Semester –II

19BTP204 **IMMUNOTECHNOLOGY** **4H-4C**

Instruction Hours / week: L:43 T: 0 P: 0 **Marks:** Internal: **40** External: **60** Total: **100**
End Semester Exam: 3 Hours

Course Objectives:

- To expose the students with the immune system of human body.

Course Outcomes (CO's):

1. The students may understanding the immune system, its components and various techniques used in bio manipulation.

UNIT –I Introduction:

History and scope, Immunity – types, Antigen and Antibody - biology, structure and functions, super antigens, antigen- antibody interactions, primary and secondary immune response. Humoral and cell mediated immunity**.**

UNIT –II Immune system:

Hematopoiesis and differentiation, Lymphocytes, Lymphoid organs**:** Primary and secondary lymphoid organs. Antigen recognition and presentation, activation of B and T lymphocytes, cytokines and their role in immune regulation. **Complement system** - Classical and alternate pathway. MHC I and II complex.

UNIT-III Transplantation:

MLR, MHC and HLA typing, bone marrow transplantation, organ transplants, immunosuppressive therapy. Hybridoma technology and monoclonal antibodies, immuno-diagnosis and application of monoclonal antibodies in biomedical research, human monoclonal antibodies and catalytic antibodies, Xeno transplantation from various species.

UNIT –IV Hyper-sensitivity reactions, auto-immune disorders. Tumor immunology:

Tumor antigens, immune response to tumours, cancer immunotherapy. Immunodeficiencies – primary and secondary.

UNIT –V Vaccines:

Vaccine technology including DNA vaccines, identification of B and T epitopes for vaccine development. Immunodiagnosis of infectious diseases, immuno screening of recombinant library.

SUGGESTED READINGS

1. Punt, J., Stranford, S., Jones, P., & Owen, J.A. (2018). *Kuby Immunology* (8th ed.). W.H. Freeman and Company, New York, United States.

2. Tizard, I.R. (2017). *Veterinary Immunology* (10th ed.). Saunders Publishers, New York, United States.

3. Abbas,A.K., Lichtman, A. H., & Pillai, S. (2017). *Cellular and Molecular Immunology* (9th ed.). Elsevier Publishers, Amsterdam, Netherlands.

4. Abbas,A.K., Lichtman, A. H., & Pillai, S. (2019). *Basic Immunology: Functions and Disorders of the Immune System* (6th ed.). Elsevier Publishers, Amsterdam, Netherlands.

5. Delves, P.J., Martin, S.J., Burton, D.R., & Roitt, I.M. (2017). *Roitt's Essential Immunology* (13th ed.). Wiley-Blackwell, New Jersey, United States.

6. Kind, T.J., Goldsby, R. A. & Osborne, B.A. (2006). *Kuby Immunology* (6th ed.). W.H. Freeman and Company, New York, United States.

7. Turgeon, M. L. (2017). *Turgeon: Immunology and Serology in Laboratory Medicine*. (6th ed.). Elsevier Publishers, Amsterdam, Netherlands.

8. Naha, S. & Narain, R. (2004). *Immunobiotechnology*. Dominant Publishers, New Delhi, India.

2019-2021 SEMESTER II

19BTP204 IMMUNOTECHNOLOGY LECTURE PLAN

S.No	Lecture Duration Period	Topics to be covered	Support Material/ Page No.
		UNIT I Introduction	
1	1	History and scope, Immunity – types	T1: 1-7
2	1	Antigen - biology, structure and functions; Super antigens	T1: 77-94; 235
3	1	Antibody - biology, structure and functions	T1: 77-94
4	1	Antigen- Antibody interactions	T1: 137-157
5	1	Primary and Secondary immune response	T1: 16-18
6	1	Humoral and cell mediated immunity.	T1: 80-90
7	1	Unit test	
		Total No of Hours Planned for Unit I = 07	
		UNIT II Immune System	
1	1	Hematopoiesis and differentiation, Lymphocytes	T1: 24-39
2	1	Lymphoid organs**:** Primary and secondary lymphoid organs.	T1: 43-52
3	1	Antigen recognition and presentation	T1: 10-12
4	1	Activation of B and T lymphocytes	T1: 254-257; 221-225
5	1	Cytokines and their role in immune regulation	T1: 276- 281
6	1	Complement system - Classical and alternate pathway	T1: 299-306
7	1	MHC I and II complex	T1: 162-171
8	1	Unit test	
		Total No of Hours Planned for Unit II = 08	
		UNIT III Transplantation	
1	1	Introduction to transplantation: MLR, MHC and HLA typing	T1: 481-482; 334; 528-530
2	1	Bone marrow transplantation, organ transplants	T1: 495-497
3	1	Immunosuppressive therapy	T1: 490-492
4	1	Hybridoma technology and monoclonal antibodies	T1: 530; 99-101
5	1	Immuno-diagnosis	T1: 531-535
6	1	Application of monoclonal antibodies in biomedical research	T1: 492
7	1	Human monoclonal antibodies and catalytic antibodies	T1: 126-129, 132
8	1	Xeno transplantation from various species	T1: 498
9	1	Unit test	
		Total No of Hours Planned for Unit III = 09	
		UNIT IV Hyper-sensitivity reactions, auto-immune disorders. Tumor immunology	
1	1	Hyper-sensitivity reactions – Type I and II	T2: 361-380
2	1	Hyper-sensitivity reactions – Type III and IV	T2: 381-386
3	1	Auto-immune disorders	T2: 183- 192
4	1	Tumor Immunology: Tumor antigens	T1: 506-512
5	1	Immune response to tumours	T1: 513-517

6	1	Cancer immunotherapy	T1: 520-522
7	1	Immunodeficiencies – primary and secondary.	T1: 18-20
8	1	Unit test	
		Total No of Hours Planned for Unit IV = 08	
		UNIT V Vaccines	
1		Passive, Active Immunization	T1: 413- 419
2	1	Vaccine technology including DNA vaccines	T1: 425- 427
3	1	Identification of B and T epitopes for vaccine development.	T1: 427- 428
4	1	Immunodiagnosis of infectious diseases	T1: 464
5	1	Immuno screening of recombinant library	T1: 129
6	1	Unit test	
7	1	ESE Question paper discussion I	
8	1	ESE Question paper discussion II	
		Total No of Hours Planned for Unit V = 08	

Support Materials

Textbooks

1. Goldsby, R.A., Kindt, T. J., Osborne, B. A., & Kuby, W.H.J. (2004). Immunology 6th ed.). USA: Freeman and Company.

2. Textbook of immunology – seemi farhat published by Asoke. K. Ghosh.

UNIT-I

SYLLABUS

Introduction: History and scope, Immunity – types, Antigen and Antibody - biology, structure and functions, super antigens, antigen- antibody interactions, primary and secondary immune response. Humoral and cell mediated immunity..

History of immunology

In Western society, it was not until the late eighteenth century that a rational approach to the origin of disease developed. Prior to the discovery that disease was the result of pathogenic organisms, it was commonly accepted that disease was a punishment from God (or the Gods), or even a witches curse. Eastern cultures perceived disease as an imbalance in the energy channels within the body. Later, the great plagues of Europe were assumed the result of virulent or noxious vapors. Nevertheless, there were intimations as early as 430 B.C. that if one survived a disease, the person thereafter became "immune" to any subsequent exposures. However, this was never recognized as evidence of some type of internal defense system until the later part of the seventeenth century.

