the second strain-B graft. Rejection of the strain-C graft proceeds according to first-set rejection kinetics, whereas the strain-B graft is rejected in an acceleratedsecond-set fashion.

T Cells Play a Key Role in Allograft Rejection

In the early 1950s, Avrion Mitchison showed in adoptive transfer experiments that lymphocytes, but not serum antibody, could transfer allograft immunity. Later studies implicated T cells in allograft rejection. For example, nude mice, which lack a thymus and consequently lack functional T cells, were found to be incapable of allograft rejection; indeed, these mice even accept xenografts. In other studies, T cells derived from an allograft-primed mouse were shown to transfer second-set allograft rejection to an unprimed syngeneic recipient, as long as that recipient was grafted with the same allogeneic tissue. Analysis of the T-cell subpopulations involved in allograft rejection has implicated both CD4+ and CD8+ populations. In one study, mice were injected with monoclonal antibodies to deplete one or both types of T cells and then the rate of graft rejection was measured. As shown in Figure 21-3, removal of the CD8+ population alone had no effect on graft survival, and the graft was rejected at the same rate as in control mice (15 days). Removal of the CD4+ T-cell population alone prolonged graft survival from 15 days to 30 days. However, removal of both the CD4+ and the CD8+ T cells resulted in long-term survival (up to 60 days) of the allografts. This study indicated that both CD4+ and CD8+ T-cells participated in rejection and that the collaboration of both subpopulations resulted in more pronounced graft rejection.

Graft Donors and Recipients Are Typed for RBC and MHC Antigens

Since differences in blood group and major histocompatibility antigens are responsible for the most intense graft-rejection reactions, various tissue-typing procedures to identify these antigens have been developed to screen potential donor and recipient cells. Initially, donor and recipient are screened for ABO blood-group compatibility. The blood-group antigens are expressed on RBCs, epithelial cells, and endothelial cells. Antibodies produced in the recipient to any of these antigens that are present on transplanted tissue will induce antibodymediated complement lysis of the incompatible donor cells. HLA typing of potential donors and a recipient can be accomplished with a microcytotoxicity test. In this test, white blood cells from the potential donors and recipient are distributed into a series of wells on a microtiter plate, and then antibodies specific for various class I and class II MHC alleles are added to different wells. After

incubation, complement is added to the wells, and cytotoxicity is assessed by the uptake or exclusion of various dyes (e.g., trypan blue or eosin Y) by the cells. If the white blood cells express the MHC allele for which a particular monoclonal antibody is specific, then the cells will be lysed upon addition of complement, and these dead cells will take up a dye such as trypan blue. HLA typing based on antibody-mediated microcytotoxicity can thus indicate the presence or absence of various MHC alleles. Even when a fully HLA-compatible donor is not available, transplantation may be successful. In this situation, a one-way mixed-lymphocyte reaction (MLR) can be used to quantify the degree of class II MHC compatibility between potential donors and a recipient. Lymphocytes from a potential donor that have been x-irradiated or treated with mitomycin C serve as the stimulator cells, and lymphocytes from the recipient serve as responder cells. Proliferation of the recipient T cells, which indicates T-cell activation, is measured by the uptake of [3H] thymidine into cell DNA. The greater the class II MHC differences between the donor and recipient cells, the more [3H] thymidine uptake will be observed in an MLR assay. Intense proliferation of the recipient lymphocytes indicates a poor prognosis for graft survival. The advantage of the MLR over microcytotoxicity typing is that it gives a better indication of the degree of TH-cell activation generated in response to the class II MHC antigens of the potential graft. The disadvantage of the MLR is that it takes several days to run the assay. If the potential donor is a cadaver, for example, it is not possible to wait for the results of the MLR, because the organ must be used soon after removal from the cadaver. In that case, the microcytotoxicity test, which can be performed within a few hours, must be relied on. The importance of MHC matching for acceptance of allografts is confirmed by data gathered from recipients of kidney transplants. The data reveal that survival of kidney grafts depends primarily on donor-recipient matching of the HLA class II antigens. Matching or mismatching of the class I antigens has a lesser effect on graft survival unless there also is mismatching of the class II antigens. A two-year survival rate of 90% is seen for kidney transplants in which one or two class I HLA loci are mismatched, while transplanted kidneys with differences in the class II MHC have only a 70% chance of lasting for this period. Those with greater numbers of mismatches have a very low survival rate at one year after transplant. As described below, HLA matching is most important for kidney and bone-marrow transplants; liver and heart transplants may survive with greater mismatching. Current understanding of the killer-inhibitory receptors (KIR) on the NK cell suggests that absence of a class I antigen recognized by the KIR molecules could lead to killing of the foreign cell. Rejection was observed in experimental bone-marrow transplants where the class I molecule recognized by the recipient NK-inhibitory receptor is absent on donor cells. The effects of such class I mismatching on solid organ grafts may be less marked. MHC identity of donor and host is not the sole factor determining tissue acceptance. When tissue is transplanted between genetically different individuals, even if their MHC antigens are identical, the transplanted tissue can be rejected because of differences at various minor histocompatibility loci. As described in Chapter 10, the major histocompatibility antigens are recognized directly by TH and TC cells, a phenomenon termed *alloreactivity*. In contrast, minor histocompatibility antigens are recognized only when they are presented in the context of self-MHC molecules. The tissue rejection induced by minor histocompatibility differences is

usually less vigorous than that induced by major histocompatibility differences. Still, reaction to these minor tissue differences often results in graft rejection. For this reason, successful transplantation even between HLA-identical individuals requires some degree of immune suppression.

Cell-Mediated Graft Rejection Occurs in Two Stages

Graft rejection is caused principally by a cell-mediated immune response to alloantigens (primarily, MHC molecules) expressed on cells of the graft. Both delayed-type hypersensitive and cell-mediated cytotoxicity reactions have been implicated. The process of graft rejection can be divided into two stages: (1) a sensitization phase, in which antigen-reactive lymphocytes of the recipient proliferate in response to allo- antigens on the graft, and (2)an effector stage, in which immune destruction of the graft takes place.

SENSITIZATION STAGE

During the sensitization phase, CD4+ and CD8+ T cells recognize alloantigens expressed on cells of the foreign graft and proliferate in response. Both major and minor histocompatibility alloantigens can be recognized. In general, the response to minor histocompatibility antigens is weak, although the combined response to several minor differences can sometimes be quite vigorous.The response to major histocompatibility antigens involves recognition of both the donor MHC molecule and an associated peptide ligand in the cleft of the MHC molecule. The peptides present in the groove of allogeneic class I MHC molecules are derived from proteins synthesized within the allogeneic cell. The peptides present in the groove of allogeneic class II MHC molecules are generally proteins taken up and processed through the endocytic pathway of the allogeneic antigen-presenting cell. A host TH cell becomes activated when it interacts with an antigen-presenting cell (APC) that both expresses an appropriate antigenic ligand–MHC molecule complex and provides the requisite co-stimulatory signal. Depending on the tissue, different populations of cells within a graft may function as APCs. Because dendritic cells are found in most tissues and because they constitutively express high levels of class II MHC molecules, dendritic cells generally serve as the major APC in grafts. APCs of host origin can also migrate into a graft and endocytose the foreign alloantigens (both major and minor histocompatibility molecules) and present them as processed peptides together with self-MHC molecules. In some organ and tissue grafts (e.g., grafts of kidney, thymus, and pancreatic islets), a population of donor APCs called *passenger leukocytes* has been shown to migrate from the graft to the regional lymph nodes. These passenger leukocytes are dendritic cells, which express high levels of class II MHC molecules (together with normal levels of class I MHC molecules) and are widespread in mammalian tissues, with the chief exception of the brain. Because passenger leukocytes express the allogeneic MHC antigens of the donor graft, they are recognized as foreign and therefore can stimulate immune activation of T lymphocytes in the lymph node. In some experimental situations, the passenger cells have been shown to induce tolerance to their surface antigens by deletion of thymic T-cell populations with receptors

specific for them. Consistent with the notion that exposure to donor cells can induce tolerance are data showing that blood transfusions from the donor prior to transplantation can aid acceptance of the graft. Passenger leukocytes are not the only cells involved in immune stimulation. For example, they do not seem to play any role in skin grafts. Other cell types that have been implicated in alloantigen presentation to the immune system include Langerhans cells and endothelial cells lining the blood vessels. Both of these cell types express class I and class II MHC antigens. Recognition of the alloantigens expressed on the cells of a graft induces vigorous T-cell proliferation in the host. This proliferation can be demonstrated in vitro in a mixedlymphocyte reaction (see Figure 21-4c). Both dendritic cells and vascular endothelial cells from an allogeneic graft induce host T-cell proliferation. The major proliferating cell is the CD4+ T cell, which recognizes class II alloantigens directly or alloantigen peptides presented by host antigen-presenting cells. This amplified population of activated TH cells is thought to play a central role in inducing the various effector mechanisms of allograft rejection.

EFFECTOR STAGE

A variety of effector mechanisms participate in allograft rejection. The most common are cell-mediated reactions involving delayed-type hypersensitivity and CTL mediated cytotoxicity; less common mechanisms are antibodyplus- complement lysis and destruction by antibody-dependent cell-mediated cytotoxicity (ADCC). The hallmark of graft rejection involving cell-mediated reactions is an influx of T cells and macrophages into the graft. Histologically, the infiltration in many cases resembles that seen during a delayed type hypersensitive response, in which cytokines produced by TDTH cells promote macrophage infiltration. Recognition of foreign class I alloantigens on the graft by host CD8+ cells can lead to CTL-mediated killing. In some cases, CD4+ T cells that function as class II MHC–restricted cytotoxic cells mediate graft rejection. In each of these effector mechanisms, cytokines secreted by TH cells play a central role. For example, IL-2, IFN-_, and TNF-_ have each been shown to be important mediators of graft rejection. IL-2 promotes T-cell proliferation and generally is necessary for the generation of effector CTLs. IFN-_ is central to the development of a DTH response, promoting the influx of macrophages into the graft and their subsequent activation into more destructive cells. TNF-_ has been shown to have a direct cytotoxic effect on the cells of a graft. A number of cytokines promote graft rejection by inducing expression of class I or class II MHC molecules on graft cells. The interferons and TNF-and TNF all increase class I MHC expression, and IFN-_ increases class II MHC expression as well. During a rejection episode, the levels of these cytokines increase, inducing a variety of cell types within the graft to express class I or class II MHC molecules. In rat cardiac allografts, for example, dendritic cells are initially the only cells that express class II MHC molecules.However, as an allograft reaction begins, localized production of IFN-_ in the graft induces vascular endothelial cells and myocytes to express class II MHC molecules as well, making these cells targets for CTL attack.

Clinical Manifestations of Graft Rejection

Graft-rejection reactions have various time courses depending upon the type of tissue or organ grafted and the immune response involved. Hyperacute rejection reactions occur within the first 24 hours after transplantation; acute rejection reactions usually begin in the first few weeks after transplantation; and chronic rejection reactions can occur from months to years after transplantation.

Pre-Existing Recipient Antibodies Mediate

Hyperacute Rejection

In rare instances, a transplant is rejected so quickly that the grafted tissue never becomes vascularized. These hyperacute reactions are caused by preexisting host serum antibodies specific for antigens of the graft. The antigen-antibody complexes that form activate the complement system, resulting in an intense infiltration of neutrophils into the grafted tissue. Theensuing inflammatory reaction causes massive blood clots within the capillaries, preventing vascularization of the graft Several mechanisms can account for the presence of preexisting antibodies specific for allogeneic MHC antigens. Recipients of repeated blood transfusions sometimes develop significant levels of antibodies to MHC antigens expressed on white blood cells present in the transfused blood. If some of these MHC antigens are the same as those on a subsequent graft, then the antibodies can react with the graft, inducing a hyperacute rejection reaction.With repeated pregnancies, women are exposed to the paternal alloantigens of the fetus and may develop antibodies to these antigens. Finally, individuals who have had a previous graft sometimes have high levels of antibodies to the allogeneic MHC antigens of that graft. In some cases, the preexisting antibodies participating in hyperacute graft rejection may be specific for blood-group antigens in the graft. If tissue typing and ABO blood-group typing are performed prior to transplantation, these preexisting antibodies can be detected and grafts that would result in hyperacute rejection can be avoided. Xenotransplants are often rejected in a hyperacute manner because of antibodies to cellular antigens of the donor species that are not present in the recipient species. Such an antigen is discussed in the Clinical Focus section of this chapter. In addition to the hyperacute rejection mediated by preexisting antibodies, there is a less frequent form of rejection termed *accelerated rejection* caused by antibodies that are produced immediately after transplantation.

Acute Rejection Is Mediated by T-Cell Response

Cell-mediated allograft rejection manifests as an acute rejection of the graft beginning about 10 days after transplantation. Histopathologic examination reveals a massive infiltration of macrophages and lymphocytes at the site of tissue destruction, suggestive of TH-cell activation and proliferation. Acute graft rejection is effected by the mechanisms described previously

Chronic Rejection Occurs Months or Years Post-Transplant

Chronic rejection reactions develop months or years after acute rejection reactions have subsided. The mechanisms of chronic rejection include both humoral and cell-mediated responses by the recipient. While the use of immunosuppressive drugs and the application of tissue-typing methods to obtain optimum match of donor and recipient have dramatically increased survival of allografts during the first years after engraftment, little progress has been made in long-term survival. The use of immunosuppressive drugs, which are described below, greatly increases the short-term survival of the transplant, but chronic rejection is not prevented in most cases. Data for rejection of kidney transplants since 1975 indicates an increase from 40% to over 80% in one-year survival of grafts. However, in the same period long-term survival has risen only slightly; as in 1975, about 50% of transplanted kidneys are still functioning at 10 years after transplant. Chronic rejection reactions are difficult to manage with immunosuppressive drugs and may necessitate another transplantation.

The Most Commonly Transplanted Organ Is the Kidney

As mentioned above, the most commonly transplanted organ is the kidney; in 2000, there were 13,258 kidney transplants performed in the United States. Major factors contributing to this number are the numerous clinical indications for kidney transplantation. Many common diseases, such as diabetes and various types of nephritis, result in kidney failure that can be alleviated by transplantation. With respect to availability, kidneys can be obtained not only from cadavers but also from living relatives or volunteers, because it is possible to donate a kidney and live a normal life with the remaining kidney. In 1999, 4457 of the 12,483 kidneys transplanted in the U.S. came from living donors. Surgical procedures for transplantation are straightforward; technically, the kidney is simpler to reimplant than the liver or heart. Because many kidney transplants have been done, patient-care procedures have been worked out in detail. Matching of blood and histocompatibility groups is advantageous in kidney transplantation because the organ is heavily vascularized, but the kidney presents no special problems that promote rejection or graft-versus-host disease (GVHD), as the bone marrow or liver do. Two major problems are faced by patients waiting for a kidney. One is the short supply of available organs, and the second is the increasing number of sensitized recipients. The latter problem stems from rejection of a first transplant, which then sensitizes the individual and leads to the formation of antibodies and activation of cellular mechanisms directed against kidney antigens. Any subsequent graft containing antigens in common with the first would be quickly rejected. Therefore, detailed tissue typing procedures must be used to ascertain that the patient has no antibodies or active cellular mechanisms directed against the potential donor's kidney. In many cases, patients can never again find a match after one or two rejection episodes. It is almost always necessary to maintain kidney-transplant patients on some form of immunosuppression, usually for their entire lives. Unfortunately, this gives rise to complications, including risks of cancer and infection as

well as other side effects such as hypertension and metabolic bone disease.

Bone-Marrow Transplants Are Used for Leukemia, Anemia, and Immunodeficiency

After the kidney, bone marrow is the most frequent transplant. Since the early 1980s, bone-marrow transplantation has been increasingly adopted as a therapy for a number of malignant and nonmalignant hematologic diseases, including leukemia, lymphoma, aplastic anemia, thalassemia major, and immunodeficiency diseases, especially severe combined immunodeficiency, or SCID. The bone marrow, which is obtained from a living donor by multiple needle aspirations, consists of erythroid, myeloid, monocytoid, megakaryocytic, and lymphocytic lineages. The graft, usually about 109 cells per kilogram of host body weight, is injected intravenously into the recipient. The first successful bone-marrow transplantations were performed between identical twins. However, development of the tissue-typing procedures described earlier now makes it possible to identify allogeneic donors who have HLA antigens identical or nearidentical to those of the recipients.While the supply of bone marrow for transplantation is not a problem, finding a matched donor may be one. In the usual procedure, the recipient of a bone-marrow transplant is immunologically suppressed before grafting. Leukemia patients, for example, are often treated with cyclophosphamide and total-body irradiation to kill all cancerous cells. The immune-suppressed state of the recipient makes graft rejection rare; however, because the donor bone marrow contains immunocompetent cells, the graft may reject the host, causing graft-versus-host disease (GVHD). GVHD affects 50%–70% of bone-marrow-transplant patients; it develops as donor T cells recognize alloantigens on the host cells. The activation and proliferation of these T cells and the subsequent production of cytokines generate inflammatory reactions in the skin, gastrointestinal tract, and liver. In severe cases, GVHD can result in generalized erythroderma of the skin, gastrointestinal hemorrhage, and liver failure. Various treatments are used to prevent GVHD in bone marrow transplantation. The transplant recipient is usually placed on a regimen of immunosuppressive drugs, often including cyclosporin A and methotrexate, in order to inhibit the immune responses of the donor cells. In another approach, the donor bone marrow is treated with anti-T-cell antisera or monoclonal antibodies specific for T cells before transplantation, thereby depleting the offending T cells. Complete T-cell depletion from donor bone marrow, however, increases the likelihood that the marrow will be rejected, and so the usual procedure now is a partial T-cell depletion.Apparently, a low level of donor T-cell activity, which results in a low-level GVHD, is actually beneficial because the donor cells kill any host T cells that survive the immunosuppression treatment. This prevents residual recipient cells from becoming sensitized and causing rejection of the graft. In leukemia patients, low-level GVHD also seems to result in destruction of host leukemic cells, thus making it less likely for the leukemia to recur.

Heart Transplantation Is a Challenging Operation

Perhaps the most dramatic form of transplantation is that of the heart; once the damaged heart

has been removed, the patient must be kept alive by wholly artificial means until the transplanted heart is in place and beating. Heart-lung machines are available to circulate and aerate the patient's blood after the heart is removed. The donor's heart must be maintained in such a manner that it will begin beating when it is placed in the recipient. It has been found that a human heart can be kept viable for a limited period in ice-cold buffer solutions that effectively short circuit the electric impulses that control the rhythmic beating, which could damage the isolated organ. The surgical methods of implanting a heart have been available for a number of years. The first heart transplant was carried out in South Africa by Dr. Christian Barnard, in 1964. Since then, the one-year survival rate for transplantation of the heart has become greater than 80%. In 2000, 2172 heart transplants were performed in the United States and about 3500 worldwide. An issue peculiar to heart transplantation has been a new type of atherosclerotic disease in the coronary arteries of the implanted organ. There is some possibility that host antibodies mediate injury to the vessels in the donated heart. Although a heart transplant may greatly benefit patients with various types of heart disease or damage, there is obviously a strict limit on the number of available hearts. Accident victims who are declared brain dead but have an intact circulatory system and a functioning heart are the normal source of these organs. HLA matching is desirable but not often possible, because of the limited supply of hearts and the urgency of the procedure.

Lung Transplants Are on the Increase

In recent years, lung transplantation, either by itself or in conjunction with heart transplantation, has been used to treat diseases such as cystic fibrosis and emphysema or acute damage to the lungs such as that caused by smoke inhalation. In 2000, 945 lung and 47 heart/lung transplants were performed. First-year survival rate for lung transplants is reporte dat about 60%.

Liver Transplants Treat Congenital Defects and Damage from Viral or Chemical Agents

The liver is a large organ that performs a number of functions related to clearance and detoxification of chemical and biological substances. Liver malfunction can be caused by damage to the organ from viral diseases such as hepatitis or by exposure to harmful chemicals, as in chronic alcoholism. Damage to the liver may correct itself and the damaged tissue can regenerate after the causative injurious agent is cleared. If the liver tissue does not regenerate, damage may be fatal. The majority of liver transplants are used as a therapy for congenital abnormalities of the liver. Because the liver is large and has a complicated circulation, re- implantation of the liver initially posed a technical problem. Techniques have been developed to overcome this major surgical challenge, and the recent one-year survival rate has risen to approximately 65%. In 2000, 4816 livers were transplanted in the United States. Increasingly, a liver from a single donor may be split and given to two recipients; normally, a child will receive the smaller portion and an adult the larger. The immunology of liver transplantation is interesting because the organ appears to resist rejection by hyperacute antibody-mediated mechanisms. It has been shown that even transplantation across blood-group barriers, which would be expected to trigger hyperacute

rejection, can be successful in the short term. However, leukocytes within the donor organ together with anti–blood-group antibodies can mediate antibody-dependent hemolysis of recipient red blood cells if there is a mismatch of the blood groups. In addition, manifestations of GVHD have occurred in liver transplants even when donor and recipient are blood-group compatible. These reactions are obviously caused by donor lymphocytes carried by the transplanted liver.

Pancreas Transplantation Offers a Cure for Diabetes Mellitus

One of the more common diseases in the United States is diabetes mellitus. This disease is caused by malfunction of insulin-producing islet cells in the pancreas. Transplantation of a pancreas could provide the appropriately regulated levels of insulin necessary to make the diabetic individual normal. Recently, one-year success rates for pancreas transplantation of about 55% have been reported. Transplantation of the complete pancreas is not necessary to restore the function needed to produce insulin in a controlled fashion; transplantation of the islet cells alone could restore function. Kidney failure is a frequent complication of advanced diabetes occurring in about 30% of diabetics, therefore kidney and pancreas transplants are indicated. In 2000, there were 420 pancreas transplants and 904 simultaneous kidney/pancreas transplants. A group at the University of Wisconsin reports that they have overcome surgical and medical barriers to the dual transplant and have achieved survival rates of 87% at one year and 78% at five years for the 381 cases in their study. Whether it is better to carry out simultaneous kidney-pancreas transplants or to transplant separately remains an issue to be resolved on a case-to-case basis.

Skin Grafts Are Used to Treat Burn Victims

Most skin transplantation in humans is done with autologous tissue. However, in cases of severe burn, grafts of foreign skin thawed from frozen deposits in tissue banks may be used. These grafts generally act as biologic dressings, because the cellular elements are no longer viable and the graft does not grow in the new host; the grafts are left in place for several days but are regularly replaced. True allogeneic skin grafting using fresh viable donor skin has been undertaken in some cases, but rejection must be prevented by the use of immunosuppressive therapy. This is not desirable because a major problem with burn victims is the high risk of infection, and immunosuppressive therapy accentuates this risk. The above list of common transplants is by no means all inclusive and is expected to grow in future years. For example, intracerebral neural-cell grafts have restored functionality in victims of Parkinson's disease. In studies conducted thus far, the source of neural donor cells was human embryos; the possibility of using those from other animal species is being tested.

Xenotransplantation May Be the Answer to the Shortage of Donor Organs

While the immune system represents a formidable barrier to the use of transplantation, there has been significant progress in overcoming this obstacle. However, there has not been comparable progress in solving the complex problem of finding organs for those who need them. The insufficient supply of available organs means that a large percentage of patients die while waiting for a transplant. The need for an alternative source of donor organs has focused attention

on xenotransplantation. The larger nonhuman primates (chimpanzees and baboons) have served as the main transplant donors, and, as discussed in the Clinical Focus section, the use of the pig as a source of organs is under serious consideration. The earliest transplants of chimpanzee kidneys into humans date back to 1964. Since that time, sporadic attempts at kidney, heart, liver, and bone-marrow transplantation from primates into humans have been made. No attempt has met with great success but several have received some attention. In 1993, T. E. Starzl performed two liver transplants from baboons into patients suffering from liver failure. Both patients died, one after 26 days and the other after 70 days. In 1994, a pig liver was transplanted into a 26-year-old suffering from acute hepatic failure. The liver functioned only 30 hours before it was rejected by a hyperacute rejection reaction. In 1995, baboon bone marrow was infused into an HIV-infected man with the aim of boosting his weakened immune system with the baboon immune cells, which do not become infected with the virus. Although there were no complications from the transplant, the baboon bone marrow did not appear to establish itself in the recipient. A major problem with xenotransplants is that immune rejection is often quite vigorous, even when recipients are treated with potent immunosuppressive drugs such as FK506 or rapamycin. The major response involves the action of humoral antibody and complement, leading to the development of a hyperacute rejection reaction. In addition to the problem of rejection, there is general concern that xenotransplantation has the potential of spreading pathogens from the donor to the recipient. These pathogens could potentially cause diseases, called xenozoonoses, which are fatal for humans. For example, certain viruses, including close relatives of HIV-1 found in chimpanzees and HIV-2 and herpesvirus B, which occur in several primate species, cause limited pathogenesis in their primate hosts but can lead to deadly infections in humans. In addition, there is the fear that primate retroviruses (see Chapter 19), such as SIV, may recombine with human variants to produce new agents of disease. The possibility of introducing new viruses into humans maybe greater for transplants from closely related species, such as primates, and less in the case of more distantly related species, such as pigs, because viruses are less likely to replicate inB cells from unrelated species.

Transplantation Antigens

The sub discipline of tumor immunology involves the study of antigens on tumor cells and the immune response to these antigens. Two types of tumor antigens have been identified on tumor cells: tumor-specific transplantation antigens (TSTAs) and tumor-associated transplantation antigens (TATAs). Tumor-specific antigens are unique to tumor cells and do not occur on normal cells in the body. They may result from mutations in tumor cells that generate altered cellular proteins; cytosolic processing of these proteins would give rise to novel peptides that are presented with class I MHC molecules, inducing a cell-mediated response by tumor-specific CTLs (Figure 22-6). Tumor-associated antigens, which are not unique to tumor cells, may be proteins that are expressed on normal cells during fetal development when the immune system is immature and unable to respond but that normally are not expressed in the adult. Reactivation of the embryonic genes that encode these proteins in tumor cells results in their expression on the

fully differentiated tumor cells. Tumor-associated antigens may also be proteins that are normally expressed at extremely low levels on normal cells but are expressed at much higher levels on tumor cells. It is now clear that the tumor antigens recognized by human T cells fall into one of four major categories:

- Antigens encoded by genes exclusively expressed by tumors
- Antigens encoded by variant forms of normal genes that have been altered by mutation
- Antigens normally expressed only at certain stages of differentiation or only by certain differentiation lineages
- Antigens that are over expressed in particular tumors Many tumor antigens are cellular proteins that give rise to peptides presented with MHC molecules; typically, these antigens have been identified by their ability to induce the proliferation of antigen-specific CTLs or helper T cells

Most Tumor Antigens Are Not Unique to Tumor Cells

The majority of tumor antigens is not unique to tumor cells but also is present on normal cells. These tumor-associated transplantation antigens may be proteins usually expressed only on fetal cells but not on normal adult cells, or they may be proteins expressed at low levels by normal cells but at much higher levels by tumor cells. The latter category includes growth factors and growth-factor receptors, as well as oncogene-encoded proteins. Several growth-factor receptors are expressed at significantly increased levels on tumor cells and can serve as tumor associated antigens. For instance, a variety of tumor cells express the epidermal growth factor (EGF) receptor at levels 100 times greater than that in normal cells. An example of an over-expressed growth factor serving as a tumor-associated antigen is a transferrin growth factor, designated p97, which aids in the transport of iron into cells. Whereas normal cells express less than 8,000 molecules of p97 per cell, melanoma cells express 50,000–500,000 molecules of p97 per cell. The gene that encodes p97 has been cloned, and a recombinant vaccinia virus vaccine has been prepared that carries the cloned gene. When this vaccine was injected into mice, it induced both humoral and cell-mediated immune responses, which protected the mice against live melanoma cells expressing the p97 antigen. Results such as this highlight the importance of identifying tumor antigens as potential targets of tumor immunotherapy.

ONCOFETAL TUMOR ANTIGENS

Oncofetal tumor antigens, as the name implies, are found not only on cancerous cells but also on normal fetal cells. These antigens appear early in embryonic development, before the immune system acquires immunocompetence; if these antigens appear later on cancer cells, they are recognized as nonself and induce an immunologic response Two well-studied oncofetal antigens are alpha-fetoprotein (AFP) and carcinoembryonic antigen (CEA). Although the serum concentration of AFP drops from milligram levels in fetal serum to nano gram levels in normal adult serum, elevated AFP levels are found in a majority of patients with liver cancer (Table 22-3). CEA is a membrane glycoprotein found on gastrointestinal and liver cells of 2- to 6-month-

old fetuses. Approximately 90% of patients with advanced colorectal cancer and 50% of patients with early colorectal cancer have increased levels of CEA in their serum; some patients with other types of cancer also exhibit increased CEA levels. However, because AFP and CEA can be found in trace amounts in some normal adults and in some noncancerous disease states, the presence of these onco fetal antigens is not diagnostic of tumors but rather serves to monitor tumor growth. If, for example, a patient has had surgery to remove a colorectal carcinoma, CEA levels are monitored after surgery. An increase in the CEA level is an indication of resumed tumor growth.

ONCOGENE PROTEINS AS TUMOR ANTIGENS

A number of tumors have been shown to express tumor associated antigens encoded by cellular oncogenes. These antigens are also present in normal cells encoded by the corresponding proto-oncogene. In many cases, there is no qualitative difference between the oncogene and proto-oncogene products; instead, the increased levels of the oncogene product can be recognized by the immune system. For example, as noted earlier, human breast-cancer cells exhibit elevated expression of the oncogene-encoded Neu protein, a growth factor receptor, whereas normal adult cells express only trace amounts of Neu protein. Because of this difference in the Neu level, anti-Neu monoclonal antibodies can recognize and selectively eliminate breast-cancer cells without damaging normal cells. TATAS ON HUMAN MELANOMA several tumor-associated transplantation antigens have been identified on human melanomas. Five of these—MAGE-1, MAGE-3, BAGE, GAGE-1, GAGE-2—are oncofetal-type antigens. Each of these antigens is expressed on a significant proportion of human melanoma tumors, as well as on a number of other human tumors, but not on normal differentiated tissues except for the testis, where it is expressed on germ-line cells. In addition, a number of differentiation antigens expressed on normal melanocytes—including tyrosinase, gp100, Melan-A or MART-1, and gp75—are over expressed by melanoma cells, enabling them to function as tumor-associated transplantation antigens. Several of the human melanoma tumor antigens are shared by a number of other tumors. About 40% of human melanomas are positive for MAGE-1, and about 75% are positive for MAGE-2 or 3. In addition to melanomas, a significant percentage of glioma cell lines, breast tumors, non-small-cell lung tumors, and head or neck carcinomas express MAGE-1, 2, or 3. These shared tumor antigens could be exploited for clinical treatment. It might be possible to produce a tumor vaccine expressing the shared antigen for treatment of a number of these tumors, as described at the end of this chapter.

Tumors Can Induce Potent Immune Responses

In experimental animals, tumor antigens can be shown to induce both humoral and cell-mediated immune responses that result in the destruction of the tumor cells. In general, the cell-mediated response appears to play the major role. A number of tumors have been shown to induce tumor-specific CTLs that recognize tumor antigens presented by class I MHC on the

tumor cells. However, as discussed below, expression of class I MHC a molecule is decreased in a number of tumors, thereby limiting the role of specific CTLs in their destruction.

NK Cells and Macrophages Are Important in Tumor Recognition

The recognition of tumor cells by NK cells is not MHC restricted. Thus, the activity of these cells is not compromised by the decreased MHC expression exhibited by some tumor cells. In some cases, Fc receptors on NK cells can bind to antibody-coated tumor cells, leading to ADCC. The importance of NK cells in tumor immunity is suggested by the mutant mouse strain called beige and by Chediak-Higashi syndrome in humans, as described in the Clinical Focus in Chapter 14. In each case, a genetic defect causes marked impairment of NK cells and an associated increase in certain types of cancer.