Although most historical accounts credit **Edward Jenner** for the development of the first **immunization** process, a previous similar procedure had become established in China by 1700. The technique was called variolation. This was derived from the name of the infective agent—the **variola virus** . The basic principal of variolation was to deliberately cause a mild infection with unmodified pathogen. The risk of death from variolation was around two to three percent. Although still a risk, variolation was a considerable improvement on the death rate for

uncontrolled infection. **Immunity** to **smallpox** was conferred by inserting the dried exudate of smallpox pustules into the nose. This technique for the transfer of smallpox, as a form of limited infection, traveled to the west from China along the traditional trade routes to Constantinople where it spread throughout Europe. Hearing of this practice, the Royal family of England had their children inoculated against the disease in 1721, but the practice aroused severe opposition as physicians felt it was far too risky.

In 1798, Edward Jenner, noticed that milkmaids were protected from smallpox if they had been first infected with **cowpox** . It was not his intention to make medical history, as his interests were mostly scholarly and involved the transfer of infections from one species to another, especially from animals to humans. However, Jenner's work led him to the conclusion, that inoculation with cowpox (a bovine analogue of smallpox) could confer immunity to smallpox. Thus, the concept of **vaccination** was initiated. (Incidentally, the Latin word for cow is *vacca*). Jenner's ideas first made him a medical as well as a social pariah, as they were in opposition to both the church and popular beliefs. Because his method was much safer then variolation, however, the use of vaccinations gradually became widely accepted and most European countries had some form of compulsory program within fifty years of Jenner's discovery.

The idea that a pathogenic organism caused disease was not fully realized until certain technological advances had occurred. Initially, **Antoni van Leeuwenhoek** 's development of the **microscope** and the subsequent realization that entities existed that were not visible to the human eye, allowed the concept of germs to be appreciated. That these organisms were the causative agent of disease was not recognized until **Louis Pasteur** developed his **germ theory of disease** .

His original interests were in **fermentation** in wine and beer, and he was the first to isolate the organisms that caused the fermentation process. Pasteur's work eventually led him to the development of **pasteurization** (heating) as a means of halting fermentation. While working with silk worms and **anthrax** , he was able to demonstrate that the same method for transferring the fermentation process also worked in transmitting disease from infected animals to unaffected animals. Finally, in 1878, Pasteur accidentally used an attenuated (weakened) chicken cholera **culture** and realized, when he repeated the experiment using a fresh culture, that the weakened form protected the chickens from the virulent form of the disease. Pasteur went on to develop an attenuated **vaccine** against **rabies** and swine erysipelas.

Pasteur was not the only proponent of the germ theory of disease. His chief competitor was **Robert Koch** . Koch was the first to isolate the anthrax microbe and, unaware of Pasteur's work, he was able to show that it caused the disease. Then in 1882, Koch was able to demonstrate that the germ theory of disease applied to human ailments as well as animals, when he isolated the microbe that caused **tuberculosis** . His "Koch's postulates" are still used to identify infective organisms.

Much of the basis for modern medicine, as well as the field of **immunology** , can be traced back to these two scientists, but the two major questions still to be answered were how did infection cause the degradation of tissue, and how did vaccines work? The first question was addressed in 1881 by **Emile Roux** and Alexander Yersin when they isolated a soluble toxin from **diphtheria** cultures. Later, **Emil von Behring** and **Shibasaburo Kitasato** were able to demonstrate passive immunity when they took serum from animals infected with diphtheria and injected into healthy

animals. These same animals were found to be resistant to the disease. Eventually these serum factors were recognized in 1930 as antibodies. However, thirty years before antibodies were finally isolated and identified, **Paul Ehrlich** and others, recognized that a specific antigen elicited the production of a specific **antibody** . Ehrlich hypothesized that these antibodies were specialized molecular structures with specific receptor sites that fit each pathogen like a lock and key. Thus, the first realization that the body had a specific defense system was introduced. In addition, sometime later, he realized that this powerful effector mechanism, used in host defense would, if turned against the host, cause severe tissue damage. Ehrlich termed this *horror autotoxicus.* Although extremely valuable, his work still left a large gap in understanding how the **immune system** fights a pathogenic challenge. The idea that specific cells could be directly involved with defending the body was first suggested in 1884 by **Élie Metchnikoff** . His field was zoology and he studied **phagocytosis** in single cell organisms. Metchnikoff postulated that vertebrates could operate in a similar manner to remove pathogens. However, it was not until the 1940s that his theories were accepted and the cell mediated, as opposed to the humoral, immune response was recognized.

The clarification of the immune response and the science of immunology did not progress in a systematic or chronological order. Nonetheless, once scientists had a basic understanding of the cellular and humoral branches of the immune system, what remained was the identification of the various components of this intricate system, and the mechanisms of their interactions. This could not have been accomplished without the concomitant development of **molecular biology** and genetics.

Milestones in the history of immunology include:

- 1798 Edward Jenner initiates smallpox vaccination.
- 1877 Paul Erlich recognizes mast cells.
- 1879 Louis Pasteur develops an attenuated chicken cholera vaccine.
- 1883 Elie Metchnikoff develops cellular theory of vaccination.
- 1885 Louis Pasteur develops rabies vaccine.
- 1891 Robert Koch explored delayed type hypersensitivity.
- 1900 Paul Erlich theorizes specific antibody formation.
- 1906 Clemens von Pirquet coined the word allergy.
- 1938 John Marrack formulates antigen-antibody binding hypothesis.
- 1942 Jules Freund and Katherine McDermott research adjuvants.
- 1949 Macfarlane Burnet & Frank Fenner formulate immunological tolerance hypothesis.
- 1959 Niels Jerne, David Talmage, Macfarlane Burnet develop clonal **selection** theory.
- 1957 Alick Isaacs & Jean Lindemann discover interferon (cytokine).
- 1962 Rodney Porter and team discovery the structure of antibodies.
- 1962 Jaques Miller and team discover thymus involvement in cellular immunity.
- 1962 Noel Warner and team distinguish between cellular and humoral immune responses.
- 1968 Anthony Davis and team discover T cell and B cell cooperation in immune response.
- 1974 Rolf Zinkernagel and Peter Doherty explore **major histocompatibility complex** restriction.
- 1985 Susumu Tonegawa, Leroy Hood, and team identify immunoglobulin genes.

- 1987 Leroy Hood and team identify genes for the T cell receptor.
- 1985 Scientists begin the rapid identification of genes for immune cells that continues to the present.

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 the immune system.

Exogenous antigen is produced outside of the host cell and enters the cell by endocytosis or phagocytosis.

Endogenous antigen is produced within the host cell itself. Two common examples are viral proteins synthesized within virus-infected host cells and unique proteins synthesized by cancerous cells.

Immunogenicity Versus Antigenicity

Immunogenicity and antigenicity are related but distinct immunologic properties that sometimes are confused. **Immunogenicity** is the ability to induce a humoral and/or cell mediated immune response:

B cells + antigen ⟶ Effector B cells + memory B cells

↓

(plasma cells)

T cells + antigen ⟶ Effector T cells + memory T cells

↓

A005A (e.g., CTLs, THs)

Although a substance that induces a specific immune response is usually called an antigen, it is more appropriately called an **immunogen. Antigenicity** is the ability to combine specifically with the final products of the above responses (i.e., antibodies and/or cell-surface receptors). Although all molecules that have the property of immunogenicity also have the property of antigenicity, the reverse is not true. Some small molecules, called *haptens,* are antigenic but incapable, by themselves, of inducing a specific immune response. In other words, they lack immunogenicity.

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 (polymers composed of a single amino acid or sugar) tend to lack immunogenicity regardless of their size.

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.

ADJUVANTS

Adjuvants (from Latin *adjuvare,* to help) are substances that, when mixed with an antigen and injected with it, enhance the immunogenicity of that antigen. Adjuvants are often used to boost the immune response when an antigen has low immunogenicity or when only small amounts of an antigen are available. For example, the antibody response of mice to immunization with BSA can be increased fivefold or more if the BSA is administered with an adjuvant. Precisely how adjuvants augment the immune response is not entirely known, but they appear to exert one or more of the following effects. Antigen persistence is prolonged. Co-stimulatory signals are enhanced. _ Local inflammation is increased. _ The nonspecific proliferation of lymphocytes is stimulated.

Aluminum potassium sulfate (alum) prolongs the persistence of antigen. When an antigen is mixed with alum, the salt precipitates the antigen. Injection of this alum precipitate results in a slower release of antigen from the injection site, so that the effective time of exposure to the antigen increases from a few days without adjuvant to several weeks with the adjuvant. The alum

precipitate also increases the size of the antigen, thus increasing the likelihood of phagocytosis. Water-in-oil adjuvants also prolong the persistence of antigen. A preparation known as **Freund's incomplete adjuvant** contains antigen in aqueous solution, mineral oil, and an emulsifying agent such as mannide monooleate, which disperses the oil into small droplets surrounding the antigen; the antigen is then released very slowly from the site of injection. This preparation is based on **Freund's complete adjuvant,** the first deliberately formulated highly effective adjuvant, developed by Jules Freund many years ago and containing heat-killed *Mycobacteria* as an additional ingredient. Muramyl dipeptide, a component of the mycobacterial cell wall, activates macrophages, making Freund's complete adjuvant far more potent than the incomplete form.

Antibodies

Antibodies 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 (Figure 4-1). 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 α, β and γ. 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 α and the β fractions of serum.

Antibodies Are Heterodimers

Antibody molecules have a common structure of four peptide chains (Figure 4-2). This structure consists of two identical **light (L) chains,** polypeptides of about 25,000 molecular weight, and two identical **heavy (H) chains,** larger CH on the heavy chain.Antibodies are glycoproteins; with few exceptions, the sites of attachment for carbohydrates are restricted to the constant region.

Immunoglobulin Fine Structure

The structure of the immunoglobulin molecule is determined by the primary, secondary, tertiary, and quaternary organization of the protein. The primary structure, the amino acid sequence, accounts for the variable and constant regions of the heavy and light chains. The secondary structure is formed by folding of the extended polypeptide chain several homologous units of about 110 amino acid residues. Within each unit, termed a domain, an intrachain disulfide bond forms +a loop of about 60 amino acids. Light chains contain one variable domain (VL), and one constant domain (CL); heavy chains contain one variable domain (VH), and either three or four constant domains (CH1, CH2, CH3, and CH4), depending on the antibody class (Figure 4-6). X-ray crystallographic analysis revealed that immunoglobulin domains are folded into a characteristic compact structure called the **immunoglobulin fold.** This structure consists of a "sandwich" of two _ pleated sheets, each containing antiparallel _ strands of amino acids, which are connected by loops of various lengths (Figure 4-7). The _ strands within a sheet are stabilized by hydrogen bonds that connect the –NH groups in one strand with carbonyl groups of an adjacent strand (see Figure 4-4). The _ strands are characterized by alternating hydrophobic and

hydrophilic amino acids whose side chains are arranged perpendicular to the plane of the sheet; the hydrophobic amino acids are oriented toward the interior of the sandwich, and the hydrophilic amino acids face outward. The two _ sheets within an immunoglobulin fold are stabilized by the hydrophobic interactions between them and by the conserved disulfide bond. An analogy has been made to two pieces of bread, the butter between them, and a toothpick holding the slices together. The bread slices represent the two _ pleated sheets; the butter represents the hydrophobic interactions between them; and the toothpick represents the intrachain disulfide bond. Although variable and constant domains have a similar structure, there are subtle differences between them. The V domain is slightly longer than the C domain and contains an extra pair of _ strands within the _- sheet structure, as well as the extra loop sequence connecting this pair of _ strands (see Figure 4-7). The basic structure of the immunoglobulin fold contributes to the quaternary structure of immunoglobulins by facilitating noncovalent interactions between domains.

Antibody Classes and Biological Activities

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 mmunoglobulin. The IgG molecule consists of two _ heavy chains and two or two light chains (see Figure 4-13a). 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 (see Table 4-2). 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 (Figure 4-14, page 92). The subtle amino 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 heavy chain domains (C_4/C_4) and their C_3/C_3 domains (see Figure 4-13e). 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 (see Figure 4-13d). 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** (Figure 4-15a, page 93). 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. Along the jejunum of the small intestine, for example, there are more than 2.5 _ 1010 IgA-secreting plasma cells—a number that surpasses the total plasma cell population of the bone marrow, lymph, and spleen combined! Every day, a human secretes from 5 g to 15 g of secretory IgA into mucous secretions. Breast milk contains secretory IgA and many other molecules that help protect the newborn against infection during the first month of

life (Table 4-3). Because the immune system of infants is not fully functional, breast-feeding plays an important role in maintaining the health of newborns.

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 presence of a serum component responsible for allergic reactions was first demonstrated in 1921 by K. Prausnitz and H. Kustner, who injected serum from an allergic person intra-dermally into a nonallergic individual. When the appropriate antigen was later injected at the same site, a wheal and flare reaction (analogous to hives) developed there. This reaction, called the **P-K reaction** (named for its originators, Prausnitz and Kustner), was the basis for the first biological assay for IgE activity.

Actual identification of IgE was accomplished by K. and T. Ishizaka in 1966. They obtained serum from an allergic in dividual 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). In this way, each of the known anti-isotype antibodies was precipitated and removed from the rabbit anti-serum. What remained was an anti-isotype antibody specific for an unidentified class of antibody. This antibody turned out to completely block the P-K reaction.

The new antibody was called IgE (in reference to the E antigen of ragweed pollen, which is a potent inducer of this class of antibody). IgE binds to Fc receptors on the membranes of blood basophils and tissue mast cells. Cross-linkage of receptorbound 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. As a result, a variety of pharmacologically active mediators are released and give rise to allergic manifestations (Figure 4-16). Localized mast-cell degranulation induced by IgE also may release mediators that facilitate a buildup of various cells necessary for antiparasitic defense.