HLA TISSUE TYPING

HLA typing of potential donors and a recipient can be accomplished with a microcytotoxicity test. In this test, white blood cells from the potential donors and recipient are distributed into a series of wells on a microtiter plate, and then antibodies specific for various class I and class II MHC alleles are added to different wells. After incubation, complement is added to the wells, and cytotoxicity is assessed by the uptake or exclusion of various dyes (e.g., trypan blue or eosin Y) by the cells. If the white blood cells express the MHC allele for which a particular monoclonal antibody is specific, then the cells will be lysed upon addition of complement, and these dead cells will take up a dye such as trypan blue. HLA typing based on antibody-mediated microcytotoxicity can thus indicate the presence or absence of various MHC alleles. Even when a fully HLA-compatible donor is not available, transplantation may be successful. In this situation, a one-way mixed-lymphocyte reaction (MLR) can be used to quantify the degree of class II MHC compatibility between potential donors and a recipient Lymphocytes from a potential donor that have been x-irradiated or treated with mitomycin C serve as the stimulator cells, and lymphocytes from the recipient serve as responder cells. Proliferation of the recipient T cells, which indicates T-cell activation, is measured by the uptake of [3H] thymidine into cell DNA. The greater the class II MHC differences between the donor and recipient cells, the more [3H] thymidine uptake will be observed in an MLR assay. Intense proliferation of the recipientlymphocytes indicates a poor prognosis for graft survival. The advantage of the MLR over microcytotoxicity typing is that it gives a better indication of the degree of TH-cell activation generated in response to the class II MHC antigens of the potential graft. The disadvantage of the MLR is that it takes several days to run the assay. If the potential donor is a cadaver, for example, it is not possible to wait for the results of the MLR, because the organ must be used soon after removal from the cadaver. In that case, the microcytotoxicity test, which can be performed within a few hours, must be relied on.

The importance of MHC matching for acceptance of allograft is confirmed by data gathered from recipients of kidney transplants. The data in Figure 21-5 reveal that survival of kidney grafts depends primarily on donor-recipient matching of the HLA class II antigens. Matching or mismatching of the class I antigens has a lesser effect on graft survival unless there also is mismatching of the class II antigens. A two-year survival rate of 90% is seen for kidney transplants in which one or two class I HLA loci are mismatched, while transplanted kidneys with differences in the class II MHC have only a 70% chance of lasting for this period. Those with greater numbers of mismatches have a very low survival rate at one year after transplant. As described below, HLA matching is most important for kidney and bone-marrow transplants; liver and heart transplants may survive with greater mismatching. Current understanding of the killer-inhibitory receptors (KIR) on the NK cell suggests that absence of a class I antigen recognized by the KIR molecules could lead to killing of the foreign cell. Rejection was observed in experimental bone-marrow transplants where the class I molecule recognized by the recipient NK-inhibitory receptor is absent on donor cells. The effects of such class I mismatching on solid organ grafts may be less marked.MHC identity of donor and host is not the sole factor determining tissue acceptance. When tissue is transplanted between genetically different individuals, even if their MHC antigens are identical, the transplanted tissue can be rejected because of differences at various minor histocompatibility loci**.** The major histocompatibility antigens are recognized directly by TH and TC cells, a phenomenon termed *alloreactivity*. In contrast, minor histocompatibility antigens are recognized only when they are presented in the context of self-MHC molecules. The tissue rejection induced by minor histocompatibility difference is usually less vigorous than that induced by major histocompatibility differences. Still, reaction to these minor tissue differences often results in graft rejection. For this reason, successful transplantation even between HLA-identical individuals requires some degree of immune suppression.

Cell-Mediated Graft Rejection Occurs in Two Stages

Graft rejection is caused principally by a cell-mediated immune response to alloantigens (primarily, MHC molecules) expressed on cells of the graft. Both delayed-type hypersensitive and cell-mediated cytotoxicity reactions have been implicated. The process of graft rejection can be divided into two stages: (1) a sensitization phase, in which antigen-reactive lymphocytes of the recipient proliferate in response to allo-typing procedures for HLA antigens. HLA typing by micro cytotoxicity. (a) White blood cells from potential donors and the recipient are added to separate wells of a microtiter plate. The example depicts the reaction of donor and recipient cells with a single antibody directed against an HLA-A antigen. The reaction sequence shows that if the antigen is present on the lymphocytes, addition of complement will cause them to become porous and unable to exclude the added dye. (b) Because cells express numerous HLA antigens, they are tested separately with a battery of antibodies specific for various HLA-A antigens. Here, donor 1 shares HLA-A antigens recognized by antisera in wells 1 and 7 with the recipient, whereas donor 2 has none of HLA-A antigens in common with the recipient. (c) Mixed

lymphocyte reaction to determine identity of class II HLA antigens between a potential donor and recipient. Lymphocytes from the donor are irradiated or treated with mitomycin C to prevent cell division and then added to cells from the recipient. If the class II antigens on the two cell populations are different, the recipient cells will divide rapidly and take up large quantities of radioactive nucleotides into the newly synthesized nuclear DNA. The amount of radioactive nucleotide uptake is roughly proportionate to the MHC class II differences between the donor and recipient lymphocytes.

Immune Response to Infectious Diseases

One of the first and most important features of host innate immunity is the barrier provided by the epithelial surfaces of the skin and the lining of the gut. The difficulty of penetrating these epithelial barriers ensures that most pathogens never gain productive entry into the host. In addition to providing a physical barrier to infection, the epithelia also produce chemicals that are useful in preventing infection. The secretion of gastric enzymes by specialized epithelial cells lowers the pH of the stomach and upper gastrointestinal tract, and other specialized cells in the gut produce antibacterial peptides .A major feature of innate immunity is the presence of the normal gut flora, which can competitively inhibit the binding of pathogens to gut epithelial cells. Innate responses can also block the establishment of infection. For example, the cell walls of some gram-positive bacteria contain a peptidoglycan that activates the alternative complement pathway, resulting in the generation of C3b, which opsonizes bacteria and enhances phagocytosis. Some bacteria produce endotoxins such as LPS, which stimulate the production of cytokines such as TNF-_, IL-1, and IL-6 by macrophages or endothelial cells. These cytokines can activate macrophages. Phagocytosis of bacteria by macrophages and other phagocytic cells is another highly effective line of innate defense. However, some types of bacteria that commonly grow intracellularly have developed mechanisms that allow them to resist degradation within the phagocyte. Viruses are well known for the stimulation of innate responses. In particular, many viruses induce the production of interferons, which can inhibit viral replication by inducing an antiviral response. Viruses are also controlled by NK cells. As described, NK cells frequently form the first line of defense against viral infections. Generally, pathogens use a variety of strategies to escaped estruction by the adaptive immune system. Many pathogens reduce their own antigenicity either by growing within host cells, where they are sequestered from immune attack, or by shedding their membrane antigens. Other pathogens camouflage themselves by mimicking the surfaces of host cells, either by expressing molecules with amino acid sequences similar to those of host cell-membrane molecules or by acquiring a covering of host membrane molecules. Some pathogens are able to suppress the immune response selectively or to regulate it so that a branch of the immune system is activated that is ineffective against the pathogen. Continual variation in surface antigens is another strategy that enables a pathogen to elude the immune system. This antigenic variation may be due to the gradual accumulation of mutations,or it may involve an abrupt change in surface antigens. Both innate and adaptive immune responses to pathogens provide critical defense, but infectious diseases, which have

plagued human populations throughout history, still cause the death of millions each year. Although widespreaduse of vaccines and drug therapy has drastically reduced mortality from infectious diseases in developed countries, such diseases continue to be the leading cause of death in theThird World. It is estimated that over 1 billion people are infected worldwide, resulting in more than 11 million deaths every year. Despite these alarming numbers, estimated expenditures for research on infectious diseases prevalent in the Third World are less than 5% of total health research expenditures worldwide. Not only is this a tragedy for these countries, but some of these diseases are beginning and a new drug-resistant strain of *Mycobacterium tuberculosis*is spreading at an alarming rate in the United States. In this chapter, the concepts described in earlier chapters,antigenicity and immune effector mechanisms, as well as vaccine development are applied to selected infectious diseases caused by viruses, bacteria, protozoa, andhelminths— the four main types of pathogens.

Viral Infections

A number of specific immune effector mechanisms, together with nonspecific defense mechanisms, are called into play to eliminate an infecting virus (Table 17-1). At the same time, the virus acts to subvert one or more of these mechanisms to prolong its own survival. The outcome of the infection depends on how effectively the host's defensive mechanisms resist the offensive tactics of the virus. The innate immune response to viral infection is primarily through the induction of type I interferons (IFN-_ and IFN-_) and the activation of NK cells. Double stranded RNA (dsRNA) produced during the viral life cycle can induce the expression of IFN-_ and IFN-_ by the infected cell. Macrophages, monocytes, and fibroblasts also are capable of synthesizing these cytokines, but the mechanisms that induce the production of type I interferons in these cells are not completely understood. IFN-_ and IFN-_ can induce an antiviral response or resistance to viral replication by binding to the IFN _/_ receptor. Once bound, IFN-_ and IFN- activate the JAK-STAT pathway, which in turn induces the transcription of several genes. One of these genes encodes an enzyme known as 2_-5_-oligo-adenylate synthetase [2-5(A) synthetase], which activates a ribonuclease (RNAse L) that degrades viral RNA. Other genes activated by IFN-_/_ binding to its receptor also contribute to the inhibition of viral replication. For example, IFN-_/_ binding induces a specific protein kinase called dsRNA-dependent protein kinase (PKR), which inactivates protein synthesis, thus blocking viral replication in infected cells (Figure 17-2). The binding of IFN-_ and IFN-_ to NK cells induces lytic activity,making them very effective in killing virally infected cells. The activity of NK cells is also greatly enhanced by IL-12, a cytokine that is produced very early in a response to viral infection.

Many Viruses are neutralized by Antibodies

Antibodies specific for viral surface antigens are often crucial in containing the spread of a virus during acute infection and in protecting against reinjection. Antibodies are particularly effective in protecting against infection if they are localized at the site of viral entry into the body.Most

viruses express surface receptor molecules that enable them to initiate infection by binding tospecific host-cell membrane molecules. For example, influenza virus binds to sialic acid residues in cellmembrane glycoproteins and glycolipids; rhinovirus binds to intercellular adhesion molecules (ICAMs); and Epstein-Barrvirus binds to type 2 complement receptors on B cells. If antibody to the viral receptor is produced, it can block infection altogether by preventing the binding of viral particles to host cells. Secretory IgA in mucous secretions plays an important role in host defense against viruses by blocking viral attachment of mucosal epithelial cells. The advantage of the attenuated oral polio vaccine, considered in Chapter 18, is that it induces production of secretory IgA, which effectively blocks attachment of poliovirus along the gastrointestinal tract. Viral neutralization by antibody sometimes involves mechanisms that operate after viral attachment to host cells.In some cases, antibodies may block viral penetration by binding to epitopes that are necessary to mediate fusion ofthe viral envelope with the plasma membrane. If the induced antibody is of a complement-activating isotype, lysis of enveloped virions can ensue. Antibody or complement can also agglutinate viral particles and function as an opsonizingagent to facilitate Fc- or C3b-receptor–mediated phagocytosis of the viral particles.

Cell-Mediated Immunity is Important for Viral Control and Clearance

Although antibodies have an important role in containing the spread of a virus in the acute phases of infection, they are not usually able to eliminate the virus once infection has occurred—particularly if the virus is capable of entering a latent state in which its DNA is integrated into host chromosomal DNA. Once an infection is established, cell-mediated immune mechanisms are most important in host defense. In general, CD8+ TC cells and CD4+ TH1 cells are the main components of cell-mediated antiviral defense, although in some cases CD4+ TC cells have also been implicated. Activated TH1 cells produce a number of cytokines, including IL-2, IFN-, and TNF that defend against viruses either directly or indirectly. IFN-_ acts directly by inducing an antiviral state in cells. IL-2 acts indirectly by assisting in the recruitment of CTL precursors into an effector population. Both IL-2 and IFN-_ activate NK cells, which play an important role in host defense during the first days of many viral infections until a specific CTL response develops. In most viral infections, specific CTL activity arises within 3–4 days after infection, peaks by 7–10 days, and then declines. Within 7–10 days of primary infection, most virions have been eliminated, paralleling the development of CTLs. CTLs specific for the virus eliminate virus-infected self-cells and thus eliminate potential sources of new virus. The role of CTLs in defense against viruses is demonstrated by the ability of virus-specific CTLs to confer protection for the specific virus on non immune recipients by adoptive transfer. The viral specificity of the CTL as well can be demonstrated with. adoptive transfer: adoptive transfer of a CTL clone specific for influenza virus strain X protects mice against influenza virus X but not against influenza virus strain Y.

Viruses Can Evade Host Defense Mechanisms

Despite their restricted genome size, a number of viruses have been found to encode proteins that interfere at various levels with specific or non specific host defenses. Presumably, the advantage of such proteins is that they enable viruses to replicate more effectively amidst host antiviral defenses. As described above, the induction of IFN-α and IFN-γ is a major innatedefense against viral infection, but some viruses have developed strategies to evade the action of IFN-_/_. These include hepatitis C virus, which has been shown to overcome the antiviral effect of the interferons by blocking or inhibiting the action of PKR. Another mechanism for evading host responses, utilized in particular by herpes simplex viruses (HSV) is inhibition of antigen presentation by infected host cells. HSV-1 and HSV-2 both express an immediate-early protein (a protein synthesized shortly after viral replication) called ICP47, which very effectively inhibits the human transporter molecule needed for antigen processing (TAP). Inhibition of TAP blocks antigen delivery to class I MHC receptors on HSV-infected cells, thus preventing presentation of viral antigen to CD8+ T cells. This results in the trapping of empty class I MHC molecules in the endoplasmic reticulum and effectively shuts down a CD8+ T-cell response to HSV-infected cells. The targeting of MHC molecules is not unique to HSV. Other viruses have been shown to down-regulate class I MHC expression shortly after infection. Two of the best characterized examples, the adenoviruses and cytomegalovirus (CMV), use distinct molecular mechanisms to reduce the surface expression of class I MHC molecules, again inhibiting antigen presentation to CD8+ T cells. Some viruses CMV, measles virus, and HIV—have been shown to reduce levels of class II MHC molecules on the cell surface, thus blocking the function of antigen-specific antiviral helper T cells. Antibody-mediated destruction of viruses requires complement activation, resulting either in direct lysis of the viral particle or opsonization and elimination of the virus by phagocytic cells. A number of viruses have strategies for evading complement-mediated destruction. Vaccinia virus, for example, secretes a protein that binds to the C4b component, inhibiting the classical complement pathway; and herpes simplex viruses have a glycoprotein component that binds to the C3b complement component, inhibiting both the classical and alternative pathways. A number of viruses escape immune attack by constantly changing their antigens. In the influenza virus, continual antigenic variation results in the frequent emergence of new infectious strains. The absence of protective immunity to these newly emerging strains leads to repeated epidemics of influenza. Antigenic variation among rhinoviruses, the causative agent of the common cold, is responsible for our inability to produce an effective vaccine for colds. Nowhere is antigenic variation greater than in the human immunodeficiency virus (HIV), the causative agent of AIDS. Estimates suggest that HIV accumulates mutations at a rate 65 times faster than does influenza virus. Because of the importance of AIDS, a section of Chapter 19 addresses this disease. A large number of viruses evade the immune response by causing generalized immunosuppression. Among these are the paramyxoviruses that cause mumps, the measles virus, Epstein-Barr virus (EBV), cytomegalovirus, and HIV. In some cases, immunosuppression is caused by direct viral infection of lymphocytes or macrophages. The virus can then either directly destroy the immune cells by cytolytic mechanisms or alter their function. In other cases, immunosuppression is the result of a cytokine imbalance. For example, EBV produces a protein, called BCRF1 that is homologous to

IL-10; like IL-10, BCRF1 suppresses cytokine production by the TH1 subset, resulting in decreased levels of IL-2, TNF, and IFN

Bacterial Infections

Immunity to bacterial infections is achieved by means of antibody unless the bacterium is capable of intracellular growth, in which case delayed-type hypersensitivity has an importantrole. Bacteria enter the body either through a number of natural routes (e.g., the respiratory tract, the gastrointestinal tract, and the genitourinary tract) or through normally inaccessible routes opened up by breaks in mucous membranes or skin. Depending on the number of organisms entering and their virulence, different levels of host defense are enlisted. If the inoculum size and the virulence are both low, then localized tissue phagocytes may be able to eliminate the bacteria with an innate, nonspecific defense. Larger inoculums or organisms with greater virulence tend to induce an adaptive, specific immune response.

Immune Responses to Extracellular and Intracellular Bacteria Can Differ

Infection by extracellular bacteria induces production of humoral antibodies, which are ordinarily secreted by plasma cells in regional lymph nodes and the submucosa of the respiratory and gastrointestinal tracts. The humoral immune response is the main protective response against extracellular bacteria. The antibodies act in several ways to protect the host from the invading organisms, including removal of the bacteria and inactivation of bacterial toxins (Figure 17-8). Extracellular bacteria can be pathogenic because they induce a localized inflammatory response or because they produce toxins. The toxins, endotoxin or exotoxin, can be cytotoxic but also may cause pathogenesis in other ways. An excellent example of this is the toxin produced by diphtheria, which exerts a toxic effect on the cell by blocking protein synthesis. Endotoxins, such as lipopolysaccharides (LPS), are generally components of bacterial cell walls, while exotoxins, such as diphtheria toxin, are secreted by the bacteria. Antibody that binds to accessible antigens on the surface of a bacterium can, together with the C3b component of complement, act as an opsonin that increases phagocytosis and thus clearance of the bacterium. In the case of some bacteria—notably, the gram-negative organisms— complement activation can lead directly to lysis of the organism. Antibody-mediated activation of the complement system can also induce localized production of immune effector molecules that help to develop an amplified and more effective inflammatory response. For example, the complement split products C3a, C4a, and C5a act as anaphylatoxins, inducing local mast-cell degranulation and thus vasodilation and the extravasation of lymphocytes and neutrophils from the blood into tissue space. Other complement split products serve as chemotactic factors for neutrophils and macrophages, thereby contributing to the buildup of phagocytic cells at the site of infection. Antibody to a bacteria toxin may bind to the toxin and neutralize it; the antibody-toxin complexes are then cleared byphagocytic cells in the same manner as any other antigenantibodycomplex. While innate immunity is not very effective against intracellular bacterial pathogens, intracellular bacteria can

activate NK cells, which, in turn, provide an early defense againstthese bacteria. Intracellular bacterial infections tend to induce a cell-mediated immune response, specifically, delayedtypehypersensitivity. In this response, cytokines secreted byCD4+ T cells are important—notably IFN-γ, which activates macrophages to kill ingested pathogens more effectively

Bacteria Can Effectively Evade Host Defense Mechanisms

There are four primary steps in bacterial infection: Attachment to host cells Proliferation Invasion of host tissue,Toxin-induced damage to host cells Host-defense mechanisms act at each of these steps, and many bacteria have evolved ways to circumvent some of these host defenses. Some bacteria have surface structures or molecules that enhance their ability to attach to host cells. A number of gram-negative bacteria, for instance, have pili (long hair like projections), which enable them to attach to the membrane of the intestinal or genitourinary tract. Other bacteria, such as *Bordetella pertussis,* secrete adhesion molecules that attach to both the bacterium and the ciliatedepithelial cells of the upper respiratory tract. Secretory IgA antibodies specific for such bacterial structures can block bacterial attachment to mucosal epithelialcells and are the main host defense against bacterial attachment. However, some bacteria (e.g., *Neisseria gonorrhoeae,Haemophilus influenzae,* and *Neisseria meningitidis*) evade the IgA response by secreting proteases that cleave secretory IgA at the hinge region; the resulting Fab and Fc fragments have a shortened half-life in mucous secretions and are not able to agglutinate microorganisms. Some bacteria evade the IgA response of the host by changing these surface antigens. In *N. gonorrhoeae,* for example, pilin, the protein component of the pili, has a highly variable structure.Variation in the pilin amino acid sequenceis generated by gene rearrangements of its coding sequence. The pilin locus consists of one or two expressed genes and 10–20 silent genes. Each gene is arranged into six regions called *minicassettes*. Pilin variation is generated by a process of gene conversion, in which one or more minicassettes from the silent genes replace a minicassette of the expression gene. This process generates enormous antigenic variation, which may contribute to the pathogenicity of *N. gonorrhoeae* by increasing the likelihood that expressed pili will bind firmly to epithelial cells. In addition, the continual changes in the pilin sequence allow the organism to evade neutralization by IgA. Some bacteria possess surface structures that serve to inhibit phagocytosis. A classic example is *Streptococcus pneumoniae,* whose polysaccharide capsule prevents phagocytosisvery effectively. There are 84 serotypes of *S. pneumoniae* that differ from one another by distinct capsular polysaccharides. During infection, the host produce antibody against the infecting serotype. This antibody protects against reinjection with the same serotype but will not protect against infection by a different serotype. In this way, *S. pneumoniae* can cause disease many times in the same individual. On other bacteria, such as *Streptococcus pyogenes,* a surface protein projection called the M protein inhibits phagocytosis. Some pathogenic staphylococci are able to assemble a protective coat from host proteins. These bacteria secrete a coagulase enzyme that precipitates a fibrin coat around them, shielding them from phagocytic cells. Mechanisms for interfering with the

complement system help other bacteria survive. In some gram-negative bacteria, for example, long side chains on the lipid A moiety of the cell-wall core polysaccharide help to resist complement mediated lysis. *Pseudomonas* secretes an enzyme, elastase that inactivates both the C3a and C5a anaphylatoxins, thereby diminishing the localized inflammatory reaction. A number of bacteria escape host defense mechanisms by their ability to survive within phagocytic cells. Some, such as*Listeria monocytogenes,* do this by escaping from the phagolysosome to the cytoplasm, which is a more favorable environment for their growth. Other bacteria, such as *Mycobacterium avium,* block lysosomal fusion with the phagolysosome; and some mycobacteria are resistant to the oxidative attack that takes place within the phagolysosome.

Immune Responses Can Contribute to Bacterial Pathogenesis

In some cases, disease is caused not by the bacterial pathogen itself but by the immune response to the pathogen. As described in Chapter 12, pathogen-stimulated overproduction of cytokines leads to the symptoms of bacterial septic shock, food poisoning, and toxic-shock syndrome. For instance, cell-wall endotoxins of some gram-negative bacteria activate macrophages, resulting in release of high levels of IL-1 and TNF-_, which can cause septic shock. In staphylococcal food poisoning and toxic-shock syndrome, exotoxins produced by the pathogens function as super antigens, which can activate all T cells that express T-cell receptors with a particular_ domain. The resulting overproduction of cytokines by activated TH cells causes many of the\ symptoms of these diseases.

Protozoan Diseases

Protozoans are unicellular eukaryotic organisms. They are responsible for several serious diseases in humans, including amoebiasis, Chagas' disease, African sleeping sickness, malaria, leishmaniasis, and toxoplasmosis. The type of immune response that develops to protozoan infection and the effectiveness of the response depend in part on the location of the parasite within the host. Many protozoans have life-cyclestages in which they are free within the bloodstream, and it is during these stages that humoral antibody is most effective. Many of these same pathogens are also capable of intracellular growth; during these stages, cell-mediated immune reactions are effective in host defense. In the development of vaccines for protozoan diseases, the branch of the immune system that is most likely to confer protection must be carefully considered.

Malaria (*Plasmodium* Species) Infects 600 Million People Worldwide

Malaria is one of the most devastating diseases in the world today, infecting nearly 10% of the world population and causing 1–2 million deaths every year. Malaria is caused by various species of the genus *Plasmodium,* of which *P. falciparum* is the most virulent and prevalent. The alarming development of multiple-drug resistance in *Plasmodium* and the increased resistance of its vector, the *Anopheles* mosquito,to DDT underscore the importance of developing new

strategies to hinder the spread of malaria.

PLASMODIUM LIFE CYCLE AND PATHOGENESIS OF MALARIA

Plasmodium progresses through a remarkable series of developmental and maturational stages in its extremely complex life cycle. Female *Anopheles* mosquitoes, which feed on blood meals, serve as the vector for *Plasmodium,* and part of the parasite's life cycle takes place within the mosquito. (Because male *Anopheles* mosquitoes feed on plant juices, they do not transmit *Plasmodium.* Human infection begins when sporozoites, one of the *Plasmodium* stages, are introduced into an individual's bloodstream as an infected mosquito takes a blood meal. Within 30 min, the sporozoites disappear from the blood as they migrate to the liver,where they infect hepatocytes.Sporozoites are long, slender cells that are covered b a 45-kDa protein called circumsporozoite (CS) antigen, which appears to mediate their adhesion to hepatocytes. Thembinding site on the CS antigen is a conserved region in the carboxyl-terminal end (called region II) that has a high degree of sequence homology with known cell-adhesion molecules. Within the liver, the sporozoites multiply extensively and undergo a complex series of transformations that culminate in the formation and release of merozoites in about a week. It has been estimated that a liver hepatocyte infected with a single sporozoite can release 5,000–10,000 merozoites. The released merozoites infect red blood cells, initiating the symptoms and pathology of malaria. Within a red blood cell, merozoites replicate and undergo successive differentiations; eventually the cell ruptures and releases new merozoites, which go on to infect more red blood cells. Eventually some of the merozoites differentiate into male and female gametocytes, which may be ingested by a female *Anopheles* mosquito during a blood meal. Within the mosquito's gut, the male and female gametocytes differentiate into gametes that fuse to form a zygote, which multiplies and differentiates into sporozoites with in the salivary gland. The infected mosquito is now set to initiate the cycle once again. The symptoms of malaria are recurrent chills, fever, and sweating. The symptoms peak roughly every 48 h, when successive generations of merozoites are released from infected red blood cells. An infected individual eventually becomes weak and anemic and shows splenomegaly. The large numbers of merozoites formed can block capillaries, causing intense headaches, renal failure, heart failure, or cerebral damage—often with fatal consequences. There is speculation that some of the symptoms of malaria may be caused not by *Plasmodium* itself but instead by excessive production of cytokines. This hypothesis stemmed from the observation that cancer patients treated in clinical trials with recombinant tumor necrosis factor (TNF) developed symptoms that mimicked malaria. The relation between TNF and malaria symptoms was studied by infecting mice with a mouse specific strain of *Plasmodium,* which causes rapid death by cerebral malaria. Injection of these mice with antibodies to TNF was shown to prevent the rapid death.

HOST RESPONSE TO PLASMODIUM INFECTION

In regions where malaria is endemic, the immune response to *Plasmodium* infection is poor.

Children less than 14 years old mount the lowest immune response and consequently are most likely to develop malaria. In some regions, the childhood mortality rate for malaria reaches 50%, and worldwide the disease kills about a million children a year. The low immune response to *Plasmodium* among children can be demonstrated by measuring serum antibody levels to the sporozoite stage. Only 22% of the children living in endemic areas have detectable antibodies to the sporozoite stage, whereas 84% of the adults have such antibodies. Even in adults, the degree of immunity is far from complete, however, and most people living in endemic regions have lifelong low-level *Plasmodium* infections.A number of factors may contribute to the low levels of immune responsiveness to *Plasmodium.* The maturational changes from sporozoite to merozoite to gametocyte allowthe organism to keep changing its surface molecules, resulting in continual changes in the antigens seen by the immune system. The intracellular phases of the life cycle in liver cells and erythrocytes also reduce the degree of immune activation generated by the pathogen and allow the organism tomultiply while it is shielded from attack. Furthermore, the most accessible stage, the sporozoite, circulates in the bloodfor only about 30 min before it infects liver hepatocytes; it is unlikely that much immune activation can occur in such a short period of time. And even when an antibody response does develop to sporozoites, *Plasmodium* has evolved a way of overcoming that response by sloughing off the surface CSantigen coat, thus rendering the antibodies ineffective.

Diseases Caused by Parasitic Worms (Helminths)

Unlike protozoans, which are unicellular and often grow within human cells, helminths are large, multicellular organisms that reside in humans but do not ordinarily multiply there and are not intracellular pathogens. Although helminthes are more accessible to the immune system than protozoans, most infected individuals carry few of these parasites; for this reason, the immune system is not strongly engaged and the level of immunity generated to helminths is often very poor. Parasitic worms are responsible for a wide variety of diseases in both humans and animals.More than a billion people are infected with *Ascaris,* a parasitic roundworm that infects the small intestine, and more than 300 million people are infected with *Schistosoma,* a trematode worm that causes a chronic debilitating infection. Several helminths are important pathogens of domestic animals and invade humans who ingest contaminated food. These helminths include *Taenia,* a tapeworm of cattle and pigs, and *Trichinella,* the roundworm of pigs that causes trichinosis. Several *Schistosoma* species are responsible for the chronic, debilitating, and sometimes fatal disease schistosomiasis(formerly known as *bilharzia*). Three species, *S. mansoni, S. japonicum,* and *S. haematobium,* are the major pathogens in humans, infecting individuals in Africa, the Middle East, South America, the Caribbean, China, Southeast Asia, and the Philippines.A rise in the incidence of schistosomiasis in recent years has paralleled the increasing worldwide use of irrigation, which has expanded the habitat of the freshwater snail that serves as the intermediate host for schistosomes. Infection occurs through contact with free-swimming infectious larvae, called cercariae, which are released from an infected snail at the rate of 300–3000 per day.When cercariae contact human skin, they secrete digestive enzymes that

help them to bore into the skin,where they shed their tails and are transformed into schistosomules. The schistosomules enter the capillaries and migrate to the lungs, then to the liver, and finally to the primary site of infection, which varies with the species. *S. mansoni* and *S. japonicum* infect the intestinalmesenteric veins; *S. haematobium* infects the veins of the urinary bladder. Once established in their final tissue site, schistosomules mature into male and female adult worms. The worms mate and the females produce at least 300 spiny eggsa day. Unlike protozoan parasites, schistosomes and otherhelminths do not multiply within their hosts. The eggs produced by the female worm do not mature into adult worms in humans; instead, some of them pass into the feces or urine and are excreted to infect more snails. The number of worms in an infected individual increases only through repeated exposure to the free-swimming cercariae, and so most infected individuals carry rather low numbers of worms. Most of the symptoms of schistosomiasis are initiated by the eggs. As many as half of the eggs produced remain in the host, where they invade the intestinal wall, liver, or bladder and cause hemorrhage. A chronic state can then develop in which the adult worms persist and the unexcreted eggs induce cell-mediated delayed-type hypersensitive reactions, resulting in large granulomas that are gradually walled off by fibrous tissue. Although the eggs are contained by the formation of the granuloma, often the granuloma itself obstructs the venous blood flow to the liver or bladder. Although an immune response does develop to the schistosomes, in most individuals it is not sufficient to eliminate the adult worms, even though the intravascular sites of schistosome infestation should make the worm an easy target forimmune attack. Instead, the worms survive for up to 20 years. The schistosomules would appear to be the forms most susceptible to attack, but because they are motile, they can evade the localized cellular buildup of immune and inflammatory cells. Adult schistosome worms also have several unique mechanisms that protect them from immune defenses. The adult worm has been shown to decrease the expression of antigens on its outer membrane and also to enclose itself in a glycolipid and glycoprotein coat derived from the host, masking the presence of its own antigens. Among the antigens observed on the adult worm are the host's own ABO blood-group antigens and histocompatibility antigens

The immune response is, of course, diminished by this covering made of the host's self-antigens, which must contribute to the lifelong persistence of these organisms. The relative importance of the humoral and cellmediatedresponses in protective immunity to schistosomiasis is controversial. The humoral response to infection with*S. mansoni* is characterized by high titers of antischistosome IgE antibodies, localized increases in mast cells and their subsequent degranulation, and increased numbers of eosinophils. These manifestations suggest that cytokines produced by a TH2-like subset are important for the immune response: IL-4, which induces B cells to classswitch to IgE production; IL-5, which induces bone-marrow precursors to differentiate into eosinophils; and IL-3, which (along with IL-4) stimulates growth of mast cells. Degranulation of mast cells releases mediators that increase the infiltration of such inflammatory cells as macrophages and eosinophils. The eosinophils express Fc receptors for IgE and IgG and bind to the antibody-coated parasite. Once bound to the parasite, an eosinophil can participate in antibodydependent cell-mediated cytotoxicity (ADCC), releasing mediators from its granules

that damage the parasite. One eosinophil mediator, called basic protein, is particularly toxic to helminths. Immunization studies with mice, however, suggest that this humoral IgE response may not provide protective immunity. When mice are immunized with *S. mansoni* vaccine, the protective immune response that develops is not an IgE response, but rather a TH1 response characterized by IFN-γ production and macrophage accumulation. Furthermore, inbred strains of mice with deficiencies in mast cells or IgE develop protective immunity from vaccination, whereas inbred strains with deficiencies in cellmediated CD4+ T-cell responses fail to develop protective immunity in response to the vaccine. These studies suggest that the CD4+ T-cell response may be the most important in immunity to schistosomiasis. It has been suggested that the ability to induce an ineffective TH2-like response may have evolved in schistosomes as a clever defense mechanism to ensure that TH2 cells produced sufficient levels of IL-10 to inhibit protective immunity mediated by the TH1-like subset in the CD4+ T response. Antigens present on the membrane of cercariae and young schistosomules are promising vaccine components because these stages appear to be most susceptible to immune attack. Injecting mice and rats with monoclonal antibodies to cercariae and young schistosomules passively transferred resistance to infection with live cercariae.When these protective antibodies were used in affinity columns to purify schistosome membrane antigens from crude membrane extracts, it was found that mice immunized and boosted with these purified antigens exhibited increased resistance to a later challenge with live cercariae. Schistosome cDNA libraries were then established and screened with the protective monoclonal antibodies to identify those cDNAs encoding surface antigens. Experiments using cloned cercariae or schistosomule antigens are presently under way to assess their ability to induce protective immunity in animal models. However, in developing an effective vaccine for schistosomiasis, a fine line separates a beneficial immune response, which at best limits the parasite load, from a detrimental response, which in itself may become pathologic.