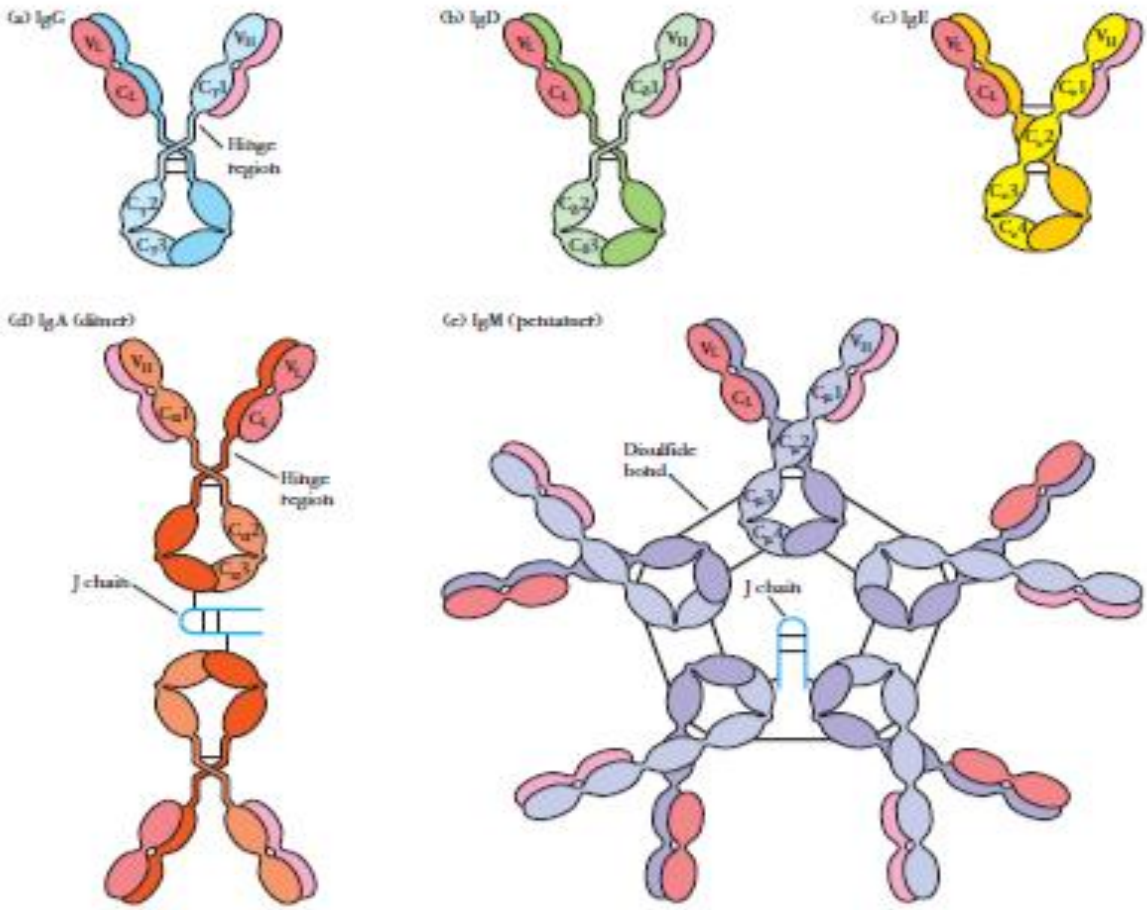


FIGURE 4-13 General structures of the five major classes of secreted antibody. Light chains are shown in shades of pink, disulfide bonds are indicated by thick black lines. Note that the IgG, IgA, and IgD heavy chains (blue, orange, and green, respectively) contain four domains and a hinge region, whereas the IgM and IgE heavy chains (purple and yellow, respectively) contain five domains but no hinge region. The polymeric forms of IgM and IgA contain a polypeptide, called the J chain, that is linked by two disulfide bonds to the Fc region in two different monomers. Serum IgM is always a pentamer; most serum IgA exists as a monomer, although dimers, trimers, and even tetramers are sometimes present. Not shown in these figures are intrachain disulfide bonds and disulfide bonds linking light and heavy chains (see Figure 4-2).

Types of Immunity

The following points highlight the three main types of immunity present in humans. The types are: 1. Innate (Natural or Nonspecific) Immunity 2. Acquired (Specific or Adaptive) Immunity 3. Active and Passive Immunity.

Type # 1. Innate (Natural or Nonspecific) Immunity:

Innate immunity (also called nonspecific or natural immunity) refers to the inborn-ability of the body to resist, and is genetically transmitted from one generation to the next. This immunity offers resistance to any microorganism or foreign material encountered by the host.

It includes general mechanisms inherited as part of the innate structure and function of each vertebrate, and acts as first line of defence. Innate immunity lacks immunological memory, i.e., it occurs to the same extent each time a microorganism or foreign material is encountered.

Types of Innate Immunity:

Innate immunity can be divided into species, racial, and individual immunity.

(i) Species Immunity:

Species immunity (species resistance) is that in which a disease affecting one species does not affect the other species. For convenience, humans do not contract cattle plague, chicken cholera, hog cholera, infectious horse anaemia, etc., while animals are not affected by many human diseases such as enteric fever, scarlet fever, syphilis, gonorrhoea, measles, etc.

Diseases of skin, to which humans are quite susceptible, are often resisted by animals because they have more hair and thicker hides. Species resistance is considered to be the result of a long evolution of interactions between the highly evolved "macro" organisms and the pathogenic microorganisms.

(ii) Racial Immunity:

Racial immunity (racial resistance) is that in which various races (breeds) show marked differences in their resistance to certain infectious diseases. A well known example is that Brahman cattle are resistant to the protozoan parasite responsible for tick fever in other breeds of cattle. Similarly, Black Africans affected by sickle cell anaemia, a genetic disease, are resistant to malaria while malaria affects other human races.

(iii) Individual Immunity:

Having the same racial background and opportunity for exposure, some individuals of the race experience fewer or less severe infections than other individuals of the same race. For convenience, children are more susceptible to diseases such as measles and chicken pox, while aged individuals are susceptible to other diseases like pneumonia.

Type # 2. Acquired (Specific or Adaptive) Immunity:

Acquired immunity (also called specific or adaptive immunity) refers to an immunity that is developed by the host in its body after exposure to a suitable antigen or after transfer of antibodies or lymphocytes from an immune donor.

Characteristics of Acquired Immunity:

Acquired immunity is highly adaptive and is capable of specifically recognizing and selectively eliminating foreign microorganisms and macromolecules, i.e., antigens.

It exhibits the following four characteristic features that distinguish it from nonspecific (innate) immunity:

(i) Specificity:

Acquired immunity is extremely antigenic specific as it acts against a particular microbial pathogen or foreign macromolecule and immunity to this antigen usually does not confer resistance to others. For convenience, the ability of the antibodies to differentiate between antigen molecules differs even by a single amino acid.

(ii) Diversity:

The acquired immune system generates tremendous diversity in its recognition molecules. As a result, it is able to specifically recognise billions of different structures on foreign antigens.

(iii) Memory:

Once the acquired immune system has recognised and responded to an antigen, it is able to respond this antigen more quickly and strongly following a subsequent exposure. This is due to the constitution of immunologic memory that makes the basis for long-term immunity in the body of the host.

(iv) Discrimination between "Self" and "Nonself":

The immune system almost always recognizes self and nonself antigens and responds only to nonself antigens. This ability to recognize self antigens from nonself ones is critical for normal functioning of the immune system. Sometimes this feature fails and, as a result, there develops autoimmune disease in the host.

Major Functions of Acquired immunity:

The acquired (specific or adaptive) immune system of the body is required to perform the following three major functions:

(i) It has to recognize any thing that is foreign to the body. The foreign material is called "nonself". The recognition system of acquired immunity is so highly specific that it is able to differentiate one pathogen from another, cancer cells, and even body's own "self" proteins from foreign "nonself" proteins.

(ii) After recognizing the foreign invader, the acquired immune system responds to this invader by recruiting its defensive molecules and cells to attack the invader. This response, called effector response, either eliminates the invader or makes it harmless to the host and thus protects the body from disease.

(iii) The acquired immune system remembers the foreign invader even after its first encounter. If the same invader attacks the previously attacked body at a later time, the system remembers the

invader and mounts a more intense and rapid memory or anamnestic response, which ones again eliminates the invader and protects the host from disease.

Components of Acquired Immunity:

Acquired immunity involves the following two major groups of cells:

(1) lymphocytes and

(2) antigen-presenting cells (APCs).

Lymphocytes are one of the many types of white blood cells (leucocytes) generated in bone marrow by the process of hematopoiesis. They migrate from bone marrow, circulate in the blood and lymphatic system, and reside in various lymphoid organs.

Lymphocytes possess antigen-binding cell-surface receptors and are responsible for the specificity, diversity, memory, and self/nonself recognition by the immune system.

In contrast, antigen-presenting cells (APCs) have class II MHC (major histocompatibility complex) molecules on their plasma membrane. These MHC molecules bind to antigen-derived peptides and present them to a group of lymphocytes, which are then activated to mount the immune response.

Collaboration between Innate and Acquired Immunities:

Although the acquired immunity develops after exposure to a suitable antigen or after transfer of antibodies or lymphocytes from an immune donor, it is not independent of innate immunity which is an inborn ability in the body.

Both the immunities function as a highly interactive and cooperative system rendering a combined response more effective than either immunity could produce by itself. It so happens because certain immune components play significant role in both types of immunities.

Following are the examples that show the interactive and cooperative roles of the two immunities:

(i) Phagocytic cells crucial to innate immunity are intimately involved in activating acquired immunity. Interactions between receptors on phagocytic cells and microbial components generate soluble factors that stimulate and direct acquired immunity facilitating the participation of the system in the elimination of the foreign invader. Acquired immune system, in turn, produces signals and components that stimulate and inhance the effectiveness of innate immunity.

(ii) Stimulated phagocytic cells involved in innate immunity also secrete cytokines that direct acquired immunity against particular intracellular microbial pathogens. In turn, some T lymphocytes of acquired immunity synthesize and secrete cytokines that increase the ability of phagocytic cells to destroy the microbial pathogens they have phagocytized during innate immune responses.

Differences between Innate and Acquired Immunities:

In contrast to their interactive and cooperative nature, the innate and acquired immunities show certain fundamental differences, which are the following:

(i) Innate immunity shows rapid response in comparison to acquired immunity the response of which is slower.

(ii) Innate immunity utilizes a pre-existing but limited repertoire of responding components, whereas the acquired immunity possesses ability to recognize a much wider repertoire of foreign substances.

(iii) Innate immunity remains constant during a response, whereas the acquired immunity possesses ability to improve during the response. It may be emphasized that due to its immunological memory, the acquired immunity operates much faster to the same pathogen during secondary exposure than the primary exposure.

These fundamental differences between innate and acquired immunity can be consolidated in the form of Table 41.2.

TABLE 41.2. Fundamental differences between innate and acquired immunities

Feature	*Innate Immunity*	*Acquired Immunity*
Response time	Rapid (hours)	Slow (days)
Specificity	Limited and fixed	Diversified and improves during the course of immune response
Response to re-exposure to pathogen	Identical to the primary response	Much more faster than the primary response

Types of Acquired Immunity:

Acquired immunity can be obtained by the host actively or passively and, on this basis, can be categorized as of two types:

(i) Active immunity and

(ii) Passive immunity.

In active immunity, there is active involvement of host's own immune system leading to the synthesis of antibodies and/or the production of immuno-competent cells (ICCs).

In passive immunity, on the contrary, the antibodies and /or the immuno-competent cells (ICCs) are transferred from one host to another. Active and passive immunities can be obtained naturally or artificially (Fig. 41.1).

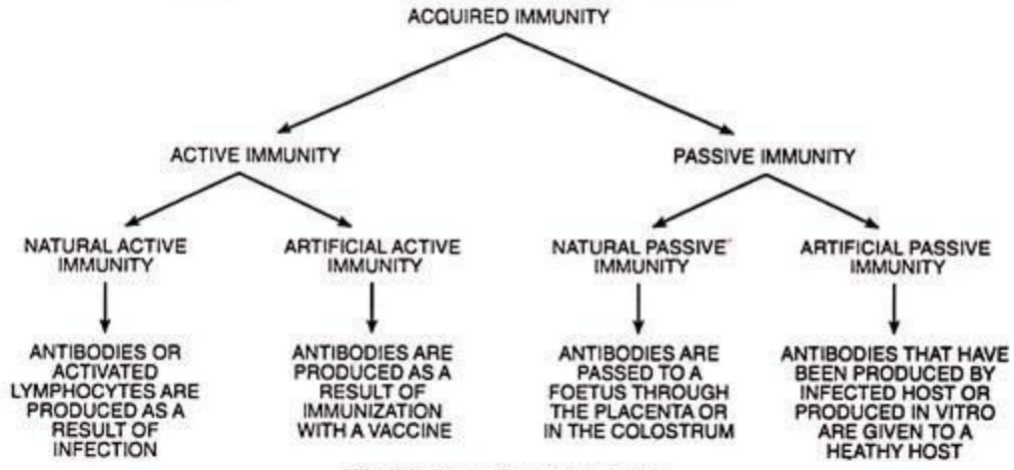


FIG. 41.1. Types of acquired immunity.

Branches or Arms of Acquired Immunity:

Acquired immunity consists of two branches or arms recognized as:

(i) Humoral and

(ii) Cellular immunity.

Humoral immunity is based on the action of soluble proteins called 'antibodies' whereas cellular immunity is based on the action of specific kinds of 'T lymphocytes'.

Type # 3. Active and Passive Immunity:

1. Active Immunity:

Active immunity, as stated earlier, refers to an immunity in which there is active involvement of host's own immune system leading to the synthesis of antibodies and/or the production of immunocompetent cells (ICCs).

There are two types of active immunity:

(i) Naturally acquired active immunity and

(ii) Artificially acquired passive immunity.

(i) Naturally Acquired Active Immunity:

This immunity develops after antigens (e.g., microbial pathogens) enter the body by natural processes such as infection and, in response, the body's immune system forms antibodies.

In some cases, the immunity may be life-long as with smallpox, measles, chickenpox, yellow fever etc. In other cases, however, the immunity may be lost after only a few years (e.g., diphtheria, tetanus) or even for lesser period (e.g., influenza, pneumonia).

(ii) Artificially Acquired Active Immunity:

When a carefully chosen antigen (e.g., vaccine, chemically altered toxins called toxoids) is intentionally introduced into a body to be immunized, the latter develops immunity that is called artificially acquired active immunity. This immunity is artificial because the antigens are intentionally or purposely introduced, and it is active because the recipient's immune system synthesizes antibodies in response.

Vaccines provide usually long-term immunity. Vaccines are now available against many infectious diseases such as cholera, tuberculosis, plague, pneumonia, rocky mountain spotted fever, smallpox, polio, tetanus, influenza, measles, rabies, yellow fever etc. Toxoids are currently available for protection against diphtheria and tetanus, the two diseases whose major effects are due to toxins.

The characteristics of naturally acquired and artificially acquired active immunities are summarized in Table 41.3.

TABLE 41.3. Summarized account of naturally acquired and artificially acquired active immunities

	Type of Active Immunity	Immunising Agents	Nature of Immunity	Origin	Effective Dose Required	Relative Duration of	Source of Antibodies	Function
1.	Naturally acquired active immunity	Antigens	Natural	Clinical or subclinical diseases	Small	Long (Life long or, in some cases, a few years)	Self	*Therapeutic
2.	Artificially acquired active immunity	Antigens	Intensional	Toxoid or vaccine	Small (months to years)	Long	Self	** Prophylactic

** *Therapeutic* = used to treat an established disease.
** *Prophylactic* = used to protect against a disease.

2. Passive Immunity:

Passive immunity, as stated earlier, refers to an immunity in which the antibodies and/or immuno-competent cells (ICCs) are transferred from one host to another.

There are two types of passive immunity:

(i) Naturally acquired passive immunity and

(ii) Artificially acquired passive immunity.

(i) Naturally Acquired Passive Immunity:

When antibodies produced in the body of an individual (called "donor") are naturally transferred into the body of other individual (called "recipient"), the latter develops immunity, called naturally acquired passive immunity, in its immune system.

This immunity is natural because the transfer of antibodies from donor to recipient occurs under natural conditions, and it is passive because the recipient does not synthesize antibodies but picks them up from the donor.

The best example of this type of immunity is the natural transfer of antibodies from the mother to the foetus across- the placenta. Certain antibodies are also transferred from mother to infant through colostrum and milk during nursing.

These antibodies, called maternal antibodies, remain with the child for about three to six months or, sometimes, twelve to fifteen months, and after the specified time the immune state disappears. The maternal antibodies generally provide resistance against whooping cough, diphtheria, german measles, diseases of respiratory and gastrointestinal tract, etc.