Emerging Infectious Diseases

A cursory glance at the current offerings in your local bookstore or video rental store brings into focus the preoccupation of the public and the press with new infectious agents. Several times a year, it seems, we hear about a new virus or bacterium that arises in a particular location and causes severe illness or death in a population. Newly described pathogens are referred to as emerging pathogens. Some of the emerging pathogens that have been described since the early 1970s appear in Table 17-4. These new pathogens are thought to have emerged within the recent past. HIV is an example of a newly emerged pathogen. In other instances, diseases that were no longer causing widespread infection suddenly began to infect an ever-larger number of individuals. These are referred to as “re-emerging infectious diseases. There emergence of these diseases should not be surprising if we consider that bacteria can adapt to living in almost any environment. If they can adapt to living at the high temperatures of the thermal vents deep within the oceans, it is not difficult to accept that they can evolve to evade antimicrobial drugs. (An additional risk from intentionally disseminated diseases is discussed in the Clinical Focus.)

Tuberculosis is a well-known re-emerging disease. Fifteen years ago, public health officials were convinced that tuberculosis would soon disappear as a major health consideration in the United States.

Then, because of a number of events, including the AIDS epidemic, thousands of infected individuals developed TB strains resistant to the conventional battery of antibiotics. These individuals then passed on the newly emerged, antibiotic-resistant strains of *M. tuberculosis* to others. While the rate of infection with *M. tuberculosis* in the United States increased sharply during the early part of the 1990s, by 1995 the incidence had begun to decline again. However, the worldwide incidence of the disease is still increasing, and the World Health Organization predicts that, between 1998 and 2020, one billion more people will become infected and over 70 million will die from this disease if preventive measures are not adopted. Another re-emerging disease is diphtheria. This disease was almost non-existent throughout Europe in recent years because of vaccination; in 1994, however, scattered cases were reported in some of the republics of the former Soviet Union. By 1995, there were over 50,000 cases reported in the same region, and thousands died from diphtheria infection. The social upheaval and instability that came with the breakup of the Soviet Union was almost certainly a major factor in the re-emergence of this disease, because of the resultant lapses in public health measures—perhaps most important was the loss of immunization programs. Since 1995, immunization programs have been re-established and the trend has reversed, with only 13,687 cases of diphtheria reported in Russian republics in 1996, 6932 in 1998, and 1573 in 2000. Other diseases have appeared seemingly from nowhere and, as far as we know, are new pathogens. These include such pathogens as the widely publicized Ebola virus and *Legionella pneumophilia,* the bacterial causative agent for Legionnaires' disease. Ebola was first recognized after an outbreak in Africa, in 1976. By 1977, the virus that causes this disease had been isolated and classified as a filovirus, a type of RNAvirus that includes Marburg virus, a close relative of Ebola. Ebola causes a particularly severe haemorrhagic fever that kills more than 50% of those infected. Because of the severity of disease and the rapid progression to death after the initial appearance of symptoms, this virus has received a great deal of attention. However, while the risk of death is very high if you are infected with Ebola, it is fairly easy to control the spread of the virus. Through isolation of infected individuals, hospital workers and medical personnel can be protected. In such ways, the spread of Ebola virus has been contained during the two most recent outbreaks. Another emerging disease recently described is Legionnaires' disease, a virulent pneumonia first reported in 221 individuals who had attended an American Legion convention in Philadelphia in 1976. Of the 221 afflicted, 34 died from the infection. The organism causing the disease was not known, but further investigation led to the identification of a bacterium that was named *Legionella pneumophilia*. This bacterium proliferates in cool, damp areas and can be found in the condensing units of large commercial air-conditioning systems. The air-conditioning system can produce an aerosol that contains the bacteria, thus spreading the infection throughout the area served by the unit. This was determined to be the source of the bacteria at the 1976 convention in Philadelphia. Because the hazard of such aerosols is now recognized, improved design of air-

conditioning and plumbing systems has greatly reduced the incidence of the disease.

Experimental Models of Immunodeficiency Include Genetically Altered Animals

Immunologists use two well-studied animal models of primary immunodeficiency for a variety of experimental purposes. One of these is the athymic, or nude, mouse; the other is the severe combined immunodeficiency, or SCID, mouse. NUDE (ATHYMIC) MICE A genetic trait designated *nu,* which is controlled by a recessive gene on chromosome 11, was discovered in certain mice. Mice homozygous for this trait (*nu/nu*) are hairless and have a vestigial thymus. Heterozygotic, *nu/_,* litter mates have hair and a normal thymus. It is not known whether the hairlessness and the thymus defect are caused by the same gene. It is possible that two very closely linked genes control these defects, which, although unrelated, appear together in this mutant mouse. A gene that controls development may be involved, since the pathway that leads to the differential development of the thymus is related to the one that controls the skin epithelial cells. The *nu/nu* mouse cannot easily survive; under normal conditions, the mortality is 100% within 25 weeks and 50% die within the first two weeks after birth. Therefore, when these animals are to be used for experimental purposes, they must be maintained under conditions that protect them from infection. Precautions include use of sterilized food, water, cages, and bedding. The cages are protected from dust by placing them in a laminar flowrack or by the use of air filters fitted over the individual cages. Nude mice lack cell-mediated immune responses, and they are unable to make antibodies to most antigens. Immunodeficiency in the nude mouse can be reversed by athymic transplant. Because they can permanently tolerate both allografts and xenografts, they have a number of practical experimental uses. For example, hybridomas or solid tumors from any origin may be grown as ascites or a implanted tumors in a nude mouse. It is known that the nude mouse does not completely lack T cells; rather, it has a limited population that increases with age. The source of these T cells is not known; an intriguing possibility is that there is an extrathymic source of mature T cells. However, it is more likely that the T cells arisefrom the vestigial thymus. The majority of cells in the circulation of a nude mouse carry T-cell receptors of the type instead of the type that prevails in the circulation of a normal mouse.

THE SCID MOUSE

In 1983, Melvin and Gayle Bosma and their colleagues described an autosomal recessive mutation in mice that gave rise to a severe deficiency in mature lymphocytes. They designated the trait SCID because of its similarity to human severe combined immunodeficiency. The SCID mouse was shown to have early B- and T-lineage cells, but there was a virtual absence of lymphoid cells in the thymus, spleen, lymph nodes, and gut tissue, the usual locations of functional T and B cells. The precursor T and B cells in the SCID mouse appeared to be unable to differentiate into mature functional B and T lymphocytes. Inbred mouse lines carrying the SCID defect have been derived and studied in great detail. The SCID mouse can neither make antibody nor carry out delayed-type hypersensitivity (DTH) or graft-rejection reactions. If the

animals are not kept in an extremely clean environment, they succumb to infection early in life. Cells other than lymphocytes develop normally in the SCID mouse; red blood cells, monocytes, and granulocytes are present and functional. SCID mice may be rendered immunologically competent by transplantation of stem cells from normal mice. The mutation in a DNA protein kinase that causes mouse SCID is a so-called "leaky" mutation, because a certain number of SCID mice do produce immunoglobulin. About half of these leaky SCID mice can also reject skin allografts. This finding suggests that the defective enzyme can function partly in T- and B-cell development, allowing normal differentiation of a small percentage of precursor cells. More recently, immunodeficient SCID-like mice have been developed by deletion of the recombination-activating enzymes (RAG-1 and RAG-2) responsible for the rearrangement of immunoglobulin or T-cell–receptor genes in both B- and T cell precursors (RAG knockout mice). This gives rise to a defect in both B and T cells of the mouse; neither can rearrange the genes for their receptor and thus neither proceeds along a normal developmental path. Because cells with abnormal rearrangements are eliminated in vivo, both B and T cells are absent from the lymphoid organs of the RAG knockout mouse. In addition to providing a window into possible causes of combined T- and B-cell immunodeficiency, the SCID mouse has proven extremely useful in studies of cellular immunology. Because its rejection mechanisms do not operate, the SCID mouse can be used for studies on cells or organs from various sources. For example, immune precursor cells from human sources may be used to reestablish the SCID mouse's immune system. These human cells can develop in a normal fashion and, as a result, the SCID mouse circulation will contain immunoglobulin of human origin. In one important application, these SCID mice are infected with HIV-1. Although normal mice are not susceptible to HIV-1 infection, the SCID mouse reconstituted with human lymphoid tissue (SCID-Hu mouse) provides an animal model in which to test therapeutic or prophylactic strategies against HIV infection of the transplanted human lymphoid tissue

Autoimmunity Can Develop Spontaneously in Animals

A number of autoimmune diseases that develop spontaneously in animals exhibit important clinical and pathologic similarities to certain autoimmune diseases in humans. Certain inbred

mouse strains have been particularly valuable models for illuminating the immunologic defects involved in the development of autoimmunity. New Zealand Black (NZB) mice and F1 hybrids of NZB and New Zealand White (NZW) mice spontaneously develop autoimmune diseases that closely resemble systemic lupus erythematosus. NZB mice spontaneously develop autoimmune hemolytic anemia between 2 and 4 months of age, at which time various auto-antibodies can be detected, including antibodies to erythrocytes, nuclear proteins, DNA, and T lymphocytes. F1 hybrid animals develop glomerulonephritis from immune-complex deposits in the kidney and die prematurely by 18 months. As in human SLE, the incidence of autoimmunity in the (NZB _NZW)F1 hybrids is greater in females. An accelerated and severe form of systemic autoimmune disease resembling systemic lupus erythematosus develops in a mouse strain called MRL/*lpr/lpr.* These mice are homozygous for a gene called *lpr,* which has been identified as a defective *fas*

gene. The *fas*-gene product is a cell-surface protein belonging to the TNF family of cysteine-rich membrane receptors. When the normal Fas protein interacts with its ligand, it transduces a signal that leads to apoptotic death of the Fas-bearing cells. This mechanism may operate in destruction of target cells by some CTLs (see Figure 14-9). Fas is known also to be essential in the death of hyperactivated peripheral CD4+ cells. Normally, when mature peripheral T cells become activated, they are induced to express both Fas antigen and Fas ligand, When Fas-bearing cells come into contact with a neighboring activated cell bearing Fas ligand, the Fas-bearing cell is induced to die. It is also possible that Fas ligand can engage Fas from the same cell, inducing a cellular suicide. In the absence of Fas, mature peripheral T cells do not die, and these activated cells continue to proliferate and produce cytokines that result in grossly enlarged lymph nodes and spleen. Defects in *fas* expression similar to that found in the *lpr* mouse are observed in humans, and these can have severe consequences. However there is no link between *fas* expression and SLE in humans, which suggests that the *lpr* mouse may not be a true model for SLE. Another important animal model is the non obese diabetic (NOD) mouse, which spontaneously develops a form of diabetes that resembles human insulin-dependent diabetes mellitus (IDDM). Like the human disease, the NOD mouse disease begins with lymphocytic infiltration into the islets of the pancreas. Also, as in IDDM, there is a strong association between certain MHC alleles and the development of diabetes in these mice. Experiments have shown that T cells from diabetic mice can transfer diabetes to non diabetic recipients. For example, when the immune system of normal mice is destroyed by lethal doses of x-rays and then is reconstituted with an injection of bone-marrow cells from NOD mice, the reconstituted mice develop diabetes. Conversely, when the immune system of still healthy NOD mice is destroyed by x-irradiation and then reconstituted with normal bone-marrow cells, the NOD mice do not develop diabetes. Various studies have demonstrated a pivotal role for CD4+ T cells in the NOD mouse, and recent evidence implicates the TH1 subset in disease development. Several other spontaneous autoimmune diseases have been discovered in animals that have served as models for similar human diseases. Among these are *Obese*-strain chickens, which develop both humoral and cell-mediated reactivity to thymoglobulin resembling that seen in Hashimoto's thyroiditis.

Autoimmunity Can Be Induced Experimentally in Animals

Autoimmune dysfunctions similar to certain human autoimmune diseases can be induced experimentally in some animals. One of the first such animal models was discovered serendipitously in 1973 when rabbits were immunized with acetylcholine receptors purified from electric eels. The animals soon developed muscular weakness similar to that seen in myasthenia gravis. This experimental autoimmune myasthenia gravis (EAMG) was shown to result when antibodies to the acetylcholine receptor blocked muscle stimulation by acetylcholine in the synapse. Within a year, this animal model had proved its value with the discovery that auto-antibodies to the acetylcholine receptor were the cause of myasthenia gravis in humans. Experimental autoimmune encephalomyelitis (EAE) is another animal model that has greatly improved understanding of autoimmunity. This is one of the best-studied models of autoimmune

disease. EAE is mediated solely by T cells and can be induced in a variety of species by immunization with myelin basic protein (MBP) or proteolipid protein (PLP) in complete Freund's adjuvant (Figure 20-7). Within 2–3 weeks the animals develop cellular infiltration of the myelin sheaths of the central nervous system, resulting in demyelization and paralysis. Most of the animals die, but others have milder symptoms, and some animals develop a chronic form of the disease that resembles chronic relapsing and remitting MS in humans. Those that recover are resistant to the development of disease from a subsequent injection of MBP and adjuvant. The mouse EAE model provides a system for testing treatments for human MS. For example, because MBP- or PLP-specific T-cell clones are found in the periphery, it is assumed that these clones must have escaped negative selection in the thymus. Recent mouse experiments have suggested that orally administered MBP may make these antigen-specific peripheral T-cell clones self-tolerant. These studies have paved the way for clinical trials in MS patients. Experimental autoimmune thyroiditis (EAT) can be induced in a number of animals by immunizing with thymoglobulin in complete Freund's adjuvant. Both humoral antibodies and TH1 cells directed against the thymoglobulin develop, resulting in thyroid inflammation. EAT appears to best mimic Hashimoto's thyroiditis. In contrast to both EAE and EAT, which are induced by immunizing with self-antigens, autoimmune arthritis (AA) is induced by immunizing rats with *Mycobacterium tuberculosis* in complete Freund's adjuvant. These animals develop an arthritis whose features are similar to those of rheumatoid arthritis in humans.

Cell-Culture Systems

The complexity of the cellular interactions that generate an immune response has led immunologists to rely heavily on various types of in vitro cell-culture systems. A variety of cells can be cultured, including primary lymphoid cells, cloned lymphoid cell lines, and hybrid cells.

Primary Lymphoid Cell Cultures

Primary lymphoid cell cultures can be obtained by isolating lymphocytes directly from blood or lymph or from various then be grown in a chemically defined basal medium (containing saline, sugars, amino acids, vitamins, trace elements, and other nutrients) to which various serum supplements are added. For some experiments, serum-free culture conditions are employed. Because in vitro culture techniques require from 10- to 100-fold fewer lymphocytes than do typical in vivo techniques, they have enabled immunologists to assess the functional properties of minor subpopulations of lymphocytes. It was by means of cell-culture techniques, for example, that immunologists were first able to define the functional differences between CD4+ Thelper cells and CD8+ T cytotoxic cells. Cell-culture techniques have also been used to identify numerous cytokines involved in the activation, growth, and differentiation of various cells involved in the immune response. Early experiments showed that media conditioned, or modified, by the growth of various lymphocytes or antigen presenting cells would support the growth of other lymphoid cells. Conditioned media contain the secreted products from actively

growing cells. Many of the individual cytokines that characterized various conditioned media have subsequently been identified and purified, and in many cases the genes that encode them have been cloned. These cytokines, which play a central role in the activation and regulation of the immune response.

Cloned Lymphoid Cell Lines

A primary lymphoid cell culture comprises a heterogeneous group of cells that can be propagated only for a limited time. This heterogeneity can complicate the interpretation of experimental results. To avoid these problems, immunologists use cloned lymphoid cell lines and hybrid cells. Normal mammalian cells generally have a finite life span in culture; that is, after a number of population doublings characteristic of the species and cell type, the cells stop dividing. In contrast, tumor cells or normal cells that have undergone transformation induced by chemical carcinogens or viruses can be propagated indefinitely in tissue culture; thus, they are said to be immortal. Such cells are referred to as cell lines. The first cell line—the mouse fibroblast L cell—was derived in the 1940s from cultured mouse subcutaneous connective tissue by exposing the cultured cells to a chemical carcinogen, methylcholanthrene, over a 4-month period. In the 1950s, another important cell line, the HeLa cell, was derived by culturing human cervical cancer cells. Since these early studies, hundreds of cell lines have been established, each consisting of a population of genetically identical (syngeneic) cells that can be grown indefinitely in culture. (SV40), Epstein-Barr virus (EBV), or human T-cell leukemia virus type 1(HTLV-1). Lymphoid cell lines differ from primary lymphoid cell cultures in several important ways: They survive indefinitely in tissue culture, show various abnormal growth properties, and often have an abnormal number of chromosomes. Cells with more or less than the normal diploid number of chromosomes for a species are said to be aneuploid. The big advantage of cloned lymphoid cell lines is that they can be grown for extended periods in tissue culture, enabling immunologists to obtain large numbers of homogeneous cells in culture. Until the late 1970s, immunologists did not succeed in maintaining normal T cells in tissue culture for extended periods. In 1978, a serendipitous finding led to the observation that conditioned medium containing a T-cell growth factor was required. The essential component of the conditioned medium turned out to be interleukin 2 (IL-2). By culturing normal T lymphocytes with antigen in the presence of IL-2, clones of antigen-specific T lymphocytes could be isolated. These individual clones could be propagated and studied in culture and even frozen for storage. After thawing, the clones continued to grow and express their original antigen specific functions. Development of cloned lymphoid cell lines has enabled immunologists to study a number of events that previously could not be examined. For example, research on the molecular events involved in activation of naive lymphocytes by antigen was hampered by the low frequency of naive B and T cells specific for a particular antigen; in a heterogeneous population of lymphocytes, the molecular changes occurring in one responding cell could not be detected

against a background of 103–106 non responding cells. Cloned T- and B-cell lines with known antigenic specificity have provided immunologists with large homogeneous cell populations in which to study the events involved in antigen recognition. Similarly, the genetic changes corresponding to different maturational stages can be studied in cell lines that appear to be "frozen" at different stages of differentiation. Cell lines have also been useful in studying the soluble factors produced by lymphoid cells. Some cell lines secrete large quantities of various cytokines; other lines express membrane receptors for particular cytokines. These cell lines have been used by immunologists to purify various cytokines and their receptors and eventually to clone their genes. With the advantages of lymphoid cell lines come a number of limitations. Variants arise spontaneously in the course of prolonged culture, necessitating frequent sub cloning to limit the cellular heterogeneity that can develop. If variants are selected in sub cloning, it is possible that two sub clones derived from the same parent clone may represent different sub populations. Moreover, any cell line derived from tumor cells or transformed cells may have unknown genetic contributions characteristic of the tumor or of the transformed state; thus, researchers must be cautious when extrapolating results obtained with cell lines to the normal situation in vivo. Nevertheless, transformed cell lines have made a major contribution to the study of the immune response, and many molecular events discovered in experiments with transformed cell lines have been shown to take place in normal lymphocytes.

Cell line	Description
L-929	Mouse fibroblast cell line; often used in DNA transfection studies and to assay tumor necrosis factor (TNF)
SP2/0	Nonsecreting mouse myeloma; often used as a fusion partner for hybridoma secretion
P3X63-Ag8.653	Nonsecreting mouse myeloma; often used as a fusion partner for hybridoma secretion
MPC 11	Mouse IgG2b-secreting myeloma
P3X63-Ag8	Mouse IgG1-secreting myeloma
MOPC 315	Mouse IgA-secreting myeloma
J558	Mouse IgA-secreting myeloma
7OZ/3	Mouse pre–B-cell lymphoma; used to study early events in B-cell differentiation
BCL 1	Mouse B-cell leukemia lymphoma that expresses membrane IgM and IgD and can be activated with mitogen to secrete IgM
CTLL-2	Mouse T-cell line whose growth is dependent on IL-2; often used to assay IL-2 production
Jurkat	Human T-cell leukemia that secretes IL-2
DO11.10	Mouse T-cell hybridoma with specificity for ovalbumin
PU 5-1.8	Mouse monocyte-macrophage line
P338 D1	Mouse monocyte-macrophage line that secretes high levels of IL-1
WEHI 265.1	Mouse monocyte line
P815	Mouse mastocytoma cells; often used as target to assess killing by cytotoxic T lymphocytes (CTLs)
YAC-1	Mouse lymphoma cells; often used as target for NK cells
HL-60	Human myeloid-leukemia cell line
COS-1	African green monkey kidney cells transformed by SV40; often used in DNA transfection studies

Hybrid Lymphoid Cell Lines

In somatic-cell hybridization, immunologists fuse normal B or T lymphocytes with tumor cells, obtaining hybrid cells, or DNA transfection studies heterokaryons, containing nuclei from both parent cells. Random loss of some chromosomes and subsequent cell proliferation yield a clone

of cells that contain a single nucleus with chromosomes from each of the fused cells; such a clone is called a hybridoma. Historically, cell fusion was promoted with Sendai virus, but now it is generally done with polyethylene glycol.Normal antigen-primed B cells can be fused with cancerous plasma cells, called myeloma cells. The hybridoma thus formed continues to express the antibody genes of thenormal B lymphocyte but is capable of unlimited growth, acharacteristic of the myeloma cell. B-cell hybridomas that secrete antibody with a single antigenic specificity, called monoclonal antibody, in reference to its derivation from a single clone, have revolutionized not only immunology but biomedicial research as well as the clinical laboratory. T-cell hybridomas can also be obtained by fusing T lymphocytes with cancerous T-cell lymphomas. Again, the resulting hybridoma continues to express the genes of the normal T cell but acquires the immortal-growth properties of the cancerous T lymphoma cell. Immunologists have generated a number of stable hybridoma cell lines representing T-helper and T-cytotoxic lineages.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: II M.Sc MB COURSE NAME: ADVANCED IMMUNOLOGY

COURSE CODE: 18MBP301 UNIT: III BATCH-2018-2020

Sl. No	Question	Option A	Option B	Option C	Option D	Correct Ans
1	On which chromosome HLA are located__	16	17	6	22	6
2	In humans the class I MHC region is about 2000kh long and it has __genes	36 genes	20 genes	45 genes	25 genes	20 genes
3	MHC - II has the transactivator called CHTA and the transcription factor called __	RFX	CHTA	Cytokines	Promotor	RFX
4	Activated T_M cell secretes _____ called as B cell stimulatory factor (BSF) which activates the B cell	lymphokine	Monokine	cytokine	Histamin	Histamin
5	B cell is triggered by the bound antigen or _____ secreted by the T_H cell	Lymphokine	Monokine	cytokine	Histamine	Histamine
6	Proliferating B cells finally produce 2 types of cells namely ____and ____	null cell & naive cell	NK cell & T cell	Memory & plasma cell	Basophil	Memory & plasma cell
7	T_H cell attaches itself to the processed antigen present on the surface of _____	Macrophage	monocyte	Mast cell	Basophil	Macrophage
8	Septic shock is the toxin manifestation of the release of large amount of ___	IL-2	Transforming growth factor b	IL-8	TNF - a	TNF - a
9	Key cytokines produced by Th_1 cells are ___	IL-2 and TFN a	IL-4 and IL-10	IL-2 and TFN a	Il-2 and TNF -2	IL-2 and TFN a
10	Major role of IL - 4 is to produce	CMI	IgE response	Enhance IgE production	suppress cytokine production	IgE response
11	Which can generate lymphokine – activated killer cells from peripheral blood mononuclear cells?	IL-5	IL-10	IL-4	IL-15	IL-4
12	Which types of cells are more susceptible to lymphotoxin killing?	Normal cells	Malignant transformed cells	T cells	platelets	Malignant transformed cells
13	Septic shock is the toxin manifestation of the	IL-2	Transforming	IL-8	TNF - a	TNF - a

	release of large amount of ___		growth factor b			
14	Key cytokines produced by Th_1 cells are ___	IL-2 and TFN a	IL-4 and IL-10	IL-2 and TFN a	Il-2 and TNF -2	IL-2 and TFN a
15	Major role of IL - 4 is to produce	CMI	IgE response	Enhance IgE production	suppress cytokine production	IgE response
16	Sabin Polio vaccine is an example of	Live virus vaccine	Killed vaccine	Subunit vaccine	Recombinant vaccine	Live virus vaccine
17	Cholera vaccine is an example of	attenuated vaccine	Subunit vaccine	DNA vaccine	Recombinant vaccine	attenuated vaccine
18	The first recombinant antigen vaccine developed is	hepatitis A	Hepatitis B	Hepatitis C	Hepatitis D	Hepatitis B
19	BCG is used as a vaccine against	Cholerae	foot and mouth disease	Tuberculosis	Typhoid	Tuberculosis
20	The immunoglobulin which acts as a surface receptor on B cells	IgG	IgD	IgA	IgE	IgD
21	The constant domain of IgM which binds to C, complement is __	CM	CM_2	CM_s	CM_4	CM_s
22	The Ig molecule composed of 5 monomer is	IgG	IgM	IgA	IgD	IgM
23	Which is major site of synthesis of complement protein	Liver	kidney	Bonemarrow	Lymphnode	Liver
24	How many major complement components present in complement system	9	6	8	10	9
25	Who coined the term complement?	Paul Ehrlich	Jules Bordet	Pasteur	Edward Jenner	Paul Ehrlich
26	Factor B when bound to C3b is susceptible to enzymatic cleavage by ___	Factor D	Properdine	Factor H	factor B	Factor D
27	Which protein is stabilizing the C_{3bBb} complex in alternative pathway	Properidine	factor D	Factor B	CVF	Properidine
28	The constant domain of IgM which binds to C, complement is __	CM	CM_2	CM_s	CM_4	CM_s

29	The Ig molecule composed of 5 monomer is	IgG	IgM	IgA	IgD	IgM
30	Which is major site of synthesis of complement protein	Liver	kidney	Bonemarrow	Lymphnode	Liver
31	Compound present in poison oak that react with T_H cells is type IV hypersensitivity reaction is __	radicals	pentadecatechol	Toxins	Tetra catechol	pentadecatechol
32	Tubercle protein is a __	Lipid	lipoprotein	protein	Glycoprotein	lipoprotein
33	Pigeon fancier's disease is a	Generalized type III	Localized type III	Delayed type reaction	Localized type II	Localized type III
34	Arthus reaction results from local	mast cell degranulation	Cytokine release	IgE production	Complement activation	Complement activation
35	Immune complexes cause hypersensitivity by stimulating	IgG production	T cells	Neutrophil invasion	Eosinophil invasion	Neutrophil invasion
36	The interferon – Gamma –Reciptor defieiency is found in patients suffering from infection with	Corynbacterium	Streptococci	Mycobacterium	Shigella	Mycobacterium
37	Primary immuno deficiency effect the	Adaptive immune function	Innate immune function	Either adaptive or Innate immunity	Neither adaptive nor innate immune function	Either adaptive or Innate immunity
38	Bruton's hypogammaglobulinemia is indicative of a deficiency of	B cell	Macrophage	T cell	Monocyte	B cell
39	Autoimmune diseases occur as a result of the development of _____that had previously been suppressed by the normal control mechanisms of the body	Null cells	NK cells	Monocytes	T cells or B cells	T cells or B cells
40	X- linked agammaglobulinemia is characterized by	Low IgG levels	Low IgM levels	Low IgA levels	Low IgE levels	Low IgG levels
41	Chronic granulomatous disease (CGE) is a	Hypocomplementemia	A defect in T	A defect in T cell	A defect in	A defect in

	result of which immunodeficiency conditions		cell number	function	neutrophil function	neutrophil function
42	Cross reactivity between S pyogens and myosin present in cardiae muscles is throught to be responsible for the ___damage observed in acute rheumatic fever that follows a streptococcal infection	Cardiac damage	Muscular damage	Neuronal damage	Respiratory damage	Cardiac damage
43	Molecular mimicry result in ___	Cross reactivity	Diffusion	Precipitation	agglutination	Cross reactivity
44	_____is a disease due to cross reactivity	Addison's disease	Diarrbborea	AIDS	Nephritis	Addison's disease
45	_____ acid protects vagina against invasion	Lactic acid	Acetic acid	Formic acid	Nitric acid	Lactic acid
46	____ is an example of for nonimmunological defense mechanism	IgE	T cells	recine	Skin	Skin
47	____ is an antibacterial and antiviral enzyme synthesized in gastric mucosa	lactoferin	lactinin	lysozyme	Transferin	lysozyme
48	____ antibacterial substance in milk	Transferin	lactiferin	Lactinin	Lysozyme	lactiferin
49	Inflammation is the body's reaction to invasion by an infections agent, antigen challenge or even just physical damage	Vasodialation	Inflammation	Opsonization	Complementation	Inflammation
50	____and S- by droxytryptamine which produce vasodialation and increased vascular permeability	Histamine	Bradykinin	C_{5a}	IL-8	Histamine
51	____ sensitizes the cells for antigen specific triggering of granule release	IgA	IgM	IgE	IgG	IgE
52	____ mechanism involves complex sequences of events that occur collectively at the site of infection and tissue injury the microbes	Artificial immune	Innate immune	Natural Immune	Acquired immune	Innate immune
53	_____system generates the mediators bradykinin and lysyl – bradykinin or kallidin	Kinin	Clotting	Complement	Tibrinolytic	Kinin
54	HIV evade the host immune mechanism	High resistant power	Hide from	Antigenic drift	Antigenic rejectio	Antigenic drift

	mainly due to ____		responses			
55	Which of the following is a chemokine?	IL-5	IL-8	IL-3	IL-7	IL-8
56	The most sensitve technique for detecting antigen or antibody is	Immunofertitin test	Radioimmuno assay	Immunochromatography	Electroimmunoblot	Radioimmuno assay
57	Radio immuno assay was developed by	Rosalyn Yalow and S.A. Berson	S.A Berson and Joseph Yalow	Jules Bordet and Rosalyn yalow	Peter Medawar and macfarlane bernet	Rosalyn Yalow and S.A. Berson
58	The antigen- antibody reaction between insoluble antigen and its antibody is called	Agglutination	Precipitation	Complement fixation	Immunoflurocens	Agglutination
59	Treatment for myasthemia gravis ____	Removal of thymus	Removal of spleen	Removal of Bonemarrow	Removal of liver	Removal of thymus
60	Autoantibodies with nuclear antigens deposited on the wall of blood vessels cause type III hypersensitivity and the disease is called ___	Multiple sclerosis	Scleroderma	Systemic lupus erythematous	Lymhadenopathy	Systemic lupus erythematous

UNIT – IV

HYPERSENSITIVE REACTIONS

Under certain circumstances, however, this inflammatory response can have deleterious effects, resulting in significant tissue damage or even death. This inappropriate immune response is termed **hypersensitivity** or **allergy.** Although the word *hypersensitivity* implies an increased response, the response is not always heightened but may, instead, be an inappropriate immune response to an antigen. Hypersensitive reactions may develop in the course of either humoral or cell-mediated responses.

The ability of the immune system to respond inappropriately to antigenic challenge was recognized early in this century. Two French scientists, Paul Portier and Charles Richet, investigated the problem of bathers in the Mediterranean reacting violently to the stings of Portuguese Man of War jellyfish. Portier and Richet concluded that the localized reaction of the bathers was the result of toxins. To counteract this reaction, the scientists experimented with the use of isolated jellyfish toxins as vaccines. Their first attempts met with disastrous results. Portier and Richet injected dogs with the purified toxins, followed later by a booster of toxins.Instead of reacting to the booster by producing antibodies against the toxins, the dogs immediately reacted with vomiting, diarrhea, asphyxia, and, in some instances, death. Clearly this was an instance where the animals "overreacted" to the antigen. Portier and Richet coined the term *anaphylaxis,* loosely translated from Greek to mean the opposite of *prophylaxis,* to describe this overreaction. Richet was subsequently awarded the Nobel Prize in Physiology or Medicine in 1913 for his work on anaphylaxis.

Gell and Coombs Classification

Several forms of hypersensitive reaction can be distinguished, reflecting differences in the effector molecules generated in the course of the reaction. In immediate hypersensitive reactions, different antibody isotypes induce different immune effector molecules. IgE antibodies, for example, induce mast-cell degranulation with release of histamine and other biologically active molecules. IgG and IgM antibodies, on the other hand, induce hypersensitive reactions by activating complement.

Three types of hypersensitivity occur within the humoral branch and are mediated by antibody or antigen-antibody complexes: IgE-mediated (type I), antibody-mediated (type II), and immune complex–mediated (type III). A fourth type of hypersensitivity depends on reactions within the cell-mediated branch, and is termed delayed-type hypersensitivity, or DTH (type IV). Each type involves distinct mechanisms, cells, and mediator molecules.

IgE-Mediated (Type I) Hypersensitivity

A type I hypersensitive reaction is induced by certain types of antigens referred to as **allergens,** and has all the hallmarks of a normal humoral response. That is, an allergen induces a humoral antibody response. What distinguishes a type I hypersensitive response from a normal humoral response is that the plasma. cells secrete IgE. This class of antibody binds with high affinity to **Fc receptors** on the surface of tissue mast cells and blood basophils. Mast cells and basophils coated by IgE are said to be sensitized. A later exposure to the same allergen cross-links the membrane-bound IgE on sensitized mast cells and basophils, causing **degranulation** of

these cells.

There Are Several Components of Type I Reactions

Several components are critical to development of type I hypersensitive reactions:

Allergens

The majority of humans mount significant IgE responses only as a defense against parasitic infections. After an individual has been exposed to a parasite, serum IgE levels increase and remain high until the parasite is successfully cleared from the body. Some persons, however, may have an abnormality called atopy, a hereditary predisposition to the development of immediate hypersensitivity reactions against common environmental antigens. The IgE regulatory defects suffered by atopic individuals allow nonparasitic antigens to stimulate inappropriate IgE production, leading to tissuedamaging type I hypersensitivity. The term *allergen* refers specifically to nonparasitic antigens capable of stimulating type I hypersensitive responses in allergic individuals.

Reaginic Antibody (IGE)

The existence of a human serum factor that reacts with allergens was first demonstrated by K. Prausnitz and H. Kustner in 1921. The local wheal and flare response that occurs when an allergen is injected into a sensitized individual is called the P-K reaction. Because the serum components responsible for the P-K reaction displayed specificity for allergen, they were assumed to be antibodies, but the nature of these P-K antibodies, or reagins, was not demonstrated for many years.

Mast Cells And Basophils

The cells that bind IgE were identified by incubating human leukocytes and tissue cells with either 125I-labeled IgE myeloma protein or 125I-labeled anti-IgE. In both cases, autoradiography revealed that the labeled probe bound with high affinity to blood basophils and tissue mast cells. Basophils are granulocytes that circulate in the blood of most vertebrates; in humans, they account for 0.5%–1.0% of the circulating white blood cells. Their granulated cytoplasm stains with basic dyes, hence the name basophil. Electron microscopy reveals a multilobed nucleus, few mitochondria, numerous glycogen granules, and electron-dense membrane-bound granules scattered throughout the cytoplasm that contain pharmacologically active mediators. Mast cell populations in different anatomic sites differ significantly in the types and amounts of allergic mediators they contain and in their sensitivity to activating stimuli and cytokines. Mast cells also secrete a large variety of cytokines that affect a broad spectrum of physiologic, immunologic, and pathologic processes.

IgE-Binding Fc Receptors

The reaginic activity of IgE depends on its ability to bind to a receptor specific for the Fc region of the _ heavy chain. Two classes of Fc_R been identified, designated Fc_RI and Fc_RII, which are expressed by different cell types and differ by 1000- fold in their affinity for IgE.

Antibody-Mediated Cytotoxic (Type II) Hypersensitivity

Type II hypersensitive reactions involve antibody-mediated destruction of cells. Antibody can activate the complement system, creating pores in the membrane of a foreign cell or it can mediate cell destruction by antibodydependent cell-mediated cytotoxicity (ADCC). In this process, cytotoxic cells with Fc receptors bind to the Fc region of antibodies on target cells and promote killing of the cells. Antibody bound to a foreign cell also can serve as an opsonin, enabling phagocytic cells with Fc or C3b receptors to bind and phagocytose the antibody-coated cell.

Transfusion Reactions Are Type II Reactions

A large number of proteins and glycoproteins on the membrane of red blood cells are encoded by different genes, each of which has a number of alternative alleles. An individual possessing one allelic form of a blood-group antigen can recognize other allelic forms on transfused blood as foreign and mount an antibody response. In some cases, the antibodies have already been induced by natural exposure to similar antigenic determinants on a variety of microorganisms present in the normal flora of the gut. This is the case with the ABO blood-group antigens. Antibodies to the A, B, and O antigens, called isohemagglutinins,are usually of the IgM class. An individual with blood type A, for example, recognizes B-like epitopes on intestinal microorganisms and produces isohemagglutinins to the B-like epitopes. This same individual does not respond to A-like epitopes on the same intestinal microorganisms because these A-like epitopes are too similar to self and a state of self-tolerance to these epitopes should exist. If a type A individual is transfused with blood containing type B cells, a **transfusion reaction** occurs in which the anti-B isohemagglutinins bind to the B blood cells and mediate their destruction by means of complement-mediated lysis. Antibodies to other blood-group antigens may result from repeated blood transfusions because minor allelic differences in these antigens can stimulate antibody production. These antibodies are usually of the IgG class.

Hemolytic Disease of the Newborn Is Caused by Type II Reactions

Hemolytic disease of the newborn develops when maternal IgG antibodies specific for fetal blood-group antigens cross the placenta and destroy fetal red blood cells. The consequences of such transfer can be minor, serious, or lethal. Severe hemolytic disease of the newborn, called **erythroblastosis fetalis,** most commonly develops when an Rh+ fetus expresses an **Rh antigen** on its blood cells that the Rh– mother does not express.

Immune Complex–Mediated (Type III) Hypersensitivity

The reaction of antibody with antigen generates immune complexes. Generally this complexing of antigen with antibody facilitates the clearance of antigen by phagocytic cells. In some cases, however, large amounts of immune complexes can lead to tissue-damaging type III hypersensitive reactions. The magnitude of the reaction depends on the quantity of immune complexes as well as their distribution within the body.When the complexes are deposited in tissue very near the site of antigen entry, a localized reaction develops.When the complexes are formed in the blood, a reaction can develop wherever the complexes are deposited.

Type III hypersensitive reactions develop when immune complexes activate the

complement system's array of immune effector molecules. Much of the tissue damage in type III reactions stems from release of lytic enzymes by neutrophils as they attempt to phagocytose immune complexes. The C3b complement component acts as an opsonin, coating immune complexes. A neutrophil binds to a C3b-coated immune complex by means of the type I complement receptor, which is specific for C3b. Because the complex is deposited on the basementmembrane surface, phagocytosis is impeded, so that lytic enzymes are released during the unsuccessful attempts of the neutrophil to ingest the adhering immune complex. Further activation of the membrane-attack mechanism of the complement system can also contribute to the destruction of tissue.In addition, the activation of complement can induce aggregation of platelets, and the resulting release of clotting factors can lead to formation of microthrombi.

Type IV or Delayed-Type Hypersensitivity (DTH)

When some subpopulations of activated TH cells encounter certain types of antigens, they secrete cytokines that induce a localized inflammatory reaction called delayed-type hypersensitivity (DTH). The reaction is characterized by large influxes of nonspecific inflammatory cells, in particular,macrophages.This type of reaction was first described in 1890 by Robert Koch, who observed that individuals infected with *Mycobacterium tuberculosis* developed a localized inflammatory response when injected intradermally with a filtrate derived from a mycobacterial culture.He called this localized skin reaction a "tuberculin reaction." Later, as it became apparent that a variety of other antigens could induce this response, its name was changed to delayed-type or type IV hypersensitivity in reference to the delayed onset of the reaction and to the tissue damage (hypersensitivity) that is often associated with it. The term *hypersensitivity* is somewhat misleading, for it suggests that a DTH response isalways detrimental. Although in some cases a DTH response does cause extensive tissue damage and is in itself pathologic, in many cases tissue damage is limited, and the response plays an important role in defense against intracellular pathogens and contact antigens. The hallmarks of a type IV reaction are the delay in time required for the reaction to developand the recruitment of macrophages as opposed to neutrophils, as found in a type III reaction.Macrophages are the major component of the infiltrate that surrounds the site of inflammation.

COMPLEMENT SYSTEM

The complement system is the major effector of the humoral branch of the immune system.

The Functions of Complement

The biological activities of the complement system affect both innate and acquired immunity and reach far beyond the original observations of antibody mediated lysis of bacteria and red blood cells. Structural comparisons of the proteins involved in complement pathways place the origin of this system in primitive organisms possessing the most rudimentary innate immune systems. By contrast, the realization that interaction of cellular receptors with complement proteins controls B-cell activities gives this system a role in the highly developed acquired immune system.

After initial activation, the various complement components interact, in a highly

regulated cascade, to carry out a number of basic functions including:

- Lysis of cells, bacteria, and viruses
- Opsonization, which promotes phagocytosis of particulate antigens
- Binding to specific complement receptors on cells of the immune system, triggering specific cell functions, inflammation, and secretion of immunoregulatory molecules
- Immune clearance, which removes immune complexes from the circulation and deposits them in the spleen and liver

The Complement Components

The proteins and glycoproteins that compose the complement system are synthesized mainly by liver hepatocytes, although significant amounts are also produced by blood monocytes, tissue macrophages, and epithelial cells of the gastrointestinal and genitourinary tracts. These components constitute 5% (by weight) of the serum globulin fraction. Most circulate in the serum in functionally inactive forms as proenzymes, or *zymogens,* which are inactive until proteolytic cleavage, which removes an inhibitory fragment and exposes the active site. The complement-reaction sequence starts with an enzyme cascade. Complement components are designated by numerals (C1–C9), by letter symbols (e.g., factor D), or by trivial names (e.g., homologous restriction factor). Peptide fragments formed by activation of a component are denoted by small letters. In most cases, the smaller fragment resulting from cleavage of a component is designated "a" and the larger fragment designated "b" (e.g., C3a, C3b; note that C2 is an exception: C2a is the larger cleavage fragment). The larger fragments bind to the target near the site of activation, and the smaller fragments diffuse from the site and can initiate localized inflammatory responses by binding to specific receptors. The complement fragments interact with one another to form functional complexes. Those complexes that have enzymatic activity are designated by a bar over the number or symbol (e.g., C4b2a, C3bBb).

Complement Activation

The early steps, culminating in formation of C5b, can occur by the classical pathway, the alternative pathway, or the lectin pathway. The final steps that lead to a membrane attack are the same in all pathways.

The Classical Pathway Begins with Antigen-Antibody Binding

Complement activation by the classical pathway commonly begins with the formation of soluble antigen-antibody complexes (immune complexes) or with the binding of antibody to antigen on a suitable target, such as a bacterial cell. IgM and certain subclasses of IgG (human IgG1, IgG2, and IgG3) can activate the classical complement pathway. The initial stage of activation involves C1, C2, C3, and C4, which are present in plasma in functionally inactive forms. Because the components were named in order of their discovery and before their functional roles had been determined, the numbers in their names do not always reflect the order in which they react.

The formation of an antigen-antibody complex induces conformational changes in the Fc portion of the IgM molecule that expose a binding site for the C1 component of the complement

system. C1 in serum is a macromolecular complex consisting of C1q and two molecules each of C1r and C1s, held together in a complex (C1qr2s2) stabilized by Ca2_ ions. The C1q molecule is composed of 18 polypeptide chains that associate to form six collagen-like triple helical arms, the tips of which bind to exposed C1q-binding sites in the CH2 domain of the antibody molecule.Each C1r and C1s monomer contains a catalytic domain and an interaction domain; the latter facilitates interaction with C1q or with each other.

Each C1 molecule must bind by its C1q globular heads to at least two Fc sites for a stable C1-antibody interaction to occur.When pentameric IgM is bound to antigen on a target surface it assumes the so-called "staple" configuration, in which at least three binding sites for C1q are exposed. Circulating IgM, however, exists as a planar configuration in which the C1q-binding sites are not exposed and therefore cannot activate the complement cascade. An IgG molecule, on the other hand, contains only a single C1q-binding site in the CH2 domain of the Fc, so that firm C1q binding is achieved only when two IgG molecules are within 30–40 nm of each other on a target surface or in a complex, providing two attachment sites for C1q. This difference accounts for the observation that a single molecule of IgM bound to a red blood cell can activate the classical complement pathway and lyse the red blood cell while some 1000 molecules of IgG are required to assure that two IgG molecules are close enough to each other on the cell surface to initiate C1q binding.

Binding of C1q to Fc binding sites induces a conformational change in C1r that converts C1r to an active serine protease enzyme, C1_r, which then cleaves C1s to a similar active enzyme, C1s. C1s has two substrates, C4 and C2. The C4 component is a glycoprotein containing three polypeptide chains α,β and β. C4 is activated when C1s hydrolyzes a small fragment (C4a) from the amino terminus of the β chain, exposing a binding site on the larger fragment (C4b). The C4b fragment attaches to the target surface in the vicinity of C1, and the C2 proenzyme then attaches to the exposed binding site on C4b, where the C2 is then cleaved by the neighboring C1_s; the smaller fragment (C2b) diffuses away. The resulting C4_b2_a complex is called C3 convertase, referring to its role in converting the C3 into an active form. The smaller fragment from C4 cleavage, C4a, is an anaphylatoxin, or mediator of inflammation, which does not participate directly in the complement cascade; the anaphylatoxins, which include the smaller fragments of C4, C3, and C5 are described below. The native C3 component consists of two polypeptide chains, α and β. Hydrolysis of a short fragment (C3a) from the amino terminus of the α chain by the C3 convertase generates C3b .A single C3 convertase molecule can generate over 200 molecules of C3b, resulting in tremendous amplification at this step of the sequence. Some of the C3b binds to C4b2a to form a trimolecular complex C4b2a3b, called C5 convertase. The C3b component of this complex binds C5 and alters its conformation, so that the C4b2a component can cleave C5 into C5a, which diffuses away, and C5b,which attaches to C6 and initiates formation of the membrane attack complex in a sequence described later. Some of the C3b generated by C3 convertase activity does not associate with C4b2a; instead it diffuses away and then coats immunecomplexes and particulate antigens, functioning as an opsonin as described in the Clinical Focus. C3b may also bind directly to cell membranes.

The Alternative Pathway Is Antibody-Independent

The alternative pathway generates bound C5b, the same product that the classical pathway generates, but it does so without the need for antigen-antibody complexes for initiation. Because no antibody is required, the alternative pathway is a component of the innate immune system. This major pathway of complement activation involves four serum proteins: C3, factor B, factor D, and properdin. The alternative pathway is initiated in most cases by cell-surfaceconstituents that are foreign to the host. For example, both gram-negative and gram-positive bacteria have cell-wall constituents that can activate the alternative pathway.

In the classical pathway, C3 is rapidly cleaved to C3a and C3b by the enzymatic activity of the C3 convertase. In the alternative pathway, serum C3, which contains an unstable thioester bond, is subject to slow spontaneous hydrolysis to yield C3a and C3b. The C3b component can bind to foreign surface antigens (such as those on bacterial cells or viral particles) or even to the host's own cells. The membranes of most mammalian cells have high levels of sialic acid, which contributes to the rapid inactivation of bound C3b molecules on host cells; consequently this binding rarely leads to further reactions on the host cell membrane. Because many foreign antigenic surfaces (e.g., bacterial cell walls, yeast cell walls, and certain viral envelopes) have only low levels of sialic acid, C3b bound to these surfaces remains active for a longer time.The C3b present on the surface of the foreign cells can bind another serum protein called factor B to form a complex stabilized by Mg2-. Binding to C3b exposes a site on factor B that serves as the substrate for an enzymatically active serum protein called factor D. Factor D cleaves the C3b-bound factor B, releasing a small fragment (Ba) that diffuses away and generating C3bBb. The C3bBb complex has C3 convertase activity and thus is analogous to the C4b2a complex in the classical pathway. The C3 convertase activity of C3bBb has a half-life of only 5 minutes unless the serum protein properdin binds to it, stabilizing it and extending the half-life of this convertase activity to 30 minutes.

The C3bBb generated in the alternative pathway can activate unhydrolyzed C3 to generate more C3b autocatalytically. As a result, the initial steps are repeated and amplified, so that more than 2- 106 molecules of C3b can be deposited on an antigenic surface in less than 5 minutes. The C3 convertase activity of C3bBb generates the C3bBb3b complex, which exhibits C5 convertase activity, analogous to the C4b23b complex in the classical pathway. The nonenzymatic C3b component binds C5, and the Bb component subsequently hydrolyzes the bound C5 to generate C5a and C5b.

BIOLOGICAL CONSEQUENCES OF COMPLEMENT ACTIVATION

Complement serves as an important mediator of the humoral response by amplifying the response and converting it into an effective defense mechanism to destroy invading microorganisms. The MAC mediates cell lysis, while other complement components or split products participate in the inflammatory response, opsonization of antigen, viral neutralization, and clearance of immune complexes.

Many of the biological activities of the complement system depend on the binding of complement fragments to complement receptors, which are expressed by various cells. In addition, some complement receptors play an important role in regulating complement activity by binding biologically active complement components and degrading them into inactive

products. The complement receptors and their primary ligands, which include various complement components and their proteolytic breakdown products.

The Membrane-Attack Complex Can Lyse a Broad Spectrum of Cells

The membrane-attack complex formed by complement activation can lyse gram-negative bacteria, parasites, viruses, erythrocytes, and nucleated cells. Because the alternative and lectin pathways of activation generally occur without an initial antigen-antibody interaction, these pathways serve as important innate immune defenses against infectious microorganisms. Therequirement for an initial antigen-antibody reaction in the classical pathway supplements these nonspecific innate defenses with a more specific defense mechanism. In some instances, the requirement for antibody in the activating event may be supplied by so-called natural antibodies, which are raised against common components of ubiquitous microbes.

Antibody and complement do play a role in host defense against viruses and are often crucial in containing viral spread during acute infection and in protecting against reinfection. Most—perhaps all—enveloped viruses are susceptible to complementmediated lysis. The viral envelope is largely derived from the plasma membrane of infected host cells and is therefore susceptible to pore formation by the membraneattack complex. Among the pathogenic viruses susceptible to lysis by complement-mediated lysis are herpesviruses, orthomyxoviruses, paramyxoviruses, and retroviruses.

The complement system is generally quite effective in lysing gram-negative bacteria. However, some gram-negative bacteria and most gram-positive bacteria have mechanisms for evading complement-mediated damage. For example, a few gram-negative bacteria can develop resistance to complement-mediated lysis that correlates with the virulence of the organism. In *Escherichia coli* and *Salmonella,* resistance to complement is associated with the smooth bacterial phenotype, which is characterized by the presence of long polysaccharide side chains in the cell-wall lipopolysaccharide (LPS) component. It has been proposed that the increased LPS in the wall of resistant strains may prevent insertion of the MAC into the bacterial membrane, so that the complex is released from the bacterial cell rather than forming a pore. Strains of *Neisseria gonorrheae* resistant to complement-mediated killing have been associated with disseminated gonococcal infections in humans. Some evidence suggests that the membrane proteins of resistant *Neisseria* strains undergo noncovalent interactions with the MAC that prevent its insertion into the outer membrane of the bacterial cells. These examples of resistant gram-negative bacteria are the exception; most gram-negative bacteria are susceptible to complement mediated lysis.

Gram-positive bacteria are generally resistant to complement- mediated lysis because the thick peptidoglycan layer in their cell wall prevents insertion of the MAC into the inner membrane. Although complement activation can occur on the cell membrane of encapsulated bacteria such as *Streptococcus pneumoniae,* the capsule prevents interaction between C3b deposited on the membrane and the CR1 on phagocytic cells. Some bacteria possess an elastase that inactivates C3a and C5a, preventing these split products from inducing an inflammatory response. In addition to these mechanisms of evasion, various bacteria, viruses, fungi, and protozoans contain proteins that can interrupt the complement cascade on their surfaces, thus mimicking the effects of the normal complement regulatory proteins C4bBP, CR1, and DAF.

Cleavage Products of Complement Components Mediate Inflammation

The complement cascade is often viewed in terms of the final outcome of cell lysis, but various peptides generated during formation of the MAC play a decisive role in the development of an effective inflammatory response. The smaller fragments resulting from complement cleavage, C3a, C4a, and C5a, called **anaphylatoxins,** bind to receptors on mast cells and blood basophils and induce degranulation, with release of histamine and other pharmacologically active mediators. The anaphylatoxins also induce smooth-muscle contraction and increased vascularpermeability. Activation of the complement system thus results in influxes of fluid that carries antibody and phagocytic cells to the site of antigen entry.

C3a, C5a, and C5b67 can each induce monocytes and neutrophils to adhere to vascular endothelial cells, extravasate through the endothelial lining of the capillary, and migrate toward the site of complement activation in the tissues. C5a is most potent in mediating these processes, effective in picomolar quantities.C3b and C4b Binding Facilitates Opsonizatio C3b is the major **opsonin** of the complement system, although C4b and iC3b also have opsonizing activity. The amplification that occurs with C3 activation results in a coating of C3b on immune complexes and particulate antigens. Phagocytic cells, as well as some other cells, express complement receptors (CR1, CR3, and CR4) that bind C3b, C4b, or iC3b. Antigen coated with C3b binds to cells bearing CR1. If the cell is a phagocyte (e.g., a neutrophil, monocyte, or macrophage), phagocytosis will be enhanced.

The Complement System Also Neutralizes Viral Infectivity

For most viruses, the binding of serum antibody to the repeating subunits of the viral structural proteins creates particulate immune complexes ideally suited for complement activation by the classical pathway. Some viruses (e.g., retroviruses, Epstein-Barr virus, Newcastle disease virus, and rubella virus) can activate the alternative, lectin, or even the classical pathway in the absence of antibody. The complement system mediates viral neutralization by a number of mechanisms. Some degree of neutralization is achieved through the formation of larger viral aggregates, simply because these aggregates reduce the net number of infectious viral particles. Although antibody plays a role in the formation of viral aggregates, in vitro studies show that the C3b component facilitates aggregate formation in the presence of as little as two molecules of antibody per virion. For example, polyoma virus coated with antibody is neutralized when serum containing activated C3 is added.

Cytokines

Cytokines are low-molecular weight regulatory proteins or glycoproteins secreted by white blood cells and various other cells in the body in response to a number of stimuli. These proteins assist in regulating the development of immune effector cells, and some cytokines possess direct effector functions of their own.

Properties of Cytokines

Cytokines bind to specific receptors on the membrane of target cells, triggering signal-transduction pathways that cultimately alter gene expression in the target cells.The susceptibility of the target cell to a particular cytokine is determined by the presence of specific membrane receptors. In general, the cytokines and their receptors exhibit very high affinity for each other,

with dissociation constants ranging from 10^{-10} to 10^{-12} M. Because their affinities are so high, cytokines can mediate biological effects at picomolar concentrations. A particular cytokine may bind to receptors on the membrane of the same cell that secreted it, exerting autocrine action; it may bind to receptors on a target cell in close proximity to the producer cell, exerting paracrine action; in a few cases, it may bind to target cells in distant parts of the body, exerting endocrine action. Cytokines regulate the intensity and duration of the immune response by stimulating orinhibiting the activation, proliferation, and/or differentiation of various cells and by regulating the secretion of antibodies or other cytokines. As described later, binding of a given cytokine to responsive target cells generally stimulates increased expression of cytokine receptors and secretion of other cytokines, which affect other target cells in turn. Thus, the cytokines secreted by even a small number of lymphocytes activated by antigen can influence the activity of numerous cells involved in the immune response. For example, cytokines produced by activated TH cells can influence the activity of B cells, TC cells, natural killer cells, macrophages, granulocytes, and hematopoietic stem cells, thereby activating an entire network of interacting cells. Cytokines exhibit the attributes of pleiotropy, redundancy, synergy, antagonism, and cascade induction, which permit them to regulate cellular activity in a coordinated, interactive way. A given cytokine that has different biological effects on different target cells has a pleiotropic action. Two or more cytokines that mediate similar functions are said to be redundant; redundancy makes it difficult to ascribe a particular activity to a single cytokine. Cytokine synergism occurs when the combined effect of two cytokines on cellular activity is greater than the additive effects of the individual cytokines. In some cases, cytokines exhibit antagonism; that is, the effects of one cytokine inhibit or offset the effects of another cytokine. Cascade induction occurs when the action of one cytokine on a target cell induces that cell to produce one or more other cytokines, which in turn may induce other target cells to produce other cytokines. The term cytokine encompasses those cytokines secreted by lymphocytes, substances formerly known as lymphokines, and those secreted by monocytes and macrophages, substances formerly known as monokines. Although these other two terms continue to be used, they are misleading because secretion of many lymphokines and monokines is not limited to lymphocytes and monocytes as these terms imply, but extends to a broad spectrum of cells and types. For this reason, the more inclusive term cytokine is preferred.Many cytokines are referred to as interleukins, a name indicating that they are secreted by some leukocytes and act upon other leukocytes. Interleukins 1–25 have been identified. There is reason to suppose that still other cytokines will be discovered and that the interleukin group will expand further. Some cytokines are known by common names, including the interferons and tumor necrosis factors. Recently gaining prominence is yet another another subgroup of cytokines, the chemokines, a group of low-molecular weight cytokines that affect chemotaxis and other aspects of leukocyte behavior. These molecules play an important role in the inflammatory response. Because cytokines share many properties with hormones and growth factors, the distinction between these three classes of mediators is often blurred. All three are secreted soluble factors that elicit their biological effects at picomolar concentrations by binding to receptors on target cells. Growth factors tend to be produced constitutively, whereas cytokines and hormones are secreted in response to discrete stimuli, and secretion is short-lived, generally ranging from a few hours to a few days. Unlike hormones, which generally act long range in an endocrine fashion, most cytokines act over a

short distance in an autocrine or paracrine fashion. In addition, most hormones are produced by specialized glands and tend to have a unique action on one or a few types of target cell. In contrast, cytokines are often produced by, and bind to, a variety of cells. The activity of cytokines was first recognized in the mid-1960s, when supernatants derived from in vitro cultures of lymphocytes were found to contain factors that could regulate proliferation, differentiation, and maturation of allogeneic immune-system cells

Cytokines Belong to Four Structural Families

Once the genes encoding various cytokines had been cloned, sufficient quantities of purified preparations became available for detailed studies on their structure and function. Cytokines generally have a molecular mass of less than 30 kDa. Structural studies have shown that the cytokines share a similar polypeptide fold, with four -helical regions (A–D) in which the firstand second helices and the third and fourth helices run roughly parallel to one another and are connected by loops.

Cytokines Have Numerous Biological Functions

Although a variety of cells can secrete cytokines, the two principal producers are the TH cell and the macrophage. Cytokines released from these two cell types activate an entire network of interacting cells. Among the numerous physiologic responses that require cytokine involvement are development of cellular and humoral immune responses, induction of the inflammatory

Cytokine*	Secreted by*†	Targets and effects
SOME CYTOKINES OF INNATE IMMUNITY		
Interleukin 1 (IL-1)	Monocytes, macrophages, endothelial cells, epithelial cells	Vasculature (inflammation); hypothalamus (fever); liver (induction of acute phase proteins)
Tumor Necrosis Factor-α (TNF-α)	Macrophages	Vasculature (inflammation); liver (induction of acute phase proteins); loss of muscle, body fat (cachexia); induction of death in many cell types; neutrophil activation
Interleukin 12 (IL-12)	Macrophages, dendritic cells	NK cells; influences adaptive immunity (promotes T_H1 subset)
Interleukin 6 (IL-6)	Macrophages, endothelial cells	Liver (induces acute phase proteins); influences adaptive immunity (proliferation and antibody secretion of B cell lineage)
Interferon α (IFN-α) (This is a family of molecules)	Macrophages	Induces an antiviral state in most nucleated cells; increases MHC class I expression; activates NK cells
Interferon β (IFN-β)	Fibroblasts	Induces an antiviral state in most nucleated cells; increases MHC class I expression; activates NK cells
SOME CYTOKINES OF ADAPTIVE IMMUNITY		
Interleukin 2 (IL-2)	T cells	T-cell proliferation; can promote AICD. NK cell activation and proliferation; B-cell proliferation
Interleukin 4 (IL-4)	T_H2 cells; mast cells	Promotes T_H2 differentiation; isotype switch to IgE
Interleukin 5 (IL-5)	T_H2 cells	Eosinophil activation and generation
Interleukin 25 (IL-25)	Unknown	Induces secretion of T_H2 cytokine profile
Transforming growth factor β (TGF-β)	T cells, macrophages, other cell types	Inhibits T-cell proliferation and effector functions; inhibits B-cell proliferation; promotes isotype switch to IgE; inhibits macrophages
Interferon γ (IFN-γ)	T_H1 cells; $CD8^+$ cells; NK cells	Activates macrophages; increases expression MHC class I and class II molecules; increases antigen presentation

response, regulation of hematopoiesis, control of cellular proliferation and differentiation, and the healing of wounds. Although the immune response to a specific antigen may include the production of cytokines, it is important to remember that cytokines act in an antigen-nonspecific manner. That is, they affect whatever cells they encounter that bear appropriate receptors and are in a physiological state that allows them to respond. Cytokines are involved in a staggeringly broad array of biological activities including innate immunity, adaptive immunity, inflammation, and hematopoiesis. Altogether, the total number of proteins with cytokine activity easily exceeds 100 and research continues to uncover new ones. An expanded list of cytokines can be found in the Appendix. It should be kept in mind that most of the listed functions have been identified from analysis of the effects of recombinant cytokines, often at non-physiologic concentrations, added individually to in vitro systems. In vivo, however, cytokines rarely, if ever, act alone.

Instead, a target cell is exposed to a milieu containing a mixture of cytokines, whose combined synergistic or antagonistic effects can have very different consequences. In addition, cytokines often induce the synthesis of other cytokines, resulting in cascades of activity. The non-specificity of cytokines seemingly conflicts with the established specificity of the immune system. What keeps the nonspecific cytokines from activating cells in a nonspecific fashion during the immune response? One way in which specificity is maintained is by careful regulation of the expression of cytokine receptors on cells. Often cytokine receptors are expressed on a cell only after that cell has interacted with antigen. In this way cytokine activation is limited to antigen-activated lymphocytes. Another means of maintaining specificity may be a requirement for direct interaction between the cytokine-producing cell and the target cell to trigger cytokine secretion, thus ensuring that effective concentrations of the cytokine are released only in the vicinity of the intended target. In the case of the TH cell, a major producer of cytokines, close cellular interaction occurs when the T-cell receptor recognizes an antigen-MHC complex on an appropriate antigen-presenting cell, such as a macrophage, dendritic cell, or B lymphocyte. Cytokines secreted at the junction of these interacting cells reach high enough local concentrations to affect the target APC but not more distant cells. In addition, the half-life of cytokines in the bloodstream or other extracellular fluids into which they are secreted is usually very short, ensuring that they act for only a limited period of time and thus over a short distance.