(ii) Artificially Acquired Passive Immunity:

Artificially acquired passive immunity is that which develops as a result of the intentional introduction of antibody-rich serum (blood plasma devoid of clotting factors) taken from diseased individual to another susceptible individual.

It was an important therapeutic device for disease treatment before the vaccines were developed and is still used for viral diseases such as hepatitis B, chicken pox, arthropod-borne encephalitis, and for bacterial diseases such as botulism, diphtheria, tetanus, staphylococcal-poisoning where toxins are involved in disease causation.

Since these diseases are very dangerous and fatal, already-made antibodies present in serum are introduced into the blood of the susceptible individual for quick response and no risk is taken for introduction of antigens. Artificially acquired passive immunity is immediate but short-lived (only for two to three weeks).

The characteristics of naturally acquired and artificially acquired passive immunities are summarized in Table 41.4.

TABLE 41.4. Summarized account of naturally acquired and artificially acquired passive immunities

Type of Active Immunity	Immunising Agents	Nature of Immunity	Origin	Effective Dose Required	Relative Duration of	Source of Antibodies	Function
1. Naturally acquired passive immunity	Antibodies	Natural	Passage across placenta between mother and foetus	Large	Short (3-6 months; sometimes 12-15 months)	Other than self	*Prophylactic
2. Artificially acquired passive immunity	Antibodies	Intensional	Serum rich with antibodies	Large	Short (2-3 weeks)	Other than self	Prophylactic

* *Prophylactic* = used to protect against a disease.

Immunity: Types, Components and Characteristics of Acquired Immunity

Immunity is the ability of the body to protect against all types of foreign bodies like bacteria, virus, toxic substances, etc. which enter the body.

Immunity is also called disease resistance. The lack of immunity is known as susceptibility.

The science dealing with the various phenomena of immunity, induced sensitivity and allergy is called immunology.

Types of Immunity:

There are two major types of immunity: innate or natural or nonspecific and acquired or adaptive.

(A) Innate or Natural or Nonspecific Immunity (L. innatus = inborn):

Innate immunity is inherited by the organism from the parents and protects it from birth throughout life. For example humans have innate immunity against distemper, a fatal disease of dogs.

As its name nonspecific suggests that it lacks specific responses to specific invaders. Innate immunity or nonspecific immunity is well done by providing different barriers to the entry of the foreign agents into our body. Innate immunity consists of four types of barriers— physical, physiological, cellular and cytokine barriers.

1. Physical Barriers:

They are mechanical barriers to many microbial pathogens. These are of two types. Skin and mucous membrane.

(a) Skin:

The skin is physical barrier of body. Its outer tough layer, the stratum corneum prevents the entry of bacteria and viruses.

(b) Mucous Membranes:

Mucus secreted by mucous membrane traps the microor¬ganisms and immobilises them. Microorganisms and dust particles can enter the respiratory tract with air during breathing which are trapped in the mucus. The cilia sweep the mucus loaded with microorganisms and dust particles into the pharynx (throat). From the pharynx it is thrown out or swallowed for elimination with the faeces.

2. Physiological Barriers:

The skin and mucous membranes secrete certain chemicals which dispose off the pathogens from the body. Body temperature, pH of the body fluids and various body secretions prevent growth of many disease causing microorganisms. Some of the important examples of physiological barriers are as follows:

(a) Acid of the stomach kills most ingested microorganisms,

(b) Bile does not allow growth of microorganisms,

(c) Cerumen (ear wax) traps dust particles, kills bacteria and repels insects,

(d) Lysozyme is present in tissue fluids and in almost all secretions except in cerebrospinal fluid, sweat and urine. Lysozyme is in good quantity in tears from eyes. Lysozyme attacks bacteria and dissolves their cell walls. Lysoenzyme is also found in saliva,

(e) Nasal Hair. They filter out microbes and dust in nose,

(f) Urine. It washes microbes from urethra,

(g) Vaginal Secretions. It is slightly acidic which discourages bacterial growth and flush microbes out of vagina,

(h) Sebum (sweat). It forms a protective acid film over the skin surface that inhibits growth of many microbes.

3. Cellular Barriers:

These are certain white blood corpuscles (leucocytes), macrophages, natural killer cells, complement system, inflammation, fever, antimicrobial substances, etc.

(i) Certain Leucocytes:

Neutrophils and monocytes are major phagocytic leucocytes.

(a) Polymorpho-nuclear Leucocytes (PMNL- neutrophils):

As they have multilobed nucleus they are normally called polymorphonuclear leucocytes (PMNL-neu- trophils). Neutrophils are short lived and are highly motile phagocytic killers.

Neutrophils are formed from stem cells in the bone marrow. Neutrophils are the most numerous of all leucocytes. They die after a few days and must therefore, be constantly replaced. Neutro¬phils constitute about 40% to 75% of the blood leucocytes in humans.

(b) Monocytes:

They are the largest of all types of leucocytes and somewhat amoeboid in shape. They have clear cytoplasm (without cytoplasmic granules). The nucleus is bean-shaped. Monocytes constitute about 2-10% of the blood leucocytes. They are motile and phagocytic in nature and engulf bacteria and cellular debris. Their life span is about 10 to 20 hours. Generally they change into macrophages after entering tissue spaces.

(ii) Macrophages:

Monocytes circulate in the bloodstream for about 8 hours, during which time they enlarge and then migrate into the tissues and differentiate into specific tissue macrophages. Macrophages are long lived and are highly motile phagocytic.

Macrophages contain more cell organelles especially lysosomes. Macrophages are of two types,
(a) Some take up residence in particular tissues becoming fixed macroph- ages and (b) whereas other remain motile and are called wandering macrophages. Wandering macrophages move by amoeboid movement throughout the tissues. Fixed mac¬rophages serve different functions in different tissues and are named to reflect their tissue location. Some examples are given below:

i. Pulmonary alveolar macrophages in the lung

ii. Histiocytes in connective tissues

iii. Kupffer cells in the liver

iv. Glomerular Mesangial cells in the kidney

v. Microglial cells in the brain

vi. Osteoclasts in bone

(iii) Natural Killer Cells (NK Cells):

Besides the phagocytes, there are natural killer cells in the body which are a type of lymphocytes and are present in the spleen, lymph nodes and red bone marrow. NK cells do not have antigen receptors like T cells and B cells. NK cells cause cellular destruction in at least two ways:

(a) NK cells produce perforins which are chemicals that when inserted into the plasma membrane of a microbe make so weak that cytolysis (breakdown of cells particu¬larly their outer membrane) occurs and creates pores in the plasma membrane of the target cells. These pores allow entry of water into the target cells, which then swell and burst. Cellular remains are eaten by phagocytes.

(b) Another function of NK cells is apoptosis which means natural cell death. It occurs naturally as part of the normal development, maintenance and renewal of cells, tissues and organs.

Thus functions of NK cells are to destroy target cells by cytolysis and apoptosis. NK cells constitute 5%-10% of the peripheral blood lymphocytes in humans.

(iv) Complement (Fig. 8.7):

Complement is a group of 20 proteins, many of which are enzyme precursors and are produced by the liver. These proteins are present in the serum of the blood (the fluid portion of the blood excluding cells and clotting factors) and on plasma membranes. They are found circulating in the blood plasma and within tissues throughout the body. They were named complement by Ehrlich because they complement the actions of other components of the immune system (e.g., action of antibody on antigen) in the fight against infection. Jules Bordet is the discoverer of complement.