Cytokine Receptors

To exert their biological effects, cytokines must first bind to specific receptors expressed on the membrane of responsive target cells. Because these receptors are expressed by many types of cells, the cytokines can affect a diverse array of cells. Biochemical characterization of cytokine receptors initially progressed at a very slow pace because their levels on the membrane of responsive cells is quite low. As with the cytokines themselves, cloning of the genes encodingcytokine receptors has led to rapid advances in the identification and characterization of these receptors. Cytokine Receptors Fall Within Five Families Receptors for the various cytokines are quite diverse structurally, but almost all belong to one of five families of receptor proteins Immunoglobulin superfamily receptors s Class I cytokine receptor family (also known as the hematopoietin receptor family) s Class II cytokine receptor family (also known as the interferon receptor family) s TNF receptor family s Chemokine receptor family Many of the cytokine- binding receptors that function in the immune and hematopoietic systems belong to the class I cytokine receptor family. The members of this receptor family have conserved amino acid sequence motifs in the extracellular domain consisting of four positionally conserved cysteine residues (CCCC) and a conserved sequence of tryptophan serine-(any amino acid)-tryptophan-serine (WSXWS, where X is the non-conserved amino acid). The receptors for all the cytokines classified as hematopoietins belong to the class I cytokine receptor family, which also is called the hematopoietin receptor family. The class II cytokine receptors possess the conserved CCCC motifs, but lack the WSXWS motif pre- sent in class I cytokine receptors. Initially only the three interferons were thought to be ligands for these receptors. However, recent work has shown that the IL-10 receptor is also a member of this group. Another feature common to most of the hematopoietin (class I cytokine) and the class II cytokine receptor families is multiple subunits,

often including one subunit that binds specific cytokine molecules and another that mediates signal transduction. Note, however, that these two functions are not always confined to one subunit or the other. Engagement of all of the class I and class II cytokine receptors studied to date has been shown to induce tyrosine phosphorylation of the receptor through the activity of protein tyrosine kinases closely associated with the cytosolic domain of the receptors.

Cytokine-Related Diseases

Defects in the complex regulatory networks governing the expression of cytokines and cytokine receptors have been implicated in a number of diseases. This section describes several diseases resulting from over expression or underexpression of cytokines or cytokine receptors. Bacterial Septic Shock Is Common and Potentially Lethal. The role of cytokine overproduction in pathogenesis can be illustrated by bacterial septic shock. This condition may develop a few hours after infection by certain gram-negative bacteria, including E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterobacter aerogenes, and Neisseria meningitidis. The symptoms of bacterial septic shock, which is often fatal, include a drop in blood pressure, fever, diarrhea, and wide- spread blood clotting in various organs. This condition afflicts about 500,000 Americans annually and causes more than 70,000 deaths. The annual cost for treating bacterial septic shock is estimated to be in excess of $5 billion. Bacterial septic shock apparently develops because bacte- rial cell-wall endotoxins stimulate macrophages to over- produce IL-1 and TNF-to levels that cause septic shock.

Cytokines produced by TH1 cells predominate in the tuberculoid patients, while cytokines produced by TH2 cells predominate in the lepromatous patients TH1 activity TH2 activity Tuberculoid Lepromatous Tuberculoid Lepromatous IL-2 IFN-IFN-IL-4 IL-5 IL-10 one study, for example, higher levels of TNF-were found in patients who died of meningitis than in those who recovered. Furthermore, a condition resembling bacterial septic shock can beproduced by injecting mice with recombinant TNF-in the absence of gram-negative bacterial infection. Several studies offer some hope that neutralization of TNF-or IL-1 activity with monoclonal antibodies or antagonists may pre- vent this fatal shock from developing in these bacterial infections. In one study, monoclonal antibody to TNF- protected animals from endotoxin induced shock. Another study has shown that injection of a recombinant IL-1 receptor antagonist (IL-1Ra), which prevents binding of IL-1 to the IL-1 receptor, resulted in a three-fold reduction in mortality. It is hoped that these experimental results will lead to clinically useful products for the treatment of bacterial septic shock in humans.

Bacterial Toxic Shock Is Caused by Superantigens

A variety of microorganisms produce toxins that act as super- antigens. As Chapter 10 described, superantigens bind simultaneously to a class II MHC molecule and to the V domain of the T-cell receptor, activating all T cells bearing a particular V domain. Because of their unique binding ability, superantigens can activate large numbers of T cells irrespective of their antigenic specificity. Although less than 0.01% of T cells respond to a given conventional antigen,

between 5% and 25% of T cells can respond to a given superantigen. The large proportion of T cells responsive to a particular superantigen results from the limited number of TCR Vgenes carried in the germ line. Mice, for example, have about 20 V genes. Assuming that each V gene is expressed with equal frequency, then each super antigen would be expected to interact with 1 in 20 T cells, or 5% of the total T-cell population. A number of bacterial superantigens have been implicated as the causative agent of several diseases such as bacterial toxic shock and food poisoning. Included among these bacterial superantigens are several enterotoxins, exfoliating toxins, and toxic-shock syndrome toxin (TSST1) from Staphylococcus aureus; pyrogenic exotoxins from Streptococcus pyrogenes; and Mycoplasma arthritidis supernatant (MAS). The large number of T cells activated by these superantigens results in excessive production of cytokines. The toxic-shock syndrome toxin, for example, has been shown to induce extremely high levels of TNF and IL-1. As in bacterial septic shock, these elevated concentrations of cytokines can induce systemic reactions that include fever, widespread blood clotting, and shock. Cytokine Activity Is Implicated in Lymphoid and Myeloid Cancers Abnormalities in the production of cytokines or their receptors have been associated with some types of cancer. For example, abnormally high levels of IL-6 are secreted by card myxoma cells (abenign heart tumor), myeloma and plasmacytoma cells, and cervical and bladder cancer cells. In myeloma cells, IL-6 appears to operate in an autocrine manner to stimulate cell proliferation. When monoclonal anti- bodies to IL-6 are added to in vitro cultures of myeloma cells, their growth is inhibited. In addition, transgenic mice that express high levels of IL-6 have been found to exhibit a massive, fatal plasma-cell proliferation, called plasmacytosis. Although these plasma cells are not malignant, the high rate of plasma-cell proliferation possibly contributes to the development of cancer.

Therapeutic Uses of Cytokines and Their Receptors.

The availability of purified cloned cytokines and soluble cytokine receptors offers the prospect of specific clinical therapies to modulate the immune response. A few cytokines notably, interferons and colony stimulating factors, such as GM-CSF, have proven to be therapeutically useful. However, despite the promise of cytokines as powerful mediators of immune and otherbiological responses, not many have made their way into clinical practice. A number of factors are likely to raise difficulties in adapting cytokines for safe and effective routine medical use. One of these is the need to maintain effective dose levels over a clinically significant period of time.

MHC

Major Histocompatibility Complex plays roles in intercellular recognition and in discrimination between self and non-self. The MHC participates in the development of both humoral and cell-mediated immune responses. While antibodies may react with antigens alone, most T cells recognize antigen only when it is combined with an MHC molecule. Furthermore, because MHC molecules act as antigen-presenting structures, the particular set of MHC molecules expressed by an individual influences the repertoire of antigens to which that individual's TH and TC cells can respond. For this reason, the MHC partly determines the response of an individual to antigens of infectious organisms, and it has therefore been implicated in the susceptibility to disease and in

the development of autoimmunity. The recent understanding that natural killer cells express receptors for MHC class I antigens and the fact that the receptor–MHC interaction may lead to inhibition or activation expands the known role of this gene family. The present chapter examines the organization and inheritance of MHC genes, the structure of the MHC molecules, and the central function that these molecules play in producing an immune response. General Organization and Inheritance of the MHC The concept that the rejection of foreign tissue is the result of an immune response to cell-surface molecules, now called histocompatibility antigens, originated from the work of Peter Gorer in the mid-1930s. Gorer was using inbred strains of mice to identify blood-group antigens. In the course of these studies, he identified four groups of genes, desiggenes"; their current designation as histocompatibility-2 (H-2) genes was in reference to Gorer's group II blood-group antigens. Although Gorer died before his contributions were recognized fully, Snell was awarded the Nobel Prize in 1980 for this work.

The MHC Encodes Three Major Classes of Molecules

The major histocompatibility complex is a collection of genes arrayed within a long continuous stretch of DNA on chromosome 6 in humans and on chromosome 17 in mice. The MHC is referred to as the HLA complex in humans and as the H-2 complex in mice. Although the arrangement of genes is somewhat different, in both cases the MHC genes are organized into regions encoding three classes of molecules: I Class I MHC genes encode glycoprotein's expressed on the surface of nearly all nucleated cells; the major function of the class I gene products is presentation of peptide antigens to TC cells. Class II MHC genes encode glycoprotein's expressed primarily on antigen-presenting cells (macrophages, dendrite cells, and B cells), where they present processed antigenic peptides to TH cells. I Class III MHC genes encode, in addition to other products, various secreted proteins that have immune functions, including components of the complement system and molecules involved in inflammation. Class I MHC molecules encoded by the K and D regions in mice and by the A, B, and C loci in humans were the first discovered, and they are expressed in the widest range of cell types. These are referred to as classical class I molecules. Additional genes or groups of genes within the H-2or HLA complexes also encode class I molecules; these genes are designated non classical class I genes. Expression of the non- classical gene products is limited to certain specific cell types. Although functions are not known for all of these gene products, some may have highly specialized roles in immunity. For example, the expression of the class I HLA- G molecules on cytotrophoblasts at the fetal-maternal interface has been implicated in protection of the fetus from being recognized as foreign (this may occur when paternal antigens begin to appear) and from being rejected by maternal TC cells. The two chains of the class II MHC molecules are en-coded by the IA and IE regions in mice and by the DP, DQ, and DR regions in humans.The terminology is somewhat confusing, since the D region in mice encodes class I MHC molecules, whereas the D region (DR, DQ, DP) in humans refers to genes encoding class II MHC molecules! Fortunately, the designation D for the general chromosomal location encoding the human class II molecules is seldom used today; the sequence of the entire MHC region is available so the more imprecise reference to region is seldom necessary. As with the class I loci, additional class II molecules encoded within this region have specialized functions in the

immune process. The class I and class II MHC molecules have common structural features and both have roles in antigen processing. By contrast, the class III MHC region, which is flanked by the class I and II regions, encodes molecules that are critical to immune function but have little in common with class I or II molecules. Class III products include the complement components C4, C2, BF, and inflammatory cytokines, including tumor necrosis factor (TNF) and heat-shock proteins

MHC Molecules and Genes

Class I and class II MHC molecules are membrane-bound glycoprotein's that are closely related in both structure and function. Both class I and class II MHC molecules have been isolated and purified and the three-dimensional structures of their extra cellular domains have been determined by x- ray crystallography. Both types of membrane glycoprotein's function as highly specialized antigen-presenting molecules that form unusually stable complexes with antigenic pep- tides, displaying them on the cell surface for recognition by T cells. In contrast, class III MHC molecules are a group of unrelated proteins that do not share structural similarity and common function with class I and II molecules.

Class I Molecules Have a Glycoprotein Heavy Chain and a Small Protein Light Chain

Class I MHC molecules contain a 45-kilodalton (kDa) β chain associated non-covalently with a 12-kDa β2-microglobulin molecule. The α chain is a transmembrane glycoprotein encoded by polymorphic genes within the A, B, and C regions of the human HLA complex and within the K and D/L regions of the mouse H-2 complex. 2-Microglobulin is a protein encoded by a highly conserved gene located on a different chromosome. Association of α chain with β2-microglobulin is required for expression of class I molecules on cell membranes. The chain is anchored in the plasma membrane by its hydrophobic transmembrane segment and hydrophilic cytoplasmic tail. Structural analyses have revealed that the α chain of class I MHC molecules is organized into three external domains, each containing approximately 90 amino acids; a transmembrane domain of about 25 hydrophobic amino acids followed by a short stretch of charged (hydrophilic) amino acids; and a cytoplasmic anchor segment of30 amino acids. The β2-microglobulin is similar in size and organization to α 3 domain; it does not contain a trans-membrane region and is non-covalently bound to the class I glycoprotein. Sequence data reveal homology between the 3 domain, β2-microglobulin, and the constant-region domains in immunoglobulin. The enzyme papain cleaves the α chain just 13 residues proximal to its transmembrane domain, releasing the extra cellular portion of the molecule, consisting of 1 α, 2 α, 3 α, and β 2-microglobulin. Purification and crystallization of the extra cellular portion revealed two pairs of interacting domains: a membrane-distal pair made up of the 1 α and α 2 domains and a membrane-proximal pair composed of the 3 α domain and 2 β -microglobulin. The α 1and α 2 domains interact to form a platform of eight antiparallel strands spanned by two long -helical regions. The structure forms a deep groove, or cleft, approximately 25 Å 10 Å 11 Å, with the long α helices as sides and the strands of the sheet as the bottom. This peptide-binding cleft is located on the top surface of the class I MHC molecule, and it is large enough to bind a peptide of 8–10 amino acids. The great surprise in the x-ray crystallographic analysis of

class I molecules was the finding of small peptides in the cleft that had cocrystallized with the protein. These peptides are, in fact, processed antigen and self-pep- tides bound to the α 1 and 2 α domains in this deep groove. The 3 domain and 2-microglobulin are organized into two pleated sheets each formed by antiparallel strands of amino acids. As described in Chapter 4, this structure, known as the immunoglobulin fold, is characteristic of immunoglobulin domains. Because of this structural similarity, which is not surprising given the considerable sequence similarity with the immunoglobulin constant regions, class I MHC molecules and 2-microglobulin are classified as members of the immunoglobulin superfamily.

The 3 domain appears to be highly conserved among class I MHC molecules and contains a sequence that interacts with the CD8 membrane molecule present on TC cells. 2-Microglobulin interacts extensively with the 3 do- main and also interacts with amino acids of the 1 and 2 domains. The interaction of 2-microglobulin and a peptide with a class I chain is essential for the class I molecule to reach its fully folded conformatio, assembly of class I molecules is believed to occur by the initial interaction of 2-microglobulin with the folding class I chain. This metastable "empty" dimer is then stabilized by the binding of an appropriate peptide to form the native trimeric class I structure consisting of the class I chain, 2-microglobulin, and a peptide. This complete molecular complex is ultimately transported to the cell surface. In the absence of 2-microglobulin, the class I MHC chain is not expressed on the cell membrane. This is illustrated by Daudi tumor cells, which are unable to synthesize 2-microglobulin

Class II Molecules Have Two Non identical Glycoprotein Chains Class II MHC molecules contain two different polypeptide chains, a 33-kDa chain and a 28-kDa chain, which associate by non-covalent interactions. Like class I chains, class II MHC molecules are membrane-bound glycoproteins that contain external domains, a transmembrane segment, and a cytoplasmic anchor segment. Each chain in a class II molecule contains two external domains: 1 and 2 domains in one chain and 1 and 2 domains in the other. The membrane-proximal 2 and 2 domains, like the membrane-proximal 3/2-microglobulin domains of class I MHC molecules, bear sequence similarity to the immunoglobulin-fold structure; for this reason, class II MHC molecules also are classified in the immunoglobulin super- family. The membrane-distal portion of a class II molecule is composed of the 1 and 1 domains and forms the antigen- binding cleft for processed antigen. X-ray crystallographic analysis reveals the similarity of class II and class I molecules, strikingly apparent when the molecules are surperimposed The peptide- binding cleft of HLA-DR1, like that in class I molecules, is composed of a floor of eight antiparallel strands and sides of antiparallel helices. However, the class II molecule lacks the conserved residues that bind to the terminal residues of short peptides and forms instead an open pocket; class I presents more of a socket, class II an open-ended groove. These functional consequences of these differences in fine structure will be explored below. An unexpected difference between crystallized class I and class II molecules was observed for human DR1 in that the latter occurred as a dimer of heterodimers, a "dimer of dimers". The dimer is oriented so that the two peptide-binding clefts face in opposite directions. While it has not yet been determined whether this dimeric form exists in vivo, the presence of CD4 binding sites on opposite sides of the class II

molecule suggests that it does. These two sites on the 2 and 2 domains are adjacent in the dimer form and a CD4 molecule binding to them may stabilize class II dimers. The Exon/Intron Arrangement of Class I and II Genes Reflects Their Domain Structure Separate exons encode each region of the class I and II proteins

This molecule crystallized as a dimer of the αβ heterodimer. The crystallized dimer is shown with one DR1 molecule in red and the other DR1 molecule in blue. The bound peptides are yellow. The two peptide-binding clefts in the Class II Molecules Have Two Non identical Glycoprotein Chains Class II MHC molecules contain two different polypeptide chains, a 33-kDa α chain and a 28-kDa β chain, which associate by non-covalent interactions. Like class I chains, class II MHC molecules are membrane-bound glycoproteins that contain external domains, a transmembrane segment, and a cytoplasmic anchor segment. Each chain in a class II molecule contains two external domains: α1 and β2 domains in one chain and 1 and 2 domains in the other. The membrane-proximal α2 and β2 domains, like the membrane-proximal α3/β2-microglobulin domains of class I MHC molecules, bear sequence similarity to the immunoglobulin-fold structure; for this reason, class II MHC molecules also are classified in the immunoglobulin super family.

The membrane-distal portion of a class II molecule is composed of the α1 and β1 domains and forms the antigen- binding cleft for processed antigen. X-ray crystallographic analysis reveals the similarity of class II and class I molecules, strikingly apparent when the molecules are surperimposed. The peptide- binding cleft of HLA-DR1, like that in class I molecules, is composed of a floor of eight antiparallel α strands and sides of antiparallel β helices. However, the class II molecule lacks the conserved residues that bind to the terminal residues of short peptides and forms instead an open pocket; class I presents more of a socket, class II an open-ended groove. These functional consequences of these differences in fine structure will be explored below. An unexpected difference between crystallized class I and class II molecules was observed for human DR1 in that the dimeric molecule face in opposite directions. latter occurred as a dimer of αβ heterodimers, a “dimer of dimers”. The dimer is oriented so that the two peptide-binding clefts face in opposite directions. While it has not yet been determined whether this dimeric form exists in vivo, the presence of CD4 binding sites on opposite sides ofthe class II molecule suggests that it does. These two sites on the α2 and β2 domains are adjacent in the dimer form and a CD4 molecule binding to them may stabilize class II dimers.

CLASS I MHC–PEPTIDE INTERACTION

Class I MHC molecules bind peptides and present them to CD8 T cells. In general, these peptides are derived from endogenous intracellular proteins that are digested in the cytosol. The peptides are then transported from the cytosol into the cisternae of the endoplasmic reticulum, where they interact with class I MHC molecules. This process, known as the cytosolic or endogenous processing pathway, is discussed in detail in the next chapter. Each type of class I MHC molecule (K, D, and L in mice or A, B, and C in humans) binds a unique set of peptides. In addition, each allelic variant of a class I MHC molecule (e.g., H-2Kk and H-2Kd) also binds a

distinct set of peptides. Be- cause a single nucleated cell expresses about 105 copies of each class I molecule, many different peptides will be ex-pressed simultaneously on the surface of a nucleated cell by class I MHC molecules.

CLASS II MHC–PEPTIDE INTERACTION

Class II MHC molecules bind peptides and present these peptides to CD4 T cells. Like class I molecules, molecules of class II can bind a variety of peptides. In general, these pep- tides are derived from exogenous proteins (either self or nonself), which are degraded within the endocytic processing pathway. Most of the peptides associated with class II MHC molecules are derived from membrane- bound proteins or proteins associated with the vesicles of the endocytic processing pathway. The membrane-bound proteins presumably are internalized by phagocytosis or by receptor-mediated endocytosis and enter the endocytic processing pathway at this point. For instance, peptides derived from digestion of membrane-bound class I MHC molecules often are bound to class II MHC molecules. Peptides recovered from class II MHC–peptide complexes generally contain 13–18 amino acid residues, some- what longer than the nonameric peptides that most commonly bind to class I molecules. The peptide-binding cleft in class II molecules is open at both ends, allowing longer peptides to extend beyond the ends, like a long hot dog in a bun. Peptides bound to class II MHC molecules maintain a roughly constant elevation on the In addition, over 30% of the peptides eluted from class II molecules contain a proline residue at position 2 and another cluster of prolines at the carboxyl-terminal end.

Human MHC Class III Genes.

Are between Class I and II The class III region of the MHC in humans and mice contains a heterogeneous collection of genes. These genes encode several complement components, two steroid 21-hydroxylases, two heat-shock proteins, and two cytokines (TNF-β and TNF-σ). Some of these class III MHC gene products play a role in certain diseases. For example, mutations in the genes encoding 21-hydroxylase have been linked to congenital adrenal hyperplasia. Interestingly, the presence of a linked class III gene cluster is conserved in all species with an MHC region.

MHC and Immune Responsiveness

Early studies by B. Benacerraf in which guinea pigs were immunized with simple synthetic antigens were the first to show that the ability of an animal to mount an immune response, ameasured by the production of serum antibodies, is determined by its MHC haplotype. Later experiments by H. McDevitt, M. Sela, and their colleagues used congenic and recombinant congenic mouse strains to map the control of immune responsiveness to class II MHC genes. In early reports, the genes responsible for this phenotype were designated Ir or immune response genes, and for this reason mouse class II products are called IA and IE. We now know that the dependence of immune responsiveness on the class II MHC reflects the central role of class II MHC molecules in presenting antigen to TH cells. Two explanations have been proposed to account for the variability in immune responsiveness observed among different haplotypes. According to the determinant-selection model, different class II MHC molecules differ in their

ability to bind processed antigen. According to the alternative holes-in-the-repertoire model, T cells bearing receptors that recognize foreign antigens closely resembling self-antigens may be eliminated during thymic processing. Since the T- cell response to an antigen involves a trimolecular complex of the T cell's receptor, an antigenic peptide, and an MHC molecule, both models may be correct. That is, the absence of an MHC molecule that can bind and present a given peptide, or the absence of T-cell receptors that can recognize a given peptide–MHC molecule complex, could result in the absence of immune responsiveness and so account for the observed relationship between MHC haplotype and immune responsiveness to exogenous antigens

MHC and Disease Susceptibility

Some HLA alleles occur at a much higher frequency in those suffering from certain diseases than in the general population. The diseases associated with particular MHC alleles include autoimmune disorders, certain viral diseases, disorders of the complement system, some neurologic disorders, and several different allergies. The association between HLA alleles and a given disease may be quantified by deter- mining the frequency of the HLA alleles expressed by individuals afflicted with the disease, then comparing these data with the frequency of the same alleles in the general population. Such a comparison allows calculation of relative risk. A relative risk value of 1 means that the HLA allele is expressed with the same frequency in the patient and general populations, indicating that the allele confers no increased risk for the disease

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: II M.Sc MB COURSE NAME: ADVANCED IMMUNOLOGY

COURSE CODE: 18MBP301 UNIT: IV BATCH-2018-2020

Sl. No	Question	Option A	Option B	Option C	Option D	Correct Ans
1	Intrapulmonary Arthus type reactions induced by	virus	Fungal toxins	Bacterial spores	Algae	Bacterial spores
2	_____ was defined as the ratio of the phagolytic activity of the patient's blood for a given bacterium to the phagocytic activity of the patients blood for a given bacterium to the phagocytic activity of blood from a normal individual	Neutralization	Qponisation	Westernblotting	Southernblotting	Qponisation
3	Neutralizaiton of bacteriophages can be demonstrated by a ____	plaque inhibition test	Plaque lysis	Plaque neutralization	Coombs test	plaque inhibition test
4	_____ test is based on the ability of circulating antitoxin to neutralize the diphtheria toxin	Neutralization test	Schick test	Antistreptolysin O test	Westernblotting	Schick test
5	_____ is an example for CFT	Weil – Felix reaction	Coombs test	Wassermann reaction	Paul Bunnell test	Wassermann reaction
6	Serum antibodies to HIV can be detected by _____ ELISA	Indirect	sandwich	Competitive	Non competitive	Indirect
7	Gamma emitting isotope used for labeling Ag is	^{125}I	^{3}H	^{32}P	^{35}S	^{125}I
8	The heptamer sequences?	5' CACAGTG3'	5'ACAAAAACC3'	5' ACAGTG3'	5'CAAAGTG3'	5' CACAGTG3'
9	The nonamer sequences?	5' ACAAAAACC3'	5'CACAGTG3'	5' TGAAGTG3'	5'AATGACC3'	5' ACAAAAACC3'
10	Molecular weight of IgG?	160	180	900	150	150
11	The only antibody capable of crossing the placenta	IgA	IgM	IgG	IgD	IgG
12	Each chain consists of series of similar although not identical aminoacids in length?	120	150	110	130	110

13	The variation which occur between immunoglobulins of different antigenic specificity are called ___	Idiotypes	Epitopes	paratopes	suppressor cells	suppressor cells
14	Anti – idotype antibodies may enhance _____ response by acting like antigens	T cell	suppressor cell	Mast cell	B cell	B cell
15	_____ of antigen also influence the nature of he immune response	Quality	Quantity	Mass	Density	Quantity
16	____ suggested that the immunoglobulin generated during an antibody response carry new idiotypes	Rosalyn yalow	Landsteiner	Niels Jerne	R.A.fisher	Niels Jerne
17	The MHC genes have been called ___	Tra genes	Immune response genes	Regulatory genes	Constitutive genes	Immune response genes
18	Some epitopes appear to selectively turn off the immune response and have been called____	Epitopes	Supressor epitopes	Idiotypes	Plasmaphoresis	Supressor epitopes
19	During an immune response ____ cells switch to the production of Ig	T cells	B cells	NK cells	Mast cells	B cells
20	_____is the technique by which blood is removed from an animal and cells and plasma are separated while the cells are retuned to the donor	Plasmopheresis	Plasmolysis	Osmosis	Diapedesis	Plasmopheresis
21	IgG antibodies polypeptide chain is of approximately _____ KDa	70KDa	40KDa	50KDa	80 KDa	50KDa
22	Major immunoglobulin in human serum is	IgG	IgM	IgE	IgA	IgG
23	Other name of alternative pathway	Embden meyeroff pathway	PZ pathway	lectin pathway	Complement pathway	PZ pathway
24	Which complement component is acted as anaphylotoxin	C_8	C_9	3_a	C_{3a}	C_{3a}

25	Precursor proteins of alternative pathway are	C_4+ C_2	C_3+ factor B	C_4+ factor B	C_2 +C_3	C_3+ factor B
26	Which of the following is initiating classical pathway	Bacterial cellwall	Ag	Ab	Ag- Ab complex	Ag- Ab complex
27	Which ion is used to stabilize $C_1qr_2s_2$ complex	Ca^{2+}	K^+	Mg^{2+}	Zn^{2+}	Ca^{2+}
28	Which of the following complements are not required for alternative pathway	C_6	C_7, C_8	C_7, C_8,C_9	C_1, C_4,C_2	C_1, C_4,C_2
29	In mouth the flushing action of saliva is complemented by the generation of _____ form	peroxidase	hydroxypleroxide	Oxidase	catalase	hydroxypleroxide
30	Squamous epithelium is composed of cells rich in ____	starch	glycogen	Glucosamine	Galactosamine	glycogen
31	____ is mouth have an inhibitory effects on most organism	saliva	lysosyme	saliva lysozyme	bile	saliva lysozyme
32	Urine kills some bacteria owing to its ____	high P^H	low p^H	Neutral P^H	basic PH	low p^H
33	____ is an antibacterial enzyme present in the tear	lysozyme	Lipase	Enolase	Esterase	lysozyme
34	_____is the directional migration of cells up a concentration gradient of a chemotactic molecule	Chemotaxis	Chemokinesis	Dialpedesis	Pavementing	Chemotaxis
35	_____ acts as link between immunological events and inflammatory system	Clotting system	Complement system	kinin system	Fibrinolytic system	Complement system
36	_____ will actively migrate up concentration gradients of certain chemotactic molecules	Phagocytes	Endocytes	C_{5a}	Chemotactic molecule	Phagocytes
37	_____ mediators such as vasoactive amines and the products of the kinin system modulate the immune response	fast acting	Newly synthesized	Slow reacting	Rapid	fast acting
38	____ mediators such as leukotribes are involved in the accumulation and activation of	fast acting	Newly synthesized	slow reacting	Rapid	Newly synthesized

	other cells					
39	Which antibody mediate Type I hypersensitivity?	IgG	IgE	IgM	IgD	IgE
40	Immune complexes directly act an basophils and platelets and produce	toxins	vasoactive amines	proteins	Antibodies	vasoactive amines
41	Examples for Localized type III reactions	Arthus reaction	Tuberculosis	Leishmaniasis	Leprosy	Arthus reaction
42	In type III hypersensitivity, immune complexes activate	Cells	Complements	Macrophages	Antibody	Complements
43	Type IV hypersensitivity is a _____ mediated reaction	Cell mediated	Antigen mediated	Antibody mediated	immune complex mediated	Cell mediated
44	Activated T_{DTH} cells secretes	Lymphokines	Leukocytes	cytokines	Lymphocytes	cytokines
45	What is a hemolytic disease of newborn?	Drug induced hemolytic anemia	Transfusion reaction	Erythroblastosis fetalis	Allergic	Erythroblastosis fetalis
46	Class of antigen responsible for type I hyper sensitivity__	Reagin	Allergin	Hapten	rhogam	Allergin
47	Antibody produced by plasma cells in Type I Hypersensitivity	Reagin	Allergin	hapten	Rhogam	Reagin
48	Which drug is used to treat anaphylactic reaction	penicillin	Cephalosporin	Epinephrin	Streptomycin	Epinephrin
49	Which antibody is used to prevent Rh incompactibility of newborn?	Reagin	Allergin	Hapten	Rhogam	Rhogam
50	Intrapulmonary Arthus type reactions induced by	virus	Fungal toxins	Bacterial spores	Algae	Bacterial spores
51	_____ was defined as the ratio of the phagolytic activity of the patient's blood for a given bacterium to the phagocytic activity of the patients blood for a given bacterium to the phagocytic activity of blood from a normal	Neutralization	Qponisation	Westernblotting	Southernblotting	Qponisation

	individual					
52	Neutralizaiton of bacteriophages can be demonstrated by a ____	plaque inhibition test	Plaque lysis	Plaque neutralization	Coombs test	plaque inhibition test
53	_____ test is based on the ability of circulating antitoxin to neutralize the diphtheria toxin	Neutralization test	Schick test	Antistreptolysin O test	Westernblotting	Schick test
54	_____ is an example for CFT	Weil – Felix reaction	Coombs test	Wassermann reaction	Paul Bunnell test	Wassermann reaction
55	Serum antibodies to HIV can be detected by _____ ELISA	Indirect	sandwich	Competitive	Non competitive	Indirect
56	Gamma emitting isotope used for labeling Ag is	^{125}I	^{3}H	^{32}P	^{35}S	^{125}I
57	The heptamer sequences?	5’ CACAGTG3’	5’ACAAAAACC3’	5’ ACAGTG3’	5’CAAAGTG3’	5’ CACAGTG3’
58	The nonamer sequences?	5’ ACAAAAACC3’	5’CACAGTG3’	5’ TGAAGTG3’	5’AATGACC3’	5’ACAAAAACC3’
59	Molecular weight of IgG?	160	180	900	150	150
60	The only antibody capable of crossing the placenta	IgA	IgM	IgG	IgD	IgG

UNIT V

Precipitation Reactions

Antibody and soluble antigen interacting in aqueous solu- tion form a lattice that eventu ally develops into a visible pre- cipitate. Antibodies that aggregate soluble antigens are called precipitins. Although formation of the soluble Ag-Ab com- plex occurs within minutes, formation of the visible precipi- tate occurs more slowly and often takes a day or two to reach completion.Formation of an Ag-Ab lattice depends on the valency of both the antibody and antigen: I The antibody must be bivalent; a precipitate will not form with monovalent Fab fragments. I The antigen must be either bivalent or polyvalent; that is, it must have at least two copies of the same epitope, or have different epitopes that react with different antibodies present in polyclonal antisera. Experiments with myoglobin illustrate the requirement that protein antigens be bivalent or polyvalent for a precip- itin reaction to occur. Myoglobin precipitates well with spe- cific polyclonal antisera but fails to precipitate with a specific monoclonal antibody because it contains multiple, distinct epitopes but only a single copy of each epitope. Myoglobin thus can form a crosslinked lattice structure with polyclonal antisera but not with monoclonal antisera. The principles that underlie precipitation reactions are presented because they are essential for an understanding of commonly used immunological assays. Although various modifications of the precipitation reaction were at one time the major types of assay used in immunology, they have been largely replaced by methods that are faster and, because they are far more sen- sitive, require only very small quantities of antigen or anti- body. Also, these modern assay methods are not limited to antigen-antibody reactions that produce a precipitate.

Precipitation Reactions in Fluids Yield a Precipitin Curve

A quantitative precipitation reaction can be performed by placing a constant amount of antibody in a series of tubes and adding increasing amounts of antigen to the tubes. At one time this method was used to measure the amount of antigen or antibody present in a sample of interest. After the precipitate forms, each tube is centrifuged to pellet the precipitate, the supernatant is poured off, and the amount of precipitate is measured. Plotting the amount of precipitate against increasing antigen concentrations yields a precipitin curve. Excess of either antibody or antigen interferes with maximal precipitation, which occurs in the so-called equivalence zone, within which the ratio of antibody to antigen is optimal. As a large multimolecular lattice is formed at equivalence, the complex increases in size and precipitates out of solution. Under conditions of antibody excess or antigen excess, extensive lattices do not form and precipitation is inhibited. Although the quantitative precipitation reaction is seldom used exper- imentally today, the principles of antigen excess, antibody ex- cess, and equivalence apply to many Ag-Ab reactions Precipitation Reactions in Gels Yield Visible Precipitin Lines Immune precipitates can form not only in solution but also in an agar matrix.When antigen and antibody diffuse toward one another in agar, or when antibody is incorporated into the agar and antigen diffuses into the antibody-containing matrix, a visible line of precipitation will form. As in a precipitation reaction in fluid, visible precipitation occurs in the region of equivalence, whereas no visible precipitate forms in regions of antibody or antigen excess. Two types of immunodiffusion re actions can be used to

determine relative concentrations of antibodies or antigens, to compare antigens, or to determine their relative purity of an antigen preparation. They are radial im-munodiffusion (the Mancini method) and double immun- odiffusion (the Ouchterlony method); both are carried out in a semisolid medium such as agar. In radial immunodiffusion, an antigen sample is placed in a well and allowed to diffuse into agar containing a suitable dilution of an antiserum. As the antigen diffuses into the agar, the region of equivalence is es- tablished and a ring of precipitation, a precipitin ring, formsaround the well. The area of the precipitin ring is proportional to the concentration of antigen. By comparing the area of the precipitin ring with a standard curve (obtained by measuring the precipitin areas of known concen-trations of the antigen), the concentration of the antigen sam-ple can be determined. In the Ouchterlony method, both antigen and antibody diffuse radially from wells toward each other, thereby establishing a concentration gradient. As equiv-alence is reached, a visible line of precipitation, a precipitin line, form

Immunoelectrophoresis Combines Electrophoresis and Double Immunodiffusion

In immunoelectrophoresis, the antigen mixture is first elec- trophoresed to separate its components by charge. Troughs are then cut into the agar gel parallel to the direction of the electric field, and antiserum is added to the troughs. Antibody and antigen then diffuse toward each other and produce lines of precipitation where they meet in appropri- ate proportions (Figure 6-6a). Immunoelectrophoresis is used in clinical laboratories to detect the presence or absence of proteins in the serum. A sample of serum is elec- trophoresed, and the individual serum components are identified with antisera specific for a given protein or im-munoglobulin class. This technique is useful indetermining whether a patient produces abnormally low amounts of one or more isotypes, characteristic of certain immunodeficiency diseases. It can also show whether a pa-tient overproduces some serum protein, such as albumin, immunoglobulin, or transferrin. The immunoelectropho- retic pattern of serum from patients with multiple myeloma, for example, shows a heavy distorted arc caused by the large amount of myeloma protein, which is monoclonal Ig and therefore uniformly charged. Because immu- noelectrophoresis is a strictly qualitative technique that only detects relatively high antibody concentrations (greater than several hundred g/ml), it utility is limited to the detection of quantitative abnormalities only when the departure fromnormal is striking, as in immunodeficiency states and im- munoproliferative disorders. A related quantitative technique, rocket electrophore-sis, does permit measurement of antigen levels. In rocket electrophoresis, a negatively charged antigen is elec-trophoresed in a gel containing antibody. The precipitate formed between antigen and antibody has the shape of a rocket, the height of which is proportional to the concen- tration of antigen in the well. One limitation of rocket electrophoresis is the need for the antigen to be negatively charged for electrophoretic movement within the agar matrix. Some proteins, immunoglobulin for example, are not sufficiently charged to be quantitatively analyzed by rocket electrophoresis; nor is it possible to measure the amounts of several antigens in a mixture at the same time.

Agglutination Reactions

The interaction between antibody and a particulate antigen results in visible clumping called agglutination. Antibodies that produce such reactions are called agglutinins. Agglutination reactions are similar in principle to precipitation reactions; they depend on the cross linking of polyvalent antigens. Just as an excess of antibody inhibits precipitation reactions, such excess can also inhibit agglutination reactions; this inhibition is called the prozone effect. Because prozone effects can be en- countered in many types of immunoassays, understanding the basis ofthis phenomenon is of general importance. Several mechanisms can cause the prozone effect. First, at high antibody concentrations, the number of antibody bind- ing sites may greatly exceed the number of epitopes. As a result, most antibodies bind antigen only univalently instead of multivalently. Antibodies that bind univalently cannot crosslink one antigen to another. Prozone effects are readily diagnosed by performing the assay at a variety of antibody (or antigen) concentrations. As one dilutes to an optimum antibody concentration, one sees higher levels of agglutina- tion or whatever parameter is measured in the assay being used. When one is using polyclonal antibodies, the prozone effect can also occur for another reason. The antiserum may contain high concentrations of antibodies that bind to the antigen but do not induce agglutination; these antibodies, called incomplete antibodies, are often of the IgG class. At high concentrations of IgG, incomplete antibodies may oc- cupy most of the antigenic sites, thus blocking access by IgM, which is a good agglutinin. This effect is not seen with agglu- tinating monoclonal antibodies. The lack of agglutinating activity of an incomplete antibody may be due to restrictedflexibility in the hinge region, making it difficult for the anti- body to assume the required angle for optimal cross-linking of epitopes on two or more particulate antigens. Alterna- tively, the density of epitope distribution or the location of some epitopes in deep pockets of a particulate antigen may make it difficult for the antibodies specific for these epitopes to agglutinate certain particulate antigens. When feasible, the solution to both of these problems is to try different antibod- ies that may react with other epitopes of the antigen that do not present these limitations.

Hemagglutination Is Used in Blood Typing

Agglutination reactions are routinely performed to type red blood cells (RBCs). In typing for the ABO bantigens, RBCs are mixed on a slide with antisera to the A or B blood-group antigens. If the antigen is present on the cells, they agglutinate, forming a visible clump on the slide. Determination of which antigens are present on donor and recipient RBCs is the basis for matching blood types for transfusions.

Bacterial Agglutination Is Used To Diagnose Infection

A bacterial infection often elicits the production of serum antibodies specific for surface antigens on the bacterial cells. The presence of such antibodies can be detected by bacterial agglutination reactions. Serum from a patient thought to be infected with a given bacterium is serially diluted in an array of tubes to which the bacteria is added. The last tube showing visible agglutination will reflect the serum antibody titer of the patient. The agglutinin titer is defined as the reciprocal

of the greatest serum dilution that elicits a positive agglutina- tion reaction. For example, if serial twofold dilutions of serum are prepared and if the dilution of 1/640 shows agglu- tination but the dilution of 1/1280 does not, then the agglu- tination titer of the patient's serum is 640. In some cases serum can be diluted up to 1/50,000 and still show agglutina- tion of bacteria. The agglutinin titer of an antiserum can be used to diagnose a bacterial infection. Patients with typhoid fever, for ex- ample, show a significant rise in the agglutination titer to Salmonella typhi. Agglutination reactions also provide a way to type bacteria. For instance, different species of the bacterium Salmonella can be distinguished by agglutination reactions with a panel of typing antisera.

Passive Agglutination Is Useful with Soluble Antigens

The sensitivity and simplicity of agglutination reactions can be extended to soluble antigens by the technique of passive hemagglutination. In this technique, antigen-coated red blood cells are prepared by mixing a soluble antigen with red blood cells that have been treated with tannic acid or chromium chloride, both of which promote adsorption of the antigen to the surface of the cells. Serum containing anti- body is serially diluted into microtiter plate wells, and the antigen-coated red blood cells are then added to each well; agglutination is assessed by the size of the characteristic spread pattern of agglutinated red blood cells on the bottom of the well, like the pattern seen in agglutination reactions Over the past several years, there has been a shift away from red blood cells to synthetic particles, such as latex beads, as matrices for agglutination reactions. Once the anti- gen has been coupled to the latex beads, the preparation can either be used immediately or stored for later use. The use of synthetic beads offers the advantages of consistency, uniformity, and stability. Furthermore, agglutination reac-tions employing synthetic beads can be read rapidly, often within 3 to 5 minutes of mixing the beads with the test sam-ple.Whether based on red blood cells or the more convenient and versatile synthetic beads, agglutination reactions are simple to perform, do not require expensive equipment, and can detect small amounts of antibody.

Radioimmunoassay

One of the most sensitive techniques for detecting antigen or antibody is radioimmunoassay (RIA). The technique was first developed in 1960 by two endocrinologists, S. A. Berson and Rosalyn Yalow, to determine levels of insulin–anti-in- sulin complexes in diabetics. Although their technique en- countered some skepticism, it soon proved its value for measuring hormones, serum proteins, drugs, and vitamins at concentrations of 0.001 micrograms per milliliter or less. In 1977, some years after Berson's death, the significance of the technique was acknowledged by the award of a Nobel Prize to Yalow. The principle of RIA involves competitive binding of ra-diolabeled antigen and unlabeled antigen to a high-affinity antibody. The labeled antigen is mixed with antibody at a concentration that saturates the antigen-binding sites of the antibody. Then test samples of unlabeled antigen of un- known concentration are added in progressively larger amounts. The antibody does not distinguish labeled from unlabeled antigen, so the two kinds of antigen compete for available binding sites on the antibody. As the concentration of unlabeled antigen increases, more labeled antigen will be displaced from the binding sites. The

decrease in the amount of radiolabeled antigen bound to specific antibody in the presence of the test sample is measured in order to determine the amount of antigen present in the test sample. The antigen is generally labeled with a gamma-emitting isotope such as 125I, but beta-emitting isotopes such as tri- tium (3H) are also routinely used as labels. The radiola- beled antigen is part of the assay mixture; the test sample may be a complex mixture, such as serum or other body fluids, that contains the unlabeled antigen. The first step in setting up an RIA is to determine the amount of antibody needed to bind 50%–70% of a fixed quantity of radioactive antigen (Ag∗) in the assay mixture. This ratio of antibody to Ag∗ is chosen to ensure that the number of epitopes presented by the labeled antigen always exceeds the total number of antibody binding sites. Consequently, unlabeled antigen added to the sample mixture will compete with ra- diolabeled antigen for the limited supply of antibody. Even a small amount of unlabeled antigen added to the assay mixture of labeled antigen and antibody will cause a de- crease in the amount ofradioactive antigen bound, and this decrease will be proportional to the amount of unlabeled antigen added. To determine the amount of labeled antigen bound, the Ag-Ab complex is precipitated to separate it from free antigen (antigen not bound to Ab), and the ra- dioactivity in the precipitate is measured. A standard curve can be generated using unlabeled antigen samples of known concentration (in place of the test sample), and from this plot the amount of antigen in the test mixture may be precisely determined.

Several methods have been developed for separating the bound antigen from the free antigen in RIA. One method in- volves precipitating the Ag-Ab complex with a secondary anti-isotype antiserum. For example, if the Ag-Ab complex contains rabbit IgG antibody, then goat anti-rabbit IgG will bind to the rabbit IgG and precipitate the complex. Another method makes use of the fact that protein A of Staphylococcus aureus has high affinity for IgG. If the Ag-Ab complex con- tains an IgG antibody, the complex can be precipitated by mixing with formalin-killed S. aureus. After removal of the complex by either of these methods, the amount of free la- beled antigen remaining in the supernatant can be measured in a radiation counter; subtracting this value from the total amount of labeled antigen added yields the amount of la- beled antigen bound.

Various solid-phase RIAs have been developed that make it easier to separate the Ag-Ab complex from the unbound antigen. In some cases, the antibody is covalently cross- linked to Sepharose beads. The amount of radiolabeled anti- gen bound to the beads can be measured after the beads have been centrifuged and washed. Alternatively, the antibody can be immobilized on polystyrene or polyvinylchloride wells and the amount of free labeled antigen in the supernatant can be determined in a radiation counter. In another ap- proach, the antibody is immobilized on the walls of mi- crotiter wells and the amount of bound antigen determined. Because the procedure requires only small amounts of sam- ple and can be conducted in small 96-well microtiter plates (slightly larger than a 3 5 card), this procedure is well suited for determining the concentration of a particular anti- gen in large numbers of samples. For example, a microtiter RIA has been widely used to screen for the presence of the he- patitis B virus. RIA screening of donor blood has sharply reduced the incidence of hepatitis B infections in re- cipients of blood

transfusions

Immunofluorescence

In 1944, Albert Coons showed that antibodies could be labeled with molecules that have the property of fluorescence. Fluorescent molecules absorb light of one wavelength (excitation) and emit light of another wavelength (emission). If antibody molecules are tagged with a fluorescent dye, or fluorochrome, immune complexes containing these fluores- cently labeled antibodies (FA) can be detected by colored light emission when excited by light of the appropriate wave- length. Antibody molecules bound to antigens in cells or tis- sue sections can similarly be visualized. The emitted light can be viewed with a fluorescence microscope, which is equipped with a UV light source. In this technique, known as im- munofluorescence, fluorescent compounds such as fluorescein and rhodamine are in common use, but other highly fluorescent substances are also routinely used, such as phyco- erythrin, an intensely colored and highly fluorescent pig- ment obtained from algae. These molecules can be conjugated to the Fc region of an antibody molecule withoutaffecting the specificity of the antibody. Each of the fluo- rochromes below absorbs light at one wavelength and emits light at a longer wavelength. Fluorescein, an organic dye that is the most widely used label for immunofluorescence procedures, absorbs blue light (490 nm) and emits an intense yellow-green luorescence (517 nm).Rhodamine, another organic dye, absorbs in the yellow-green range (515 nm) and emits a deep redfluorescence (546 nm). Because it emits fluorescence at a longer wavelength than fluorescein, it can be used in two-color immunofluorescence assays. An antibody specific to one determinant is labeled with fluorescein, and an antibody recognizing a different antigen is labeled with rhodamine. The location of the fluorescein-tagged antibody will be visible by its yellow- green color, easy to distinguish from the red color emitted where the rhodamine-tagged antibody has bound. By conjugating fluorescein to one antibody and rhodamine to another antibody, one can, for example, visualize simultaneously two different cell-membrane antigens on the same cell.

Phycoerythrin is an efficient absorber of light (~30-fold greater than fluorescein) and a brilliant emitter of red fluorescence, stimulating its wide use as a label for immunofluorescence. Fluorescent-antibody staining of cell membrane mole- cules or tissue sections can be direct or indirect. In direct staining, the specific antibody (the primary anti- body) is directly conjugated with fluorescein; in indirect staining, the primary antibody is unlabeled and is detected with an additional fluorochrome-labeled reagent. A num- ber of reagents have been developed for indirect staining. The most common is a fluorochrome-labeled secondary antibody raised in one species against antibodies of another species, such as fluorescein-labeled goat anti-mouse im- munoglobulin. Indirect immunofluorescence staining has two advan- tages over direct staining. First, the primary antibody does not need to be conjugated with a fluorochrome. Because the supply of primary antibody is often a limiting factor, indirect methods avoid the loss of antibody that usually occurs dur- ing the conjugation reaction. Second, indirect methods in- crease the sensitivity of staining because multiple molecules of the fluorochrome reagent bind to each

primary antibody molecule, increasing the amount of light emitted at the loca- tion of each primary antibody molecule. Immunofluorescence has been applied to identify a num- ber of subpopulations of lymphocytes, notably the CD4and CD8 T-cell subpopulations. The technique is also suit- able for identifying bacterial species, detecting Ag-Ab com- plexes in autoimmune disease, detecting complement components in tissues, and localizing hormones and other cellular products stained in situ. Indeed, a major application of the fluorescent-antibody technique is the localization of antigens in tissue sections or in subcellular compartments. Because it can be used to map the actual location of target antigens, fluorescence microscopy is a powerful tool for relat- ing the molecular architecture of tissues and organs to their overall gross anatomy.

Immunoelectron Microscopy

The fine specificity of antibodies has made them powerful tools for visualizing specific intracellular tissue components by immunoelectron microscopy. In this technique, an elec- tron-dense label is either conjugated to the Fc portion of a specific antibody for direct staining or conjugated to an anti- immunoglobulin reagent for indirect staining. A number of electron-dense labels have been employed, including ferritin and colloidal gold. Because the electron-dense label absorbs electrons, it can be visualized with the electron microscope as small black dots. Inthe case of immunogold labeling, dif- ferent antibodies can be conjugated with gold particles of different sizes, allowing identification of several antigens within a cell by the different sizes of the electron-dense gold particles attached to the antibodies.

1. Overview of ELISA in Relation to Other Disciplines

This chapter examines what areas of science are needed to allow optimal use of ELISA and notes their relationships. This information is useful for students and those instructing students. Diagrams, with brief descriptions of key points, are used to illustrate such relationships. Inherent in this exercise are considerations of the exact requirements by the operators in using the ELISA. Attention to increasing knowledge in those areas highlighted is essential both in developmental work to produce a working ELISA and in the ultimate
value of any test devised. A good deal of attention should be directed at defining, as clearly as possible, the objectives for the ELISA. The development of a diagnostic test for a specific disease requires that all other data pertaining to the biology of that disease, e.g., antigenicity and structure of the agent, antibody production in different animals following infection, qualitative assessment of antibodies by different assays, and availability of standard or control sera, are known. Some attention must be paid to the laboratory facilities available, e.g., equipment, reagents already developed, small laboratory animals, experimental large animals, cash to buy commercial products, and trained personnel. In this way, the chances of producing a sustainable test to solve the defined problem are significantly greater than when a test is developed by a dabbling technique with poor or no forward planning.

Figure 1 emphasizes that we are considering the heterogeneous ELISA involving separation

steps and a solid phase. Four major advantages of ELISA are promoted, all of which add to the reasons that this form of ELISA has been, and will continue to be, successful.

Figure 2 deals with the systematics of the ELISA and shows the various stages needed and factors important in those stages.

Figure 3 emphasizes that using the equipment to perform ELISAs requires skills, and that both physical and mental processes are needed. **Figure 3** also indicates that instruments need to be maintained for optimal performance.

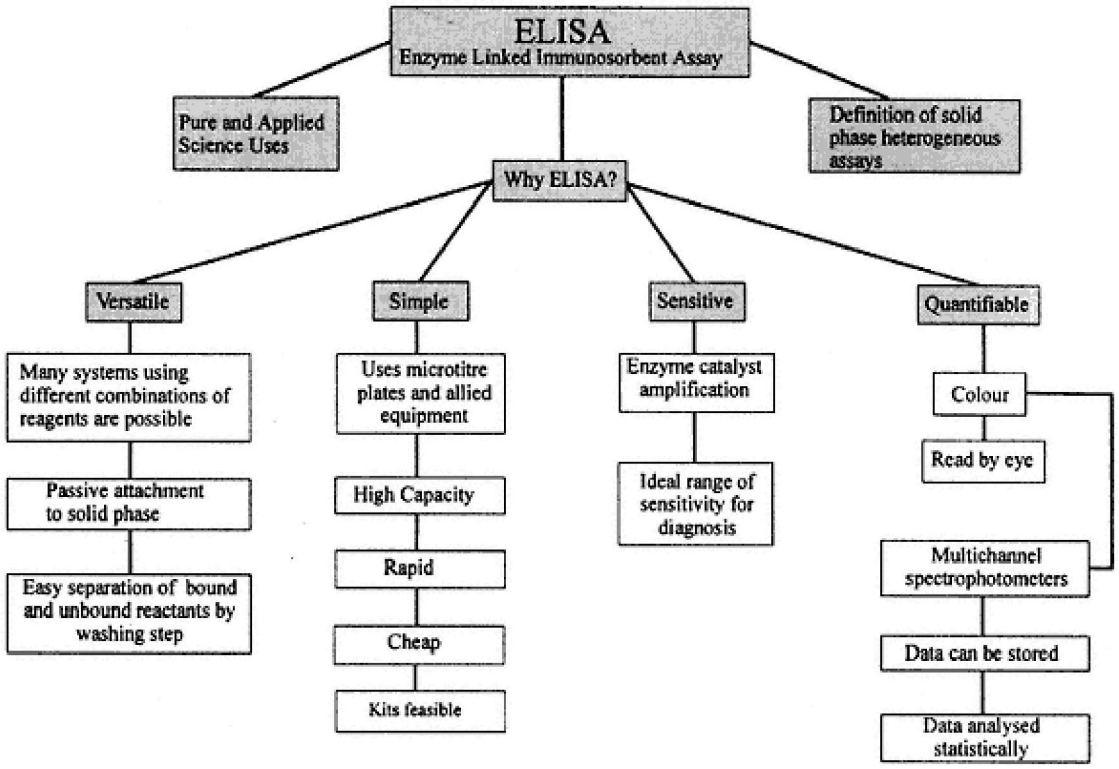


Fig. 1.
Scheme showing features of ELISA that makes it advantageous for a wide range of applications.

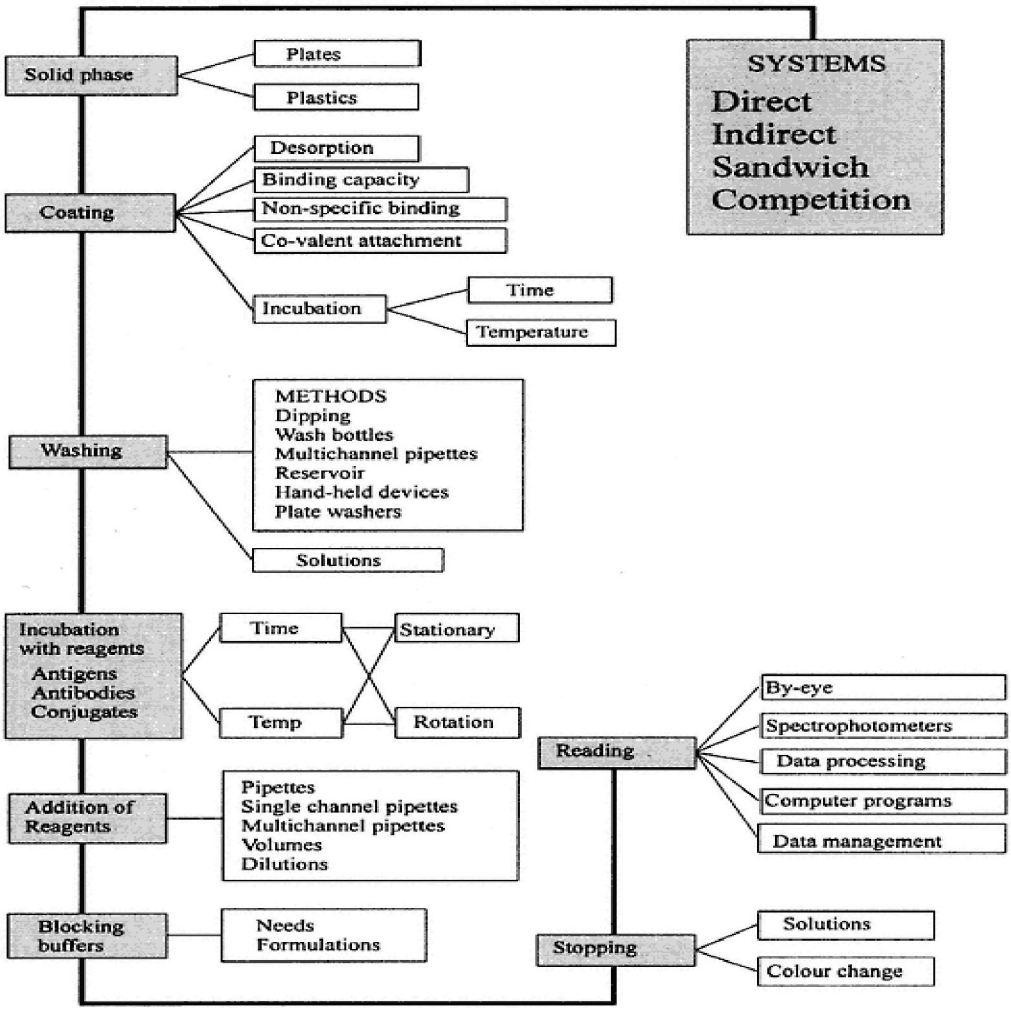
Figure 4 deals with some of the enzymatic systems in the ELISA, and illustrates areas that need to be understood in order to allow optimal performance to be maintained. Understanding enzyme kinetics, catalysis reactions, hazards, and buffer formulation (pH control) are all essential.

Figure 5 illustrates the use of ELISA's in binding and inhibition/competition interactions to allow an understanding of a problem. It is essential that the chemical and physical nature of antibodies and antigens are understood, particularly in cases of developmental work. As full an

understanding of the antigenic properties of agents being examined is needed to allow maximum exploitation of ELISA, particularly if the results are ever to be understood.

Figure 6 deals with data processing and analysis. Various essential statistical parameters must be elucidated, if data are to be interpreted. This is true in understanding how to calculate the variance in a result, and also for examining populations. Such studies actually define any ELISA's performance, allowing confidence in results to be measured, thereby allowing a meaning to be placed on results. The concepts of controlling assays with references to standards is also needed.

Figure 7 extends the use of statistical understanding into epidemiological needs. A common use of ELISA is to provide data on populations studied. The areas of sampling (size, number, and so forth) are vital when planning disease control strategies. These simplified overviews should be used as reference points when considering the development and specific use of any ELISA. They



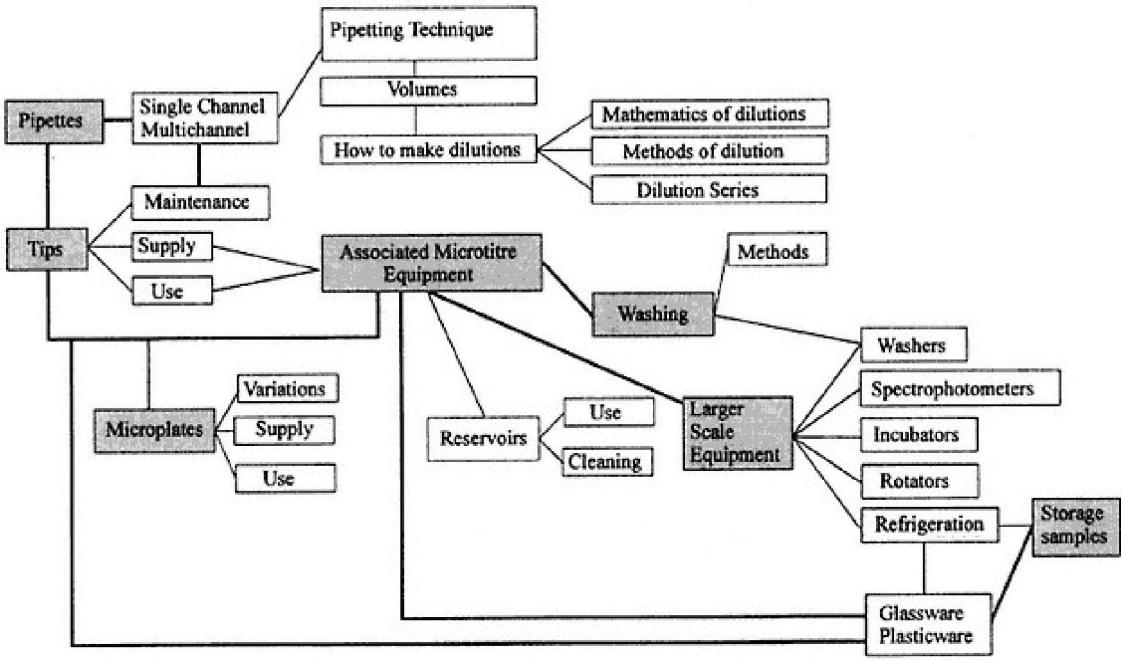


Fig. 3.
Scheme relating equipment needs and skills for ELISA.

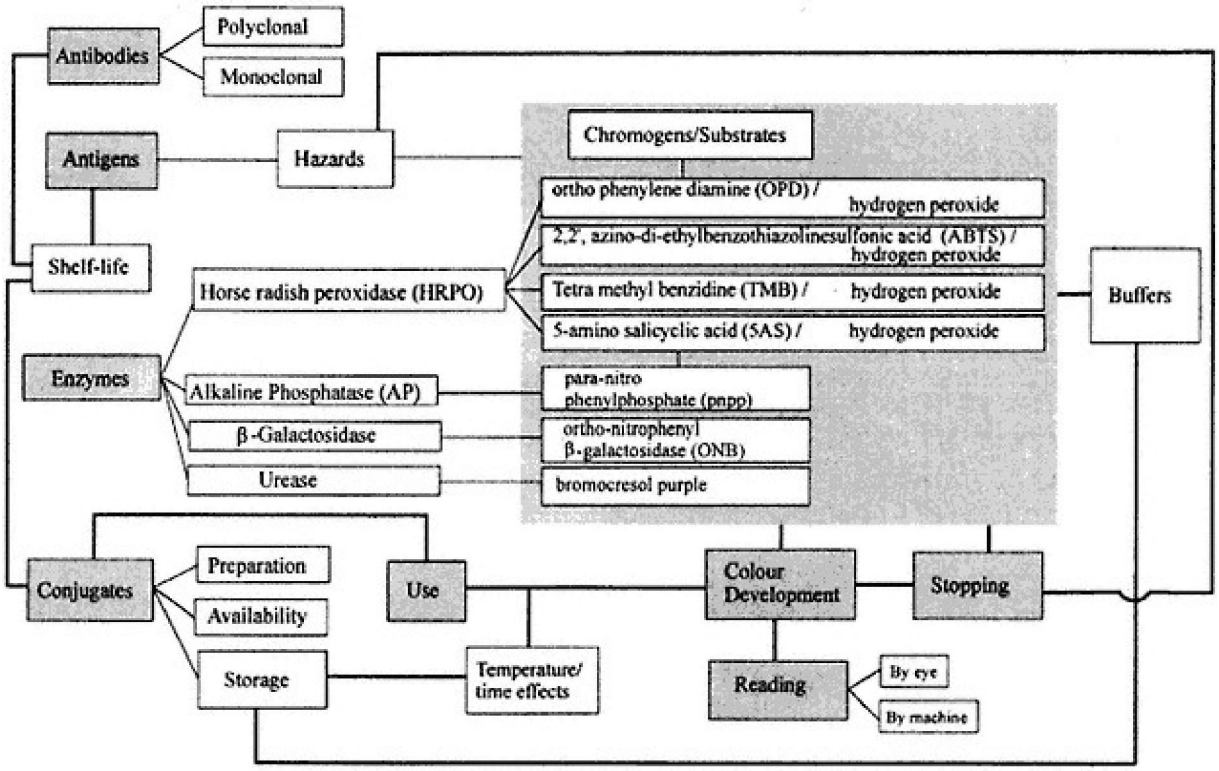


Fig. 4.
Relationships of enzyme systems to components of ELISA. readers with limited exposure to ELISA, particularly after studying the details in later chapters. They are also useful for trainers in establishing areas of competence in students.