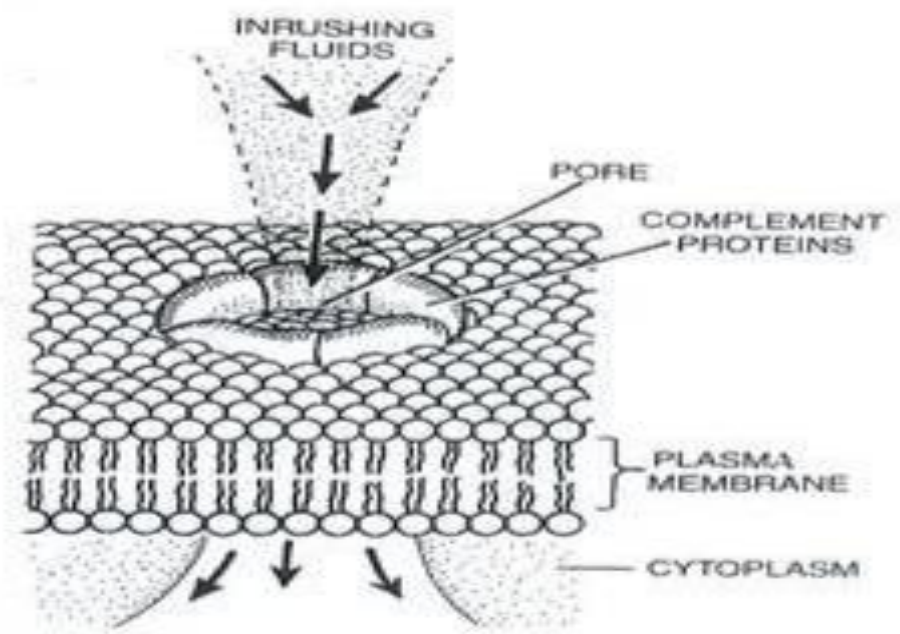


Fig. 8.7. Complement proteins creating a hole in the plasma membrane.

Complement proteins create pores in the plasma membrane of the microbes. Water enters the microbes. The latter burst and die. The proteins of complement system destroy microbes by (i)

cytolysis (ii) inflammation and (iii) phagocytosis. These proteins also prevent excessive damage of the host tissues.

(v) Inflammation:

Inflammation is a defensive response of the body to tissue damage. The conditions that may produce inflammation are pathogens, abrasions (scraping off) chemical irritations, distortion or disturbances of cells, and extreme temperatures. The signs and symptoms of inflammation are redness, pain, heat and swelling.

Inflammation can also cause the loss of function in the injured area, depending on the site and extent of the injury. Inflammation is an attempt to dispose of microbes, toxins, or foreign material at the site of injury to prevent their spread to other tissues, and to prepare the site for tissue repair. Thus, it helps restore tissue homeostasis.

Broken mast cells release histamine. Histamine causes dilation of capillaries and small blood vessels. As a result more blood flows to that area making it red and warm and fluid (plasma) takes out into the tissue spaces causing its swelling. This reaction of the body is called inflammatory response.

(vi) Fever:

Fever may be brought about by toxins produced by pathogens and a protein called endogenous pyrogen (fever producing substance), released by macrophages. When enough pyrogens reach the brain, the body's thermostat is reset to a higher temperature, allowing the temperature of the entire body to rise.

Mild fever strengthens the defence mechanism by activating the phagocytes and by inhibiting the growth of microbes. A very high temperature may prove dangerous. It must be quickly brought down by giving antipyretics.

4. Cytokine Barriers:

Cytokines (Chemical messengers of immune cells) are low molecular weight proteins that stimulate or inhibit the differentiation, proliferation or function of immune cells. They are involved in the cell to cell communication. Kinds of cytokines include interleukins produced by leucocytes, lymphocytes produced by lymphocytes, tumour necrosis factor and interferon's (IFNs). Interferon's protect against viral infection of cells.

(B) Acquired Immunity (= Adaptive or Specific Immunity):

The immunity that an individual acquires after the birth is called acquired or adaptive or specific immunity. It is specific and mediated by antibodies or lymphocytes or both which make the antigen harmless.

It not only relieves the victim of the infectious disease but also prevents its further attack in future. The memory cells formed by B cells and T cells are the basis of acquired immunity. Thus acquired immunity consists of specialized B and T lymphocytes and Antibodies.

Characteristics of Acquired Immunity:

(i) Specificity:

It is the ability to differentiate between various foreign molecules (for¬eign antigens).

(ii) Diversity:

It can recognise a vast variety of foreign molecules (foreign antigens).

(iii) Discrimination between Self and Non-self:

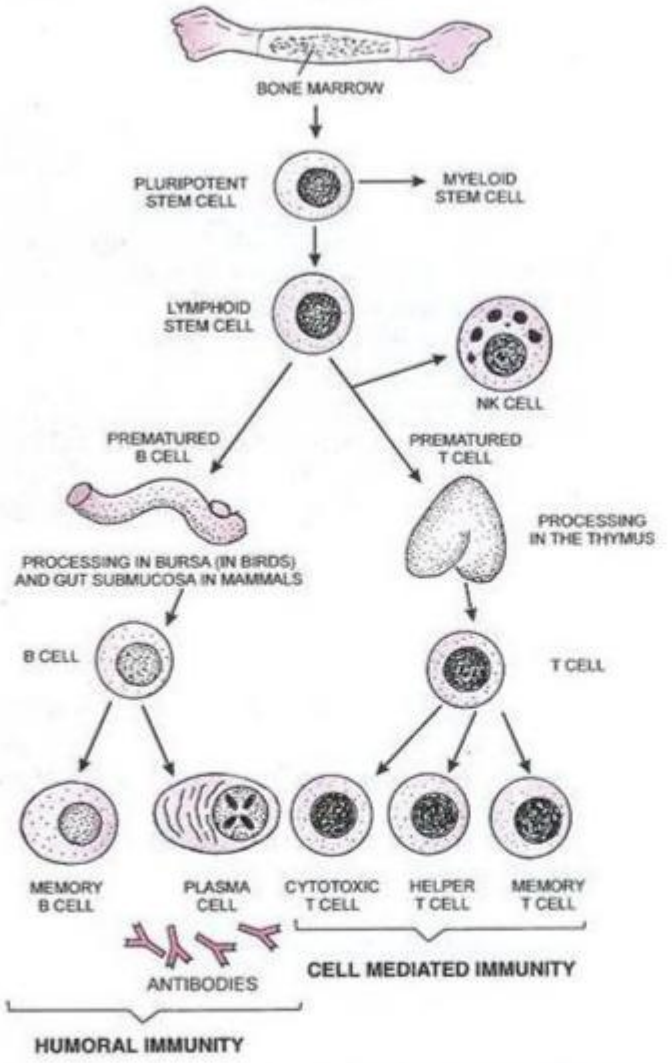
It can recognise and respond to foreign molecules (non-self) and can avoid response to those molecules that are present within the body (self) of the animal.

(iv) Memory:

When the immune system encounters a specific foreign agent, (e.g., a microbe) for the first time, it generates immune response and eliminates the invader. This is called first encounter. The immune system retains the memory of the first encounter. As a result, a second encounter occurs more quickly and abundantly than the first encounter.

The cells of the immune system are derived from the pluripotent stem cells in the bone marrow. Pluripotent means a cell that can differentiate into many different types of tissue cells. The pluripotent stem cells can form either myeloid stem cells or lymphoid stem cells.

Myeloid stem cells give rise to monocytes, macrophages and granulocytes (neutrophils eosinophil's, and basophils). RBCs and blood platelets (lymphoid stem cells) form B lymphocytes (B cells), T lymphocytes (T-cells) and natural killer (NK) cells.



Development of B and T lymphocytes. Both arise from bone marrow precursors. Natural killer (NC) cells are a third population of lymphocytes that are distinct from T cells and B cells.