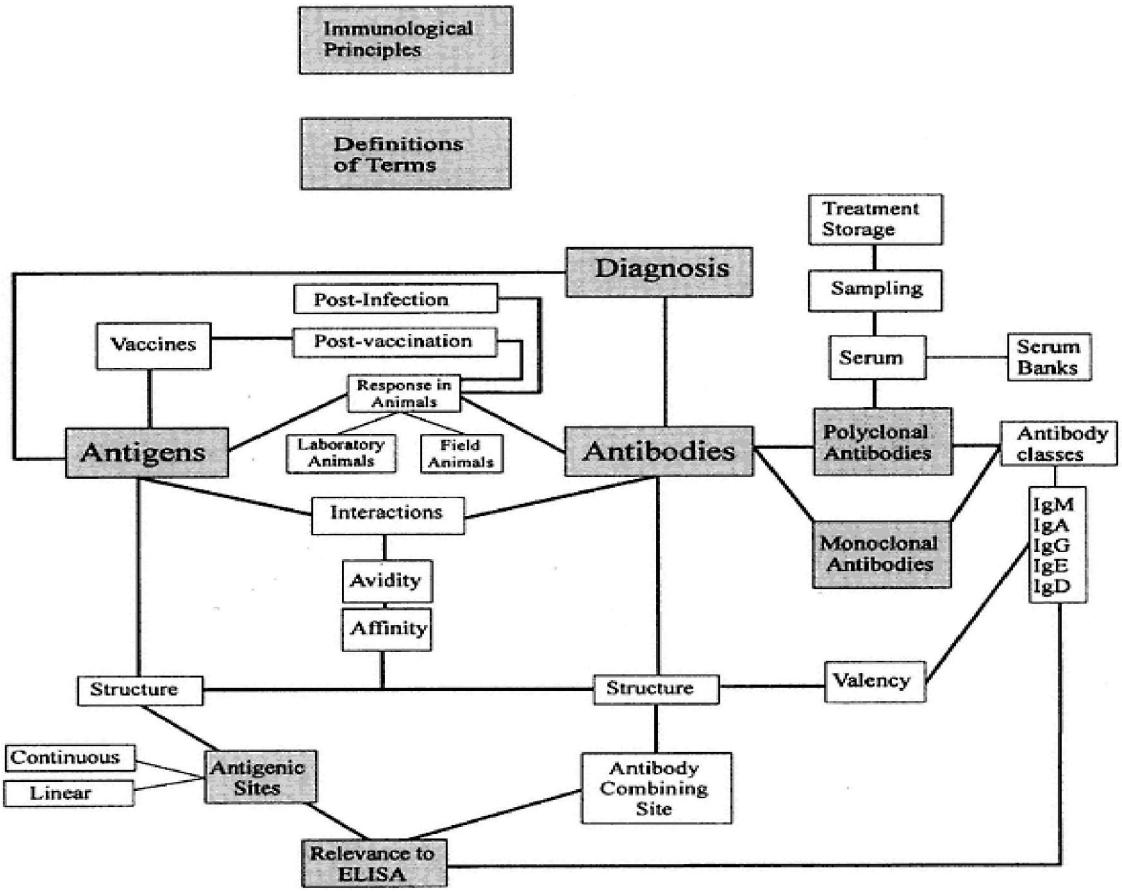


Fig. 5.
Requires features in immunological understanding in order to establish ELISA.

These are the key points to keep in mind at this early stage when considering then use of ELISA:
1. The ELISA is a tool to solve a problem.
2. Any problem should be defined, as clearly as possible, with reference to all previous work defining the specific agent involved and related agents.
3. Other methods for analyzing the problem should be reviewed, particularly when tests are already established. This has implications if the ELISA is to replace existing tests.
4. The capacity for testing has to be addressed. For example, when an ELISA may be used on a large scale (kit), then sufficient reagents, standard sera, conjugates (batches), and antigen preparations must be available. Research leading to successful assays in which reagents are difficult to prepare on a large scale, require extensive expertise to formulate, or are reliant on a specific limited batch of a commercial reagent are not sustainable.

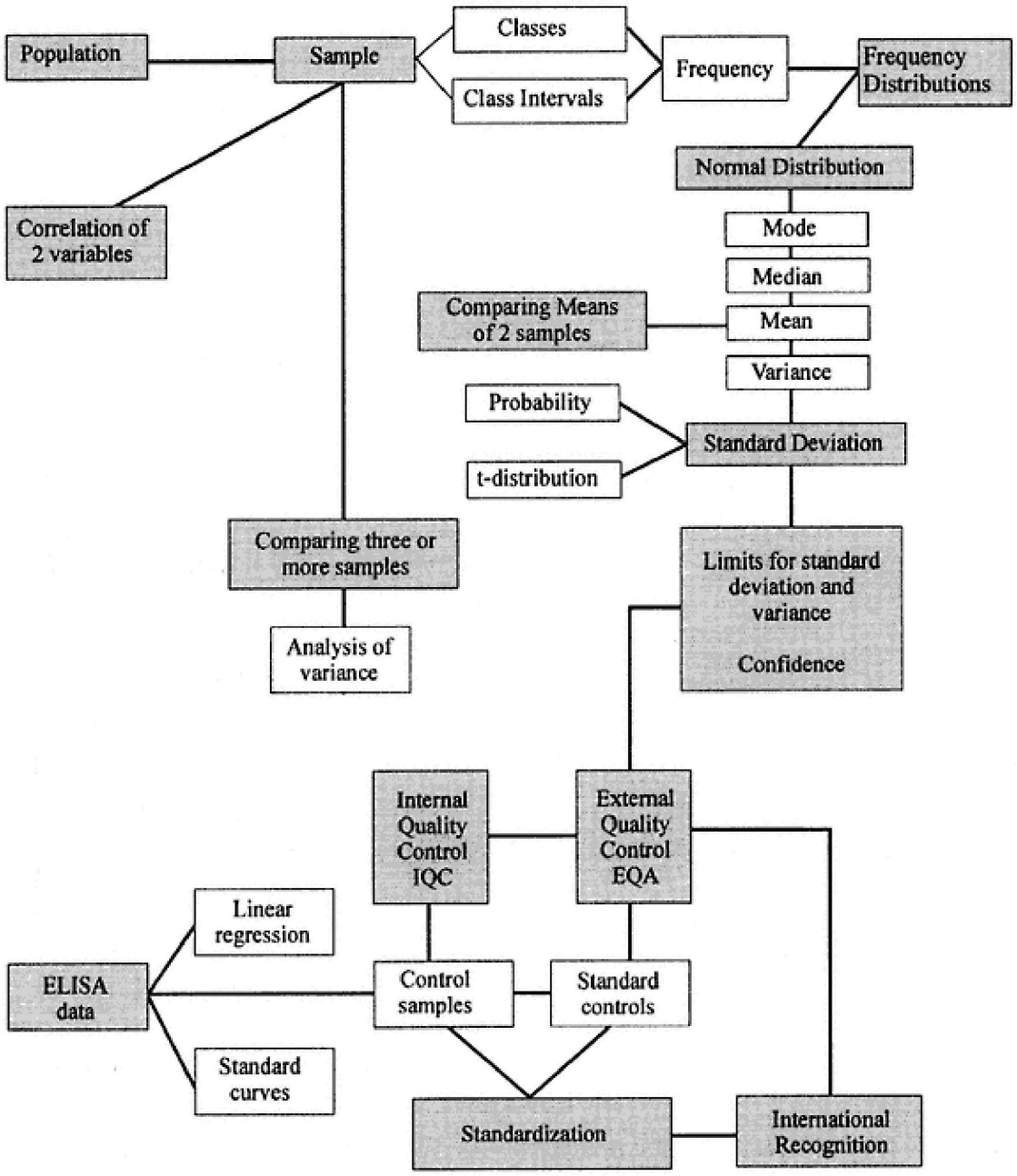


Fig. 6.
Important statistical factors needed to make use of ELISA. Note the links to quality control (internal) and the establishment of confidence in test results. Increasingly, assays need international recognition.

5. When a test may be of use to a wider group of scientists, the possible conditions (laboratory facilities, expertise) should be considered when developing assays. Such technology transfer factors are relevant, particularly in laboratories in developing countries.

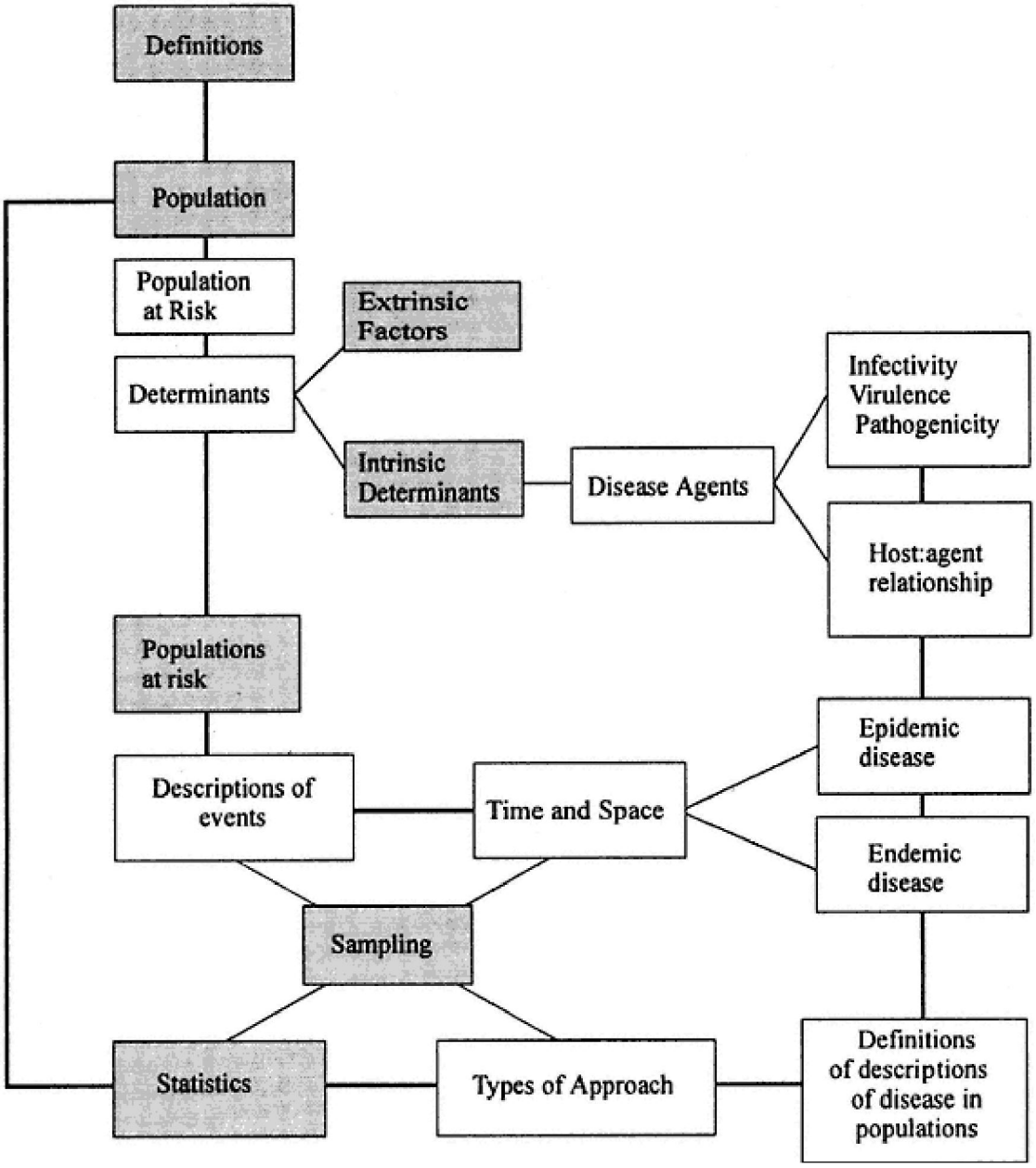


Fig. 7.

Scheme relating basic areas in epidemiology that need to be understood in the context of data obtained from ELISA. Note the strong link with statistics/sampling, which is inherent in the test design.The knowledge and skills required to both perform ELISA and make use of the data have to be gained through a variety of sources, including textbooks. As with all other techniques, the ultimate benefit is not the technique in itself, but the meaningful gathering and analysis of the data. One factor not included in all these examples is that of common sense: the ability to really consider what one is doing, and why, and not to overlook the simplicity of what is needed by being blinded by the technology for its own sake. Most problems are relatively simple to examine after some clear thought. Thus, the good ELISA person will consider the problem first, obtain the necessary technical skills and equipment to perform a test, and then obtain data that is from a planned perspective. As much data from all other tests and the scientific literature should also be sought. This is true for an assay developer, as well as a person using a supplied, predetermined kit. The skills required by the use of a kit are no less than those of the developer; indeed, a kit in the hands of an unskilled worker is often useless. The majority (90%) of problems observed in the practice of ELISA are operator faults caused by lack of common sense, failure to appreciate the need to stick to instructions, sloppy technique, or poorly maintained equipment. Most of the remaining percentage is caused by poor-quality water.

2. Systems in ELISA

This chapter defines the terms and examines the configurations used for most applications of ELISA. Such a chapter is important because the possibilities inherent in the systems of ELISA must be understood in order to maximize their versatility in assay design. All heterogeneous systems have three basic parameters:

1. One reactant is attached to a solid phase, usually a plastic microtiter plate with an 8 ¡Á 12-well format.
2. Separation of bound and free reagents, which are added subsequently to the solid phase¨Cattached substance, is by a simple washing step.
3. Results are obtained through the development of color.

1. Definition of Terms

Immunoassays involve tests using antibodies as reagents. Enzyme immunoassays make use of enzymes attached to one of the reactants in an immunoassay to allow quantification through the development of color after the addition of a suitable substrate/chromogen. As indicated, ELISAs involve the stepwise addition and reaction of reagents to a solid phase-bound substance, through incubation and separation of bound and free reagents using washing steps. An enzymatic reaction is utilized to yield color and to quantify the reaction, through the use of an enzyme-labeled reactant.

Table 1 gives definitions of terms used in ELISA. These terms are greatly amplified throughout the subsequent text.

Solid phase - Usually a microtiter plate well. Specially prepared ELISA plates are commercially available. These have an 8 X 12 well format and can be used with a wide variety of specialized equipment designed for rapid manipulation of samples including multichannel pipets.

Adsorption - The process of adding an antigen or antibody, diluted in buffer, so that it attaches passively to the solid phase on incubation. This is a simple way for immobilization of one of the reactants in the ELISA and one of the main reasons for its success.Washing - The simple flooding and emptying of the wells with a buffered solution to separate bound (reacted) from unbound (unreacted) reagents in the ELISA. Again, this is a key element to thesuccessful exploitation of the ELISA.Antigens - A protein or carbohydrate that when injected into animals elicits the production of antibodies. Such antibodies can react specifically with the antigen used and therefore can be used to detect that antigen.Antibodies - Produced in response to antigenic stimuli. These are mainly protein in nature. In turn, antibodies are antigenic. Antispecies antibodies - Produced when proteins (including antibodies) from one species are injected into another species. Thus, guinea pig serum injected into a rabbit elicits the production of rabbit anti¨Cguinea pig antibodies.Enzyme - A substance that can react at low concentration as a catalyst to promote a specific reaction. Several specific enzymes are commonly used in ELISA with their specific substrates.Enzyme conjugate - An enzyme that is attached irreversibly to a protein, usually an antibody. Thus, an example of antispecies enzyme conjugate is rabbit antiguinea linked to horseradish peroxidase. Substrate A chemical compound with which an enzyme reacts specifically. This reaction is used, in some way, to produce a signal that is read as a color reaction (directly as a color change of the substrate or indirectly by its effect on another chemical).Chromophore - A chemical that alters color as a result of an enzyme interaction with substrate.Stopping - The process of stopping the action of an enzyme on a substrate. It has the effect of stopping any further change in color in the ELISA.Reading - Measurement of color produced in the ELISA. This is quantified using special spectrophotometers reading at specific wavelengths for the specific colors obtained with particularenzyme/chromophore systems. Tests can be assessed by eye.

2. Basic Systems of ELISA

This section describes the principles involved in the many configurations possible in ELISA. The terminology here may not always agree with that used by others, and care is needed in defining assays by name only. The specific assay parameters must always be examined carefully in the literature. The

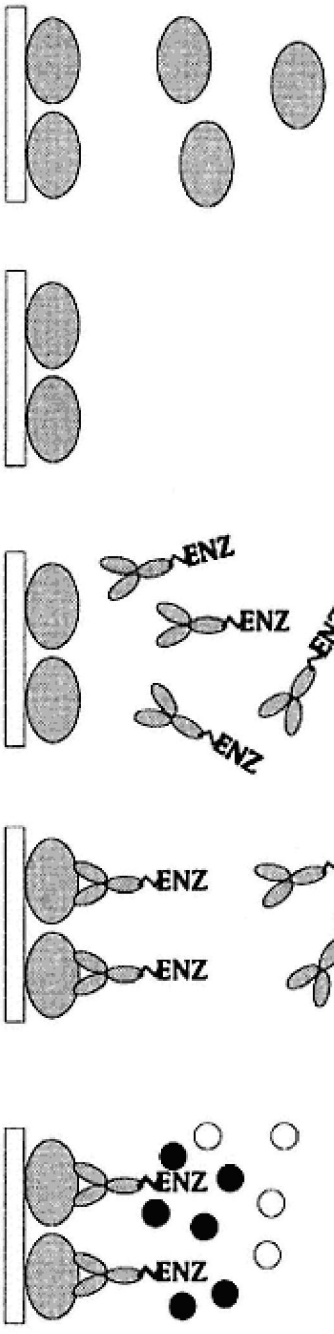
Table 2
Definition of Symbols or Terms Used to Describe Assays

Symbol/term	Definition
▨	Solid-phase microtiter well
---	Attachment to solid phase by passive adsorption
Ag	Antigen
Ab	Antibody
AB	Antibody (different species donor than Ab)
Anti-Ab	Antispecies antiserum against species from donor Ab
Anti-AB	Antispecies antiserum against species from donor AB
**Enz	Enzyme linked to reactant
S	Substrate/chromophore system
WASH	Washing step
°C	Incubation
READ	Read color in spectrophotometer
+	Addition of reagents
◆◆	Binding of reagents
STOP	Stopping of color development

following set of definitions attempts to clear up the myriad of published approaches to describing the systems used in a few words such as "double-sandwich competitive ELISA" and "indirect sandwich inhibition ELISA." The aim is to have a clear approach. Three main methods form the basis to all ELISAs:

1. Direct ELISA
2. Indirect ELISA
3. Sandwich ELISA

All three systems can be used to form the basis of a group of assays called competition or inhibition ELISAs. The systems (arrangement and use of reagents in the test), are illustrated herein through the use of symbols (as defined in **Table 2**), as well as in terms. In this way, it is hoped that the reader will gain a clear idea of the various systems and their relative advantages and disadvantages. A key feature of the flexibility of ELISA is that more than one system can be used to measure the same thing. This allows some scope to adapt assays to suit available reagents as well as to note areas of improvement through the identification of the need to prepare additional reagents¡ªe.g., that monoclonal antibodies (mAbs) may be needed to give an assay the required specificity, or that a particular antispecies conjugate against a subclass of immunoglobulin (Ig) is required.



(i)

Antigen is added to the solid phase and adsorbs passively on incubation.

(ii)

After incubation, any non-bound antigen is washed away leaving the 'coated' solid phase.

(iii)

Antibodies specific for the antigen and labeled with an enzyme (conjugate) are added, and incubated.

(iv)

The conjugate binds with antigen on solid phase.

Any unbound (free) conjugate is washed away.

(v)

A substrate/chromophore solution is added and the enzyme catalyses the reaction to give a colored product.

The reaction is terminated after a certain time (Stopped) and the colour quantified (read) using a spectrophotometer.

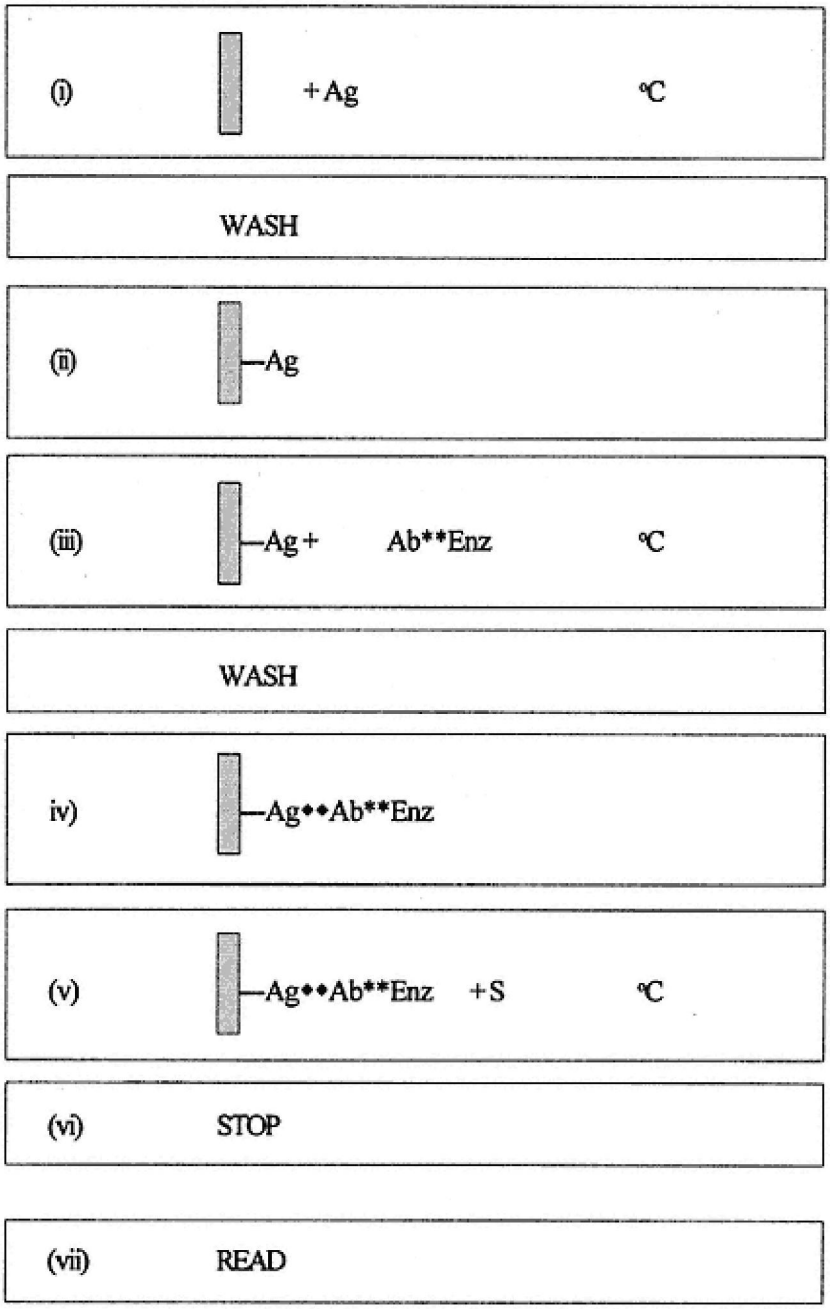
Fig. 1.

Direct ELISA. Antigen is attached to the solid phase by passive adsorption. After washing, enzyme-labeled antibodies are added. After an incubation period and washing, a substrate system is added and color is allowed to develop. Practical details of the various stages, e.g., solid phase, buffers, incubation, and conjugates are dealt with in detail in Chapters 3 and 4.

2.1
Direct ELISA

Direct ELISA can be regarded as the simplest form of the ELISA, and is illustrated in **Fig. 1** and in the following diagram.

Direct ELISA



Antigen is diluted in a buffer (stage i), commonly a high pH (9.6) carbonate/ bicarbonate buffer

or neutral phosphate-buffered saline (PBS). The key is that the buffer contains no other proteins that might compete with the target antigen for attachment to the plastic solid phase. Antigens are mainly protein in nature and will attach passively to the plastic during a period of incubation. The temperature and time of the incubation is not so critical, but standardization of conditions is vital, and the use of incubators at 37¡ãC is favored (since they are widely available in laboratories). After incubation, any excess antigen is removed by a simple washing step (stage ii), by flooding and emptying the wells, using a neutral buffered solution (e.g., PBS). Antibodies conjugated with an enzyme can now be added (stage iii), and are directed specifically against antigenic sites on the solid phase¨Cbound reagent. The conjugated antibodies are diluted in a buffer containing some substance that inhibits passive adsorption of protein, but that still allows immunological binding. Such substances either are other proteins, which are added at a high concentration to compete for the solid-phase sites with the antibody protein, or are detergents at low concentration termed *blocking* agents, and the buffers they help formulate, which are termed *blocking buffers*. On incubation, antibodies bind to the antigen. Again, a simple washing step is then used to remove unbound antibodies (stage iv). Stage v involves the addition of a suitable substrate or substrate/chromogen combination for the particular enzyme attached to the antibodies. The objective is to allow development of a color reaction through enzymatic catalysis. The reaction is allowed to progress for a defined period, after which the reaction is stopped (stage vi) by altering the pH of the system, or adding an inhibiting reactant. Finally, the color is quantified by the use of a spectrophotometer reading (stage vii) at the appropriate wavelength for the color produced. This kind of system has severe limitations when used only in this form but has assumed great importance as the "target" system in competition and inhibition assays, particularly when mAbs are conjugated and/or highly defined antigens are used.

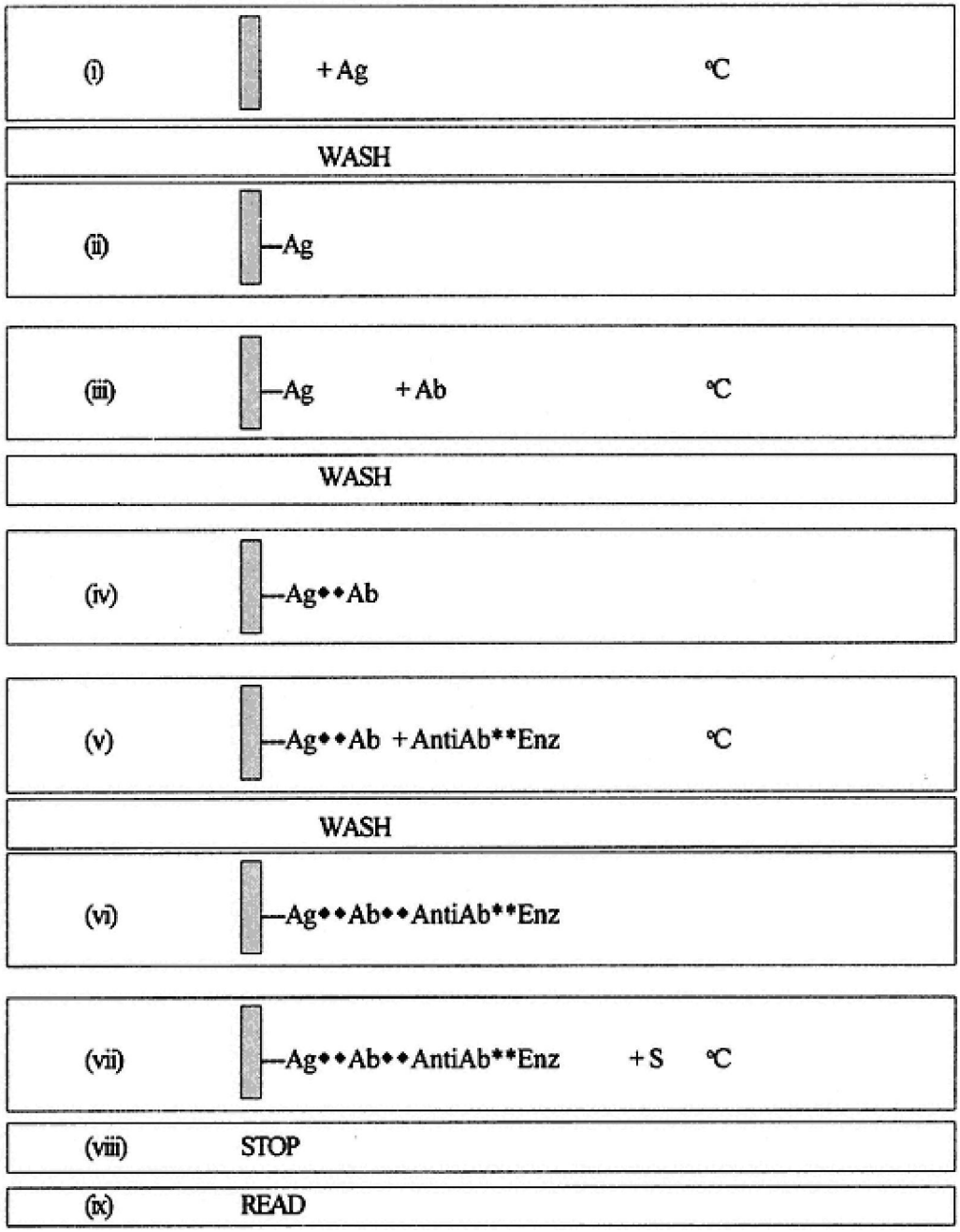
2.2

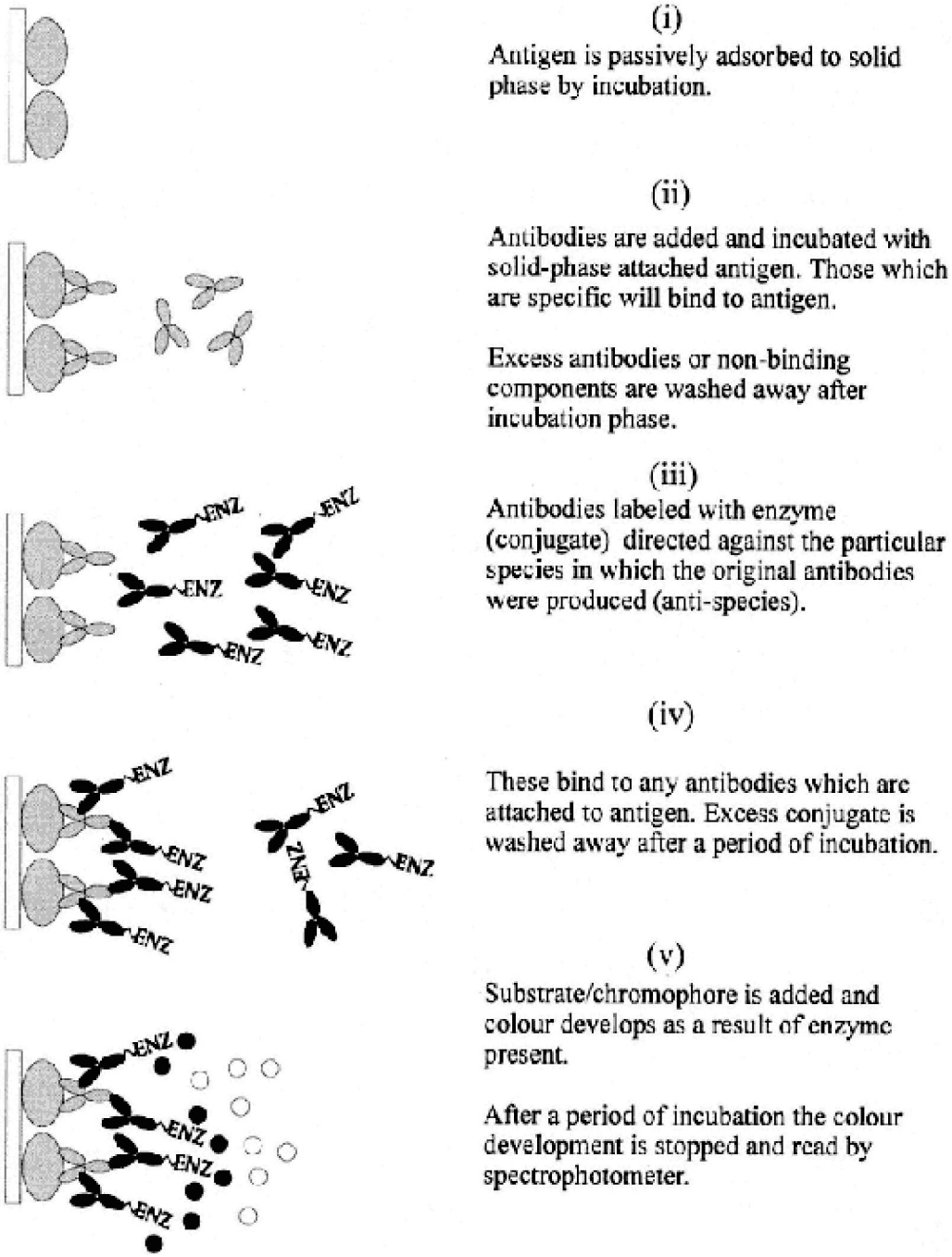
Indirect ELISA

Indirect ELISA is illustrated in the following diagram and in **Fig. 2**. Stages i and ii are similar to the direct system. Stage iii involves the addition of unlabeled detecting antibodies, which are diluted in a buffer to prevent nonspecific attachment of proteins in antiserum to solid phase (blocking buffer). This is followed by incubation and washing away of excess (unbound) antibodies, to achieve specific binding (stage iv). Stage v is the addition of the conjugate (enzyme-labeled), antispecies antibodies, diluted in blocking buffer, again followed by incubation and washing to achieve binding of conjugate (stage vi). Substrate/chromophore is then added to the bound conjugate (stage vii) and color develops, which is then stopped (stage viii) and read (stage ix) in a spectrophotometer. The indirect system is similar to the direct system in that antigen is directly attached to the solid phase and targeted by added antibodies (detecting antibodies). However, these added antibodies are not labelled with enzyme but are themselves targeted by antibodies linked to enzyme. Such antibodies are produced against the immunoglobulins of the species in which the detecting antibodies are produced and are termed antispecies conjugates. Thus, if the detecting antibodies were produced in rabbits, the enzyme-labeled antibodies would have to be antirabbit Igs in nature. This allows great flexibility in use of

antispecies conjugates in that different specificities of conjugate can be used to detect particular immunoglobulins binding in the assay, and there are literally thousands of commercially available conjugates available. For example, the antispecies conjugate could be anti-IgM, anti IgG1, IgG2, and so on.

Indirect ELISA





(i)
Antigen is passively adsorbed to solid phase by incubation.

(ii)
Antibodies are added and incubated with solid-phase attached antigen. Those which are specific will bind to antigen.

Excess antibodies or non-binding components are washed away after incubation phase.

(iii)
Antibodies labeled with enzyme (conjugate) directed against the particular species in which the original antibodies were produced (anti-species).

(iv)
These bind to any antibodies which are attached to antigen. Excess conjugate is washed away after a period of incubation.

(v)
Substrate/chromophore is added and colour develops as a result of enzyme present.

After a period of incubation the colour development is stopped and read by spectrophotometer.

Fig. 2.
Indirect ELISA. Antibodies from a particular species react with antigen attached to the solid

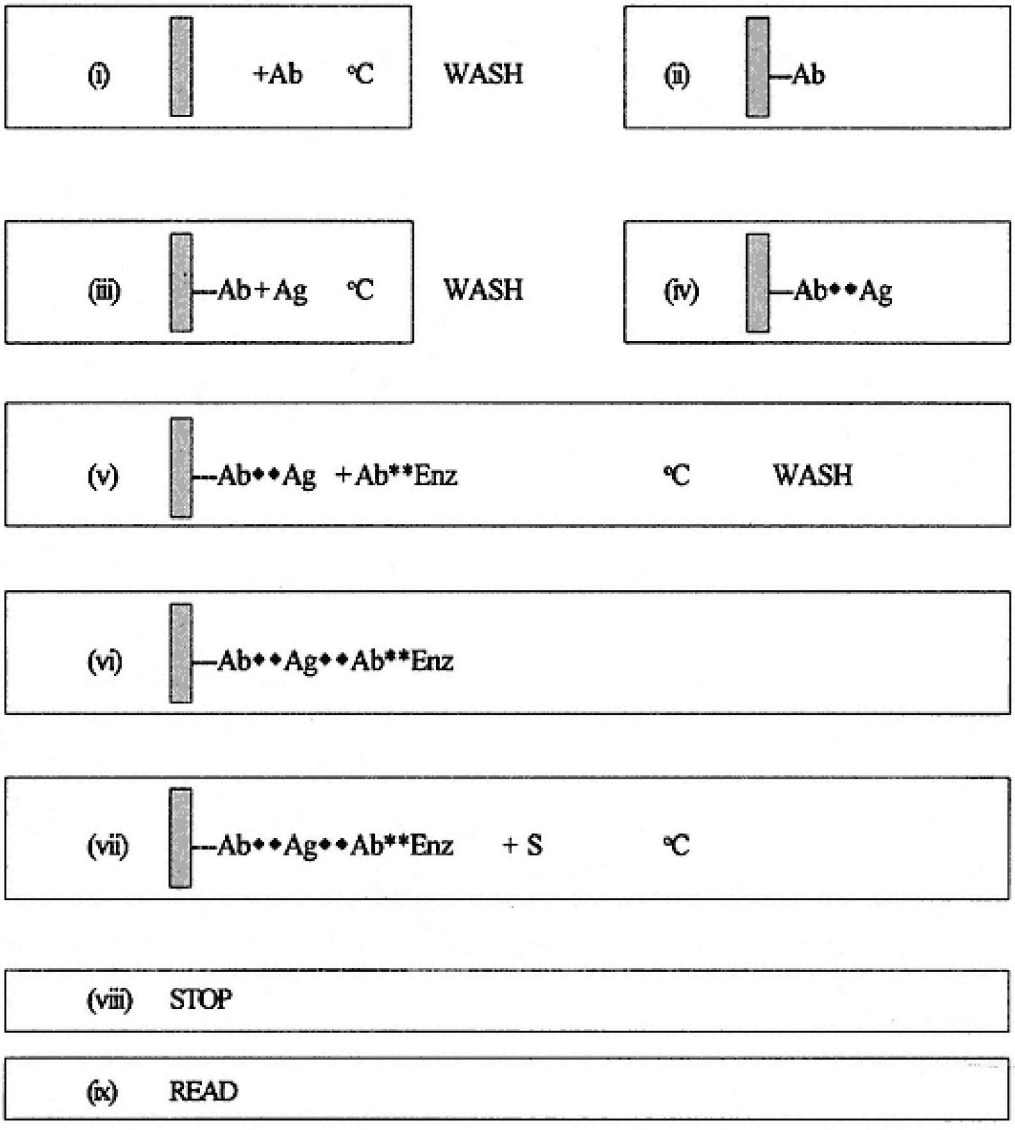
phase. Any bound antibodies are detected by the addition of an antispecies antiserum labeled with enzyme. This is widely used in diagnosis.

The indirect system offers the advantage that any number of antisera can be examined for binding to a given antigen using a single antispecies conjugate. Such systems have been heavily exploited in diagnostic applications, particularly when examining (screening) large numbers of samples. One problem that such systems have is the varying degree of nonspecific binding in individual sera. This tends to widen the dispersion (variability) in assay results and, therefore, increases the need to process many sera to assess confidence.

2.3
Sandwich ELISA
Sandwich ELISA can be divided into two systems, which have been named the direct sandwich ELISA and the indirect sandwich ELISA.

2.3.1
Direct Sandwich ELISA
The direct sandwich ELISA illustrated as follows and in **Fig. 3**.

Direct Sandwich ELISA



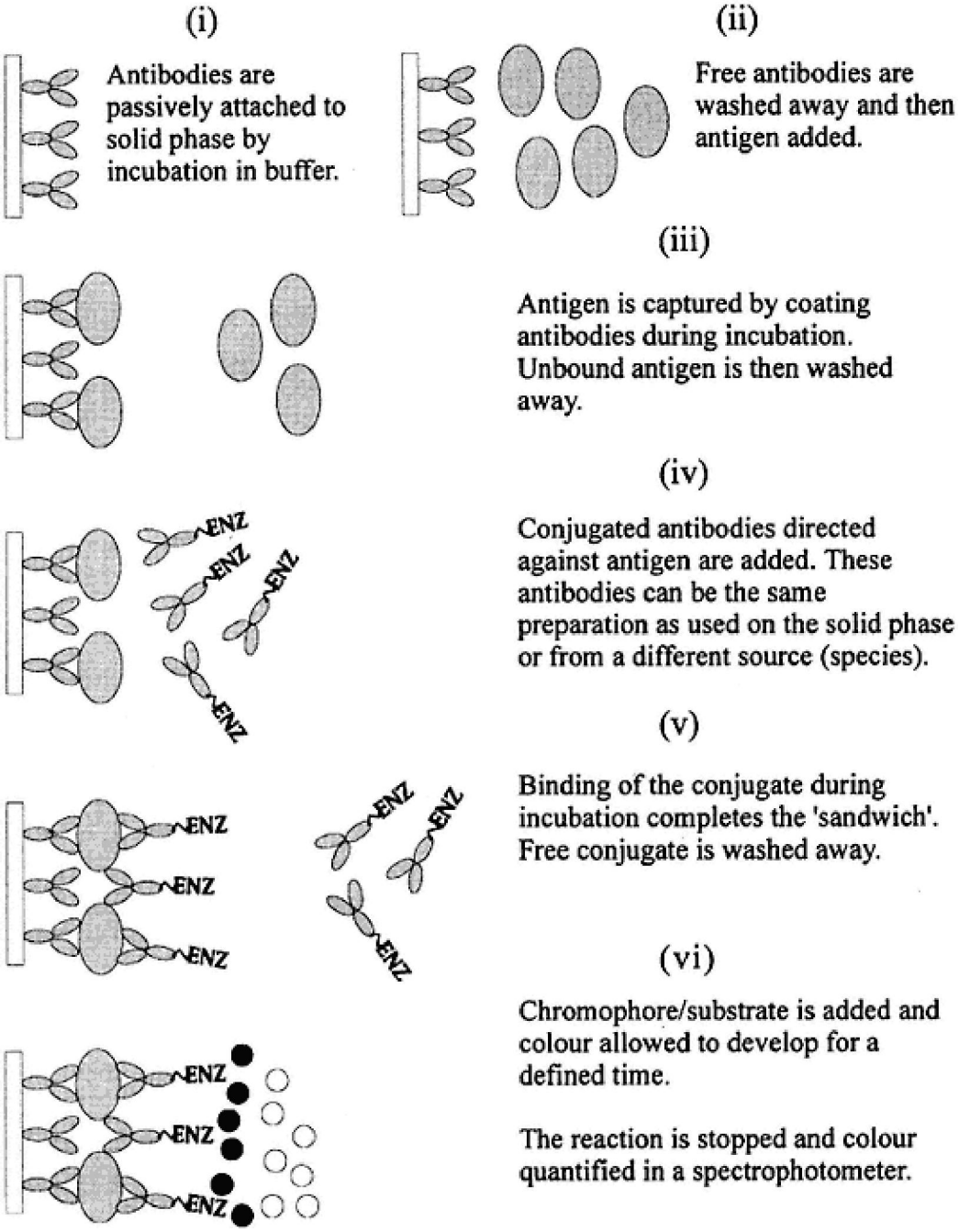

(i)
Antibodies are passively attached to solid phase by incubation in buffer.
(ii)
Free antibodies are washed away and then antigen added.
(iii)
Antigen is captured by coating antibodies during incubation. Unbound antigen is then washed away.
(iv)
Conjugated antibodies directed against antigen are added. These antibodies can be the same preparation as used on the solid phase or from a different source (species).
(v)
Binding of the conjugate during incubation completes the 'sandwich'. Free conjugate is washed away.
(vi)
Chromophore/substrate is added and colour allowed to develop for a defined time.
The reaction is stopped and colour quantified in a spectrophotometer.
ENZ

Fig. 3.
Direct sandwich ELISA. This system exploits antibodies attached to a solid phase to capture antigen. The antigen is then detected using serum specific for the antigen. The detecting antibody is labeled with enzyme. The capture antibody and the detecting antibody can be the same serum

or from different animals of the same species or from different species. The antigen must have at least two different antigenic sites.

The direct sandwich ELISA, involves the passive attachment of antibodies to the solid phase (stages I and ii). These antibodies (capture antibodies) then bind antigen(s) that are added in stage iii. The antigen(s) are diluted in a blocking buffer to avoid nonspecific attachment to the solid phase. Here, the components of the blocking buffer should not contain any antigens that might bind to the capture antibodies. After incubation and washing, an antibody¨Cantigen complex is attached to the solid phase (stage iv). The captured antigen (sometimes referred to as trapped) is then detected by the addition and incubation of enzyme-labeled specific antibodies in blocking buffer (stage v). Thus, this is a direct conjugate binding with the antigenic targets on the captured antigen. This second antibody can be the same as that used for capture, or be different in terms of specific animal source or species in which it was produced. After incubation and washing (stage vi), the bound enzyme is developed by the addition of substrate/chromogen (stage vii), then stopped (stage viii), and finally read using a spectrophotometer (stage ix). Since a single enzyme-conjugated antibody is used, the system is limited to the specificities and properties inherent in that particular antibody set. This limits the versatility of the test¡ªe.g., each antibody preparation used must be labeled (for different antigens)¡ªin the same way as the direct ELISA was limited to single antibody preparations.

The system also is limited in that antigens must have at least two antigenic sites (epitopes), since both the capture and the detecting antibodies need to bind. This can limit the assay to relatively large antigenic complexes. The capture antibody (on the solid phase), and the detecting antibody, can be against different epitopes on an antigen complex. This can be helpful in orienting the antigenic molecules so that there is an increased chance that the detecting antibodies will bind. It can also be an advantage when investigating small differences between antigenic preparations by the use of different detecting antibodies and a common capture antibody, and more versatile and hence appropriate systems are dealt with in

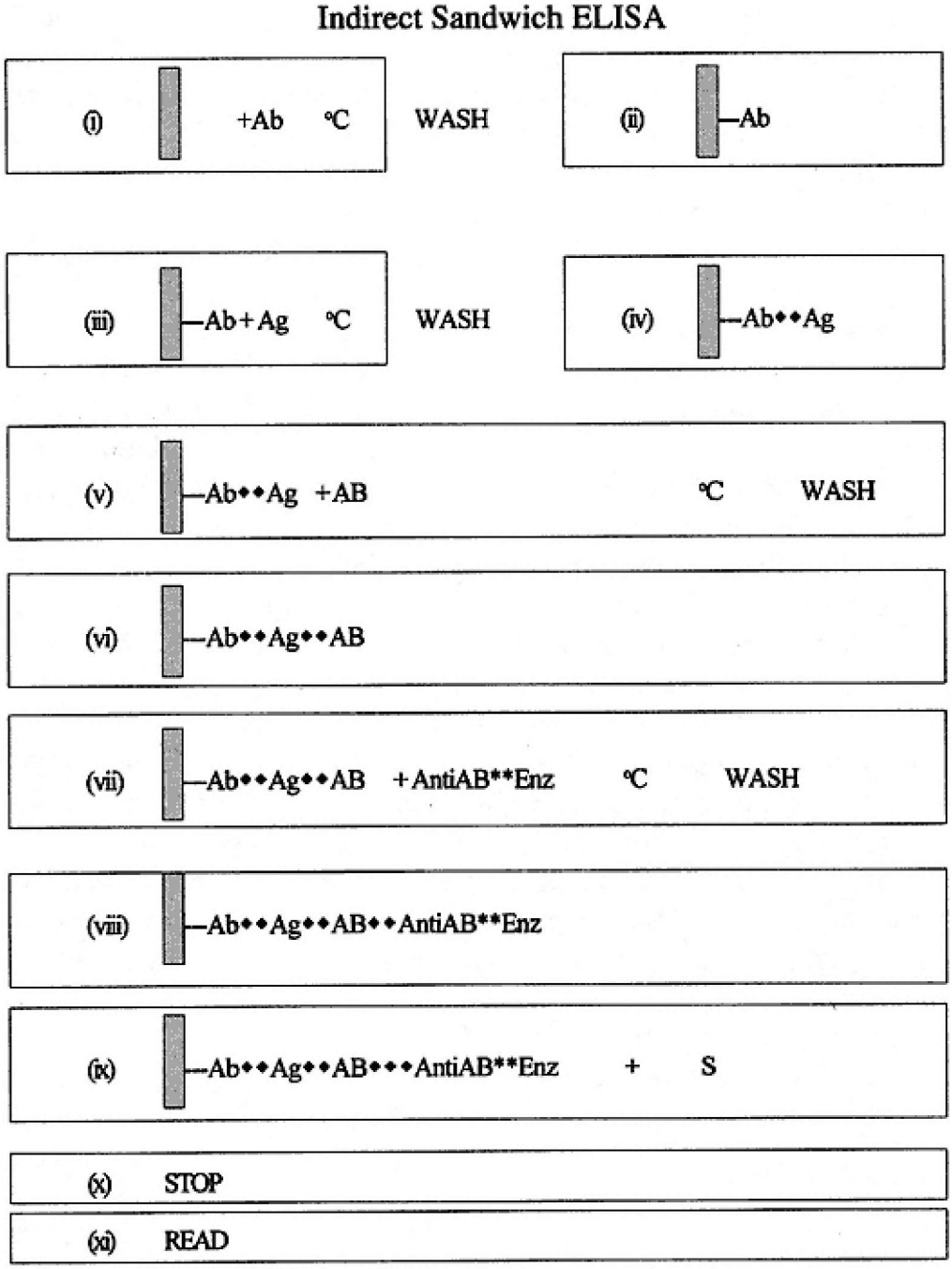
Subheading 2.3.2.

The use of exactly the same antibodies for capture and detection (e.g., mAbs) can lead to problems whereby there is a severe limitation of available binding sites for the detector. The size and the spatial relationship (topography) of the epitopes on the antigenic target is also critical and can greatly affect the assay.

2.3.2
Indirect Sandwich ELISA
Indirect sandwich ELISA is illustrated as follows and in Fig. 4. In indirect sandwich ELISA assay stages i¨Civ are quite similar to those of the direct sandwich ELISA. Thus, antibodies are passively attached to the solid phase and antigen(s) are captured. However, stage v involves the

addition of detecting antibodies. In this case, the antibodies are not labeled with enzyme. After incubation and washing (stage vi), the detecting antibodies are themselves detected by addition and incubation with an antispecies enzyme conjugate (stage vii). The bound conjugate is then

processed as described in the other systems (stages xiii¨Cix). The advantage to this assay is that any number of different sources of antibodies (samples) can be added to the captured antigen, provided that the species in which it was produced is not the same as the capture antibody. More specifically, the enzyme conjugated antispecies antibody does not react with the antibodies used to capture the antigen. It is possible to use the same species of antibody if immunochemical techniques are used to select and produce particular forms of antibodies and with attention to the specificity of the enzyme conjugate used. Thus, as an example, the capture antibody could be processed to a bivalent molecule without the Fc portion (also called F(ab')2 fraction). The detecting antibodies could be untreated. The enzyme conjugate could then be



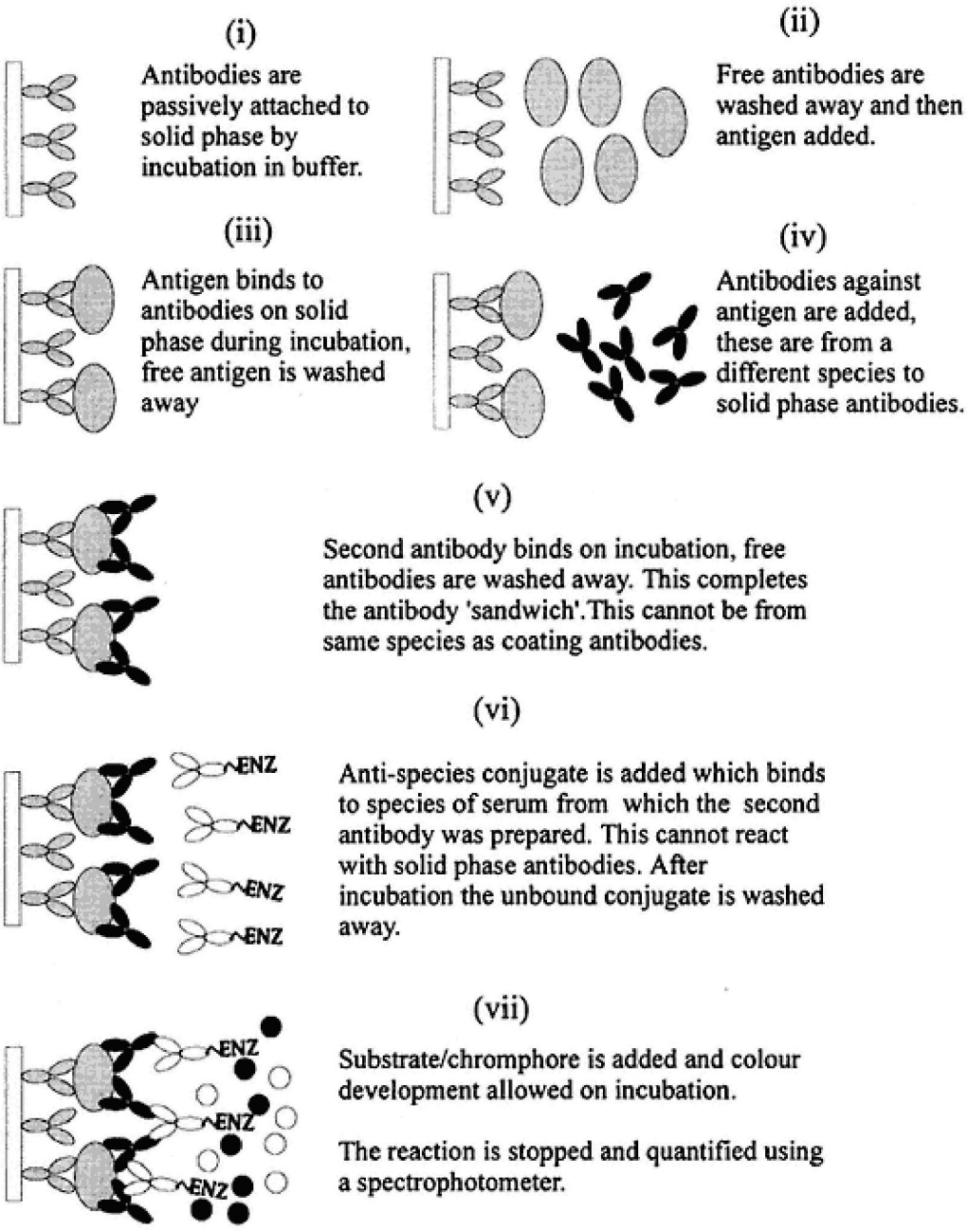


Fig. 4.
Indirect sandwich ELISA. The antigen is captured by a solid-phase antibody. Antigen is then detected using antibodies from another species. This in turn is bound by an antispecies conjugate. Thus, the species of serum for the coating and detecting antibodies must be different; the antispecies cojugate cannot react with the coating antibodies. an antispecies anti-Fc portion of the Ig molecule. Thus, the conjugate would react only with antibodies containing Fc

(andtherefore not the capture molecules). The need to devise such assays depends on the reagents available.

It may be that a mAb is available that confers a desired specificity as compared to polyclonal sera or that one wishes to screen a large number of mAbs against an antigen that must be captured (it may be at a low concentration or in a mixture of other antigens). In this the case use of F(ab')2 polyclonal sera is unsuccessful; therefore, the preparation of fragments for the capture antibody is worthwhile, and in fact, relatively easy-to-use kits are available for this purpose. The use of a commercially available antimouse Fc completes the requirements.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: II M.Sc MB COURSE NAME: ADVANCED IMMUNOLOGY

COURSE CODE: 18MBP301 UNIT: V BATCH-2018-2020

Sl. No	Question	Option A	Option B	Option C	Option D	Correct Ans
1	Key cells involved in mediating delayed hypersensitivity are	Neutrophils	Immune complex	T cells	Mast cell	T cells
2	In severe combined immuno deficiency (SCID) disorder ___ is mainly affected	homozygotes	Heterozygotes	Triploids	Tetrads	homozygotes
3	Digeorge syndrome in humans is due to the lack of ____	Thymus	Bone marrow	Spleen	Platelets	Thymus
4	AIDS Causes	Marked reduction in T – helper cells	Marked increase in T helper cells	Marked reduction in T-suppressor cells	Marked increase in T- suppressor cells	Marked reduction in T – helper cells
5	The receptor through which HIV infects is	CD_2	CD_3	CD_4	CD_5	CD_4
6	An auto immune disease that affect pancreatic beta cells is	Rheumatoid arthritis	Grave's diseases	Diabetes Mellitus	Pernicious anemia	Diabetes Mellitus
7	_____ is an autoimmune disease in the joints	Grave's disease	Diabetes mellitus	Rheumatoid arthritis	Good pasteur's	Rheumatoid arthritis
8	Autoimmune disease signified by the loss of B_{12} adsorption	Ulcerative colitis	Pernicious anemia	Good pasteurs syndrome	Goitre	Pernicious anemia
9	_____is the disease produced when the antibodies formed against thyroglobulin and thyroid peroxide involve in the uptake of iodine	Hashimoto's thyroiditis	Autoimmune anemias	Myasthemia gravis	Addison's disease	Hashimoto's thyroiditis
10	The disease produced when the antibodies block the normal binding of acetyl choline and results in the weakening of the skeletal muscles ___	Scleroderma	Graves disease	Myathemia gravis	Multiple sclerosis	Myathemia gravis
11	Auto immune thyroiditis associated with auto immunity to gastric pavietal cells, and linked with auto immunity to adrenal cortex the	Good pasteur's	Addison's disease	Sderoderma	SLE	Addison's disease

	disease called ___					
12	MHC class II molecules that associates with the auto immune cause	Juvenile Rheumatoid arthritis	Rheumatoid arthritis	Auto immune anemia	Good pasteur's	Juvenile Rheumatoid arthritis
13	Vaccination was discovered by	Louis Pasteur	Edward Jenner	Robert koch	Charles Richet	Edward Jenner
14	Toxoid can induce	Antibodies	Antigen	Antiglobulins	Haptens	Antibodies
15	Inactivated exotoxins are called	Intoxicants	Antitoxins	Toxoids	Attenuated vaccines	Toxoids
16	NK cell are ____	Specific	Nonspecific	Act on B cell	phagocytic	Nonspecific
17	RNA virus cause cancer by converting RNA into DNA by	Protease	polymerase	DNA	Reverse transcriptase	Reverse transcriptase
18	Tumor cells are embryonic cells that undergo __	Dedifferentiation	Differentiation	Disintegration	Disproportion	Dedifferentiation
19	The antiglobulin or coombs test was devised in	1942	1943	1945	1949	1945
20	Kahn test for syphilis is an example of __	Ring test	slide test	Tube flocculation test	Precipitation	Tube flocculation test
21	Latex agglutination is widely used for the detection of __	CRP	ASO	RA	HGC	All
22	Name of technique in which AB is adsorbed to carrier particle for the estimation of Ag	Hemagglutination	Bacterial agglutination	Reverse passive agglutination	passive agglutination	Reverse passive agglutination
23	The name of technique in which Ag is absorbed to carrier particle for the estimation of Ab is ___	passive agglutination	Reserve passive agglutination	bacterial antigens	Hemogglutination	passive agglutination
24	Selective theory suggest that cells express ___	Membrane receptors	Surface receptors	T cell receptors	Side chain receptors	Side chain receptors
25	RAST is used to determine concentration of	IgA	IgA	IgD	IgE	IgE
26	RIST is a type of	Liquid phase RIA	Solid phase RIA	Radio immuno	Stationary	Solid phase RIA

				precipitation		
27	The binder – ligand assay is otherwise known as	Qpsonization	Immunoflouresence	ELISA	RIA	RIA
28	CFT can be used to detect ___ amount of antigen or antibody	<5mg	>1 mg	<1 mg	5mg	<1 mg
29	The strength of interaction between a single Ag- binding site on an Ab and single epitope is called ___	Avidity	Affinity	Hapten	paratope	Affinity
30	The interaction between Ab and a soluble Ag in aqueous solution forms a lattice that eventually develops into visible reaction called__	cross reactivity	immunodiffusion	precipitation reaction	Neutralization reaction	precipitation reaction
31	The principle involved in the WIDAL test is__	Flocculation	RIA	Neutralization	Agglutination	Agglutination
32	Enzyme commonly used in ELISA is ____	b galactosidase	catalase	Perocidase	Horse raddish peroxidase	Horse raddish peroxidase
33	Color intensity ofAg andAb can be read in spectrophotometer and also in _____	EIA reader	RIA reader	ELISA reader	Fraction reader	ELISA reader
34	Amount of sample is detected by _____of color product	Adsorbance	Intensity	Transmittance	Concentration	Intensity
35	Clone selection theory suggest _____expression	Lymphocyte	Granulocyte	Tissue	Blood	Lymphocyte
36	Lattic hypothesis was proposed by ___	Marrack	Pasteur	Robert Koch	Paul Ehrlich	Marrack
37	The hypothesis proposed by Marrack is also called ___	Zone phenomenon	Clonal theory	Selective theory	Instructional theory	Zone phenomenon
38	Immunofluoresence is routinely used as sensitive method for _____	Diagnosis of hepatitis	Diagnosing malaria	Diagnostic rabies	Diagnostic of dengue	Diagnostic rabies
39	In widal test ___and ___antigens are used	H and B	H and O	O and A	AB	H and O

40	____ tubes are used for H agglutination test in widal test	Felix tubes	Round bottom tube	clonal dreyer's tube	Capillary tube	clonal dreyer's tube
41	Pregnancy test ____ coated with human chorionic gonadotripix (HCG) are used	Latex particle	Latex precipitation	Latex agglutination	Carrier	Latex particle
42	____ is an electron dense substances from horse that can be conjugated with an Ab	Ferricidin	Fernine	Forritia	Fluorescien	Forritia
43	Gamma emitting isotope used for labeling Ag is	^{125}I	^{3}H	^{32}P	^{35}S	^{125}I
44	Radioimmuno assay permits the measurement of moieties upto _ quantities	Miilligram	Micro gram	Nano gram	Pico gram	Pico gram
45	The binder – ligand assay is otherwise known as	Qpsonization	Immunoflouresence	ELISA	RIA	RIA
46	RIA was first discovered in	1949	1995	1959	1956	1959
47	The name of a competitive binding assay in which fixed amount of Ab and radiolabelled Ag react in the presence of unlabelled Ag	Opsonization	ELISA	RIA	Immunofluoresence	RIA
48	The absorption of small wave length and the emission of longer wavelength is a special property of	radioactivity	Fluorescent	Absorbance	Optical density	Fluorescent
49	The antibody molecules bound to antigens in cells or tissues section were visualized by	Radioactivity	Immunofluoresence	Absorbance	Optical density	Immunofluoresence
50	____ is an intensely colored and highly fluorescent pigment obtained from algae	Phycoerythrin	pyoverdin	phycocyanin	All	All
51	____is an organic dye that absorbs blue light and emits yellow – green	Fluorescin	phycocyanin	Pyoverdin	pyoerythrin	pyoerythrin
52	____is an organic dye that absorbs yellow green and emits deep red	Rhodamine	Fluorescien	Pyocyanin	pyoverdin	pyoverdin
53	RIA was first discovered in	1949	1995	1959	1956	1959

54	The name of a competitive binding assay in which fixed amount of Ab and radiolabelled Ag react in the presence of unlabelled Ag	Opsonization	ELISA	RIA	Immunofluoresence	RIA
55	RAST is used to determine concentration of	IgA	IgA	IgD	IgE	IgE
56	RIST is a type of	Liquid phase RIA	Solid phase RIA	Radio immuno precipitation	Stationary	Solid phase RIA
57	Name of serological reaction that uses standard dose response or caliberating curve	ELISA	RIA	Immunoelectroblot technique	Immunochromato graphic test	RIA
58	Antibodies that aggregate soluble antigens are called ____	Precipitinogen	Agglutinin	Precipitinin	Agglutinogen	Precipitinin
59	Precipitation reactions in gel are called _	Immunoflurorescence	RIA	Gel electrophoresis	Immunodiffusion	Immunodiffusion
60	The antiglobulin or coombs test was devised in	1942	1943	1945	1949	1945