Components of Acquired Immunity:

Acquired immunity has two components: humeral immunity or Antibody mediated immune system (AMIS) and cellular immunity or cell mediated immune system (CMIS).

I. Antibody Mediated Immune System (AMIS) or Humoral Immunity:

It consists of antibodies (specialised proteins produced in the body in response to antigen) that circulate in the body fluids like blood plasma and lymph. The word 'humor' pertains to fluid. B lymphocytes (B cells) produce antibodies that regulate humoral immunity. The T-lymphocytes themselves do not secrete anti-bodies but help B lymphocytes produce them.

Certain cells of the bone marrow produce B lymphocytes and mature there. Since B lymphocytes produce antibodies, therefore, this immunity is called antibody mediated or humoral immunity. Humoral immunity or antibody-mediated immune system (AMIS) provides defence against most extracellular bacterial pathogens and viruses that infect through the respiratory and intestinal tract.

Formation of Plasma B cells and Memory B cells:

When antibodies on B cell's surface bind antigens (any substances that cause antibodies formation) the B cell is activated and divides, producing a clone (descendants of a single cell) of daughter B cells. These clones give rise to plasma B cells and memory B cells. This phenomenon is called clonal selec¬tion.

(a) Plasma B Cells (Effector B cells):

Some of the activated B cells enlarge, divide and differentiate into a clone of plasma cells. Although plasma cells live for only a few days, they secrete enormous amounts of antibody during this period.

(b) Memory B Cells:

Some activated B cells do not differentiate into plasma cells but rather remain as memory cells (Primed cells). They have a longer life span. The memory cells remain dormant until activated once again by a new quantity of the same antigen.

Role of AMIS:

The AMIS protects the body from (i) viruses (ii) some bacteria and (iii) toxins that enter the body fluids like blood and lymph.

II. Cell-Mediated Immune System (CMIS) or T-Cell Immunity:

A healthy person has about a trillion lymphocytes. Lymphocytes are of two types: T lymphocytes or T cells and B lymphocytes or B cells. As we know both types of lymphocytes and other cells of the immune system are produced in the bone marrow. The process of production of cells of immune system in the bone marrow is called haematopoiesis.

Because T lymphocytes (T cells) mature in the thymus, this immunity is also called T- cell immunity.

The T-cells play two important functions—effector and regulatory.

The effector function includes cytolysis (destruction of cells by immune processes) of cells infected with microbes and tumour cells and lymphokine production. The regulatory functions are either to increase or to suppress other lymphocytes and accessory cells.

Types of T-cells and their Functions:

1. Helper T cells (TH):

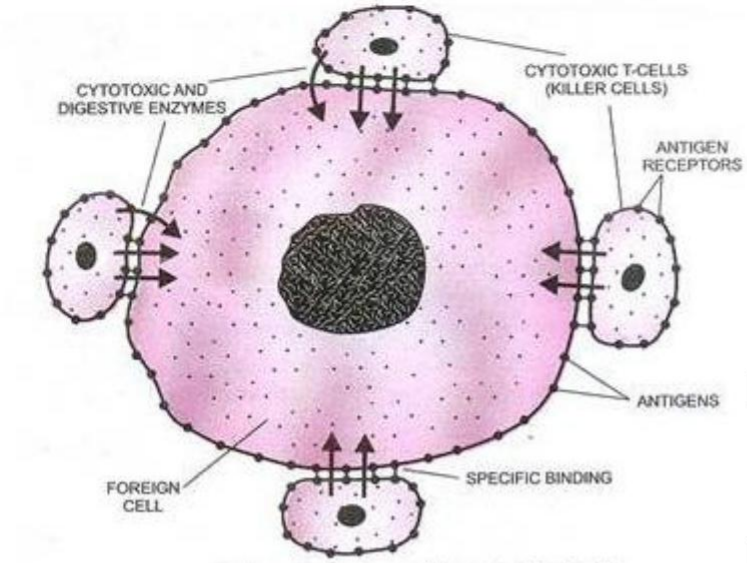
TH cells are most numerous of the T cells. They help in the functions of immune system. They produce a growth factor that stimulates B-cell proliferation and differentiation and also stimulates antibody production by plasma cells; enhance activity of cytotoxic T cells.

2. Cytotoxic T cells (Tc) or Killer cells:

These cells are capable of killing microorganisms and even some of the body's own cells directly hence they are called killer cells. The antigen receptors on the surfaces of the cytotoxic cells cause specific binding with antigens present on the surface of foreign cell.

Cell after binding, the cytotoxic T cell secretes hole-forming proteins, called perforins, that punch large round holes in the membrane of the foreign cell. Then fluid flows quickly into the cell from the interstinal space. In addition, the cytotoxic T cell releases cytotoxic substances directly into the foreign cell. Almost immediately, the foreign cell becomes greatly swollen and it usually dissolves shortly thereafter.

Thus they destroy body cells infected by viruses and attack and kill bacteria, fungi, parasites and cancer cells.



Killing of a foreign cell by cytotoxic T-cells.

3. Memory T Cells (Primed Cells):

These cells are also formed by T-lymphocytes as a result of exposure to antigen and remain in the lymphatic tissue (e.g., spleen, lymph nodes). They recognize original invading antigens even years after the first encounter.

These cells keep ready to attack as soon as the same pathogens infect the body again. They proliferate and differentiate into cytotoxic T cells, helper T cells, suppressor T cells, and additional memory cells.

4. Suppressor Cells (Regulatory T cells (TR)):

These cells are capable of suppressing the functions of cytotoxic and helper T cells. They also inhibit the immune system from attacking the body's own cells. It is believed that suppressor cells regulate the activities of the other cells. For this reason, the suppressor cells are classified as regulatory T cells.

Natural Killer (NK) Cells:

NK cells attack and destroy target cells, participate in antibody dependent cell mediated cytotoxicity. They can also attack parasites which are much larger than bacteria.

Types of Acquired Immunity:

Acquired (= Adaptive) Immunity is of two types: active immunity and passive immunity.

1. Active Immunity:

In this immunity person's own cells produce antibodies in response to infection or vaccination. It is slow and takes time in the formation of antibodies. It is long lasting and is harmless. Active immunity may be natural or artificial.

(a) A person who has recovered from an attack of small pox or measles or mumps develops natural active immunity.

(b) Artificial active immunity is the resistance induced by vaccines. Examples of vaccines are as follows: Bacterial vaccines, (a) Live- BCG vaccine for tuberculosis, (b) Killed vaccines- TAB vaccine for enteric fever. Viral vaccines, (a) Live – sabin vaccine for poliomyelitis, MMR

vaccine for measles, mumps, rubella, (b) Killed vaccines- salk vaccine for poliomyelitis, neural and non-neural vaccines for rabies. Bacterial products. Toxoids for Diphtheria and Tetanus.

2. Passive Immunity:

When ready-made antibodies are directly injected into a person to protect the body against foreign agents, it is called passive immunity. It provides immediate relief. It is not long lasting. It may create problems. Passive immunity may be natural or artificial.

(a) Natural passive immunity is the resistance passively transferred from the mother to the foetus through placenta. IgG antibodies can cross placental barrier to reach the foetus. After birth, immunoglobulin's are passed to the new-born through the breast milk. Human colostrum (mother's first milk) is rich in IgA antibodies. Mother's milk contains antibodies which protect the infant properly by the age of three months.

(b) Artificial passive immunity is the resistance passively transferred to a recipient by administration of antibodies. This is done by administration of hyper-immune sera of man or animals. Serum (pi. sera) contains antibodies. For example, anti-tetanus serum (ATS) is prepared in horses by active immunisation of horses with tetanus toxoid, bleeding them and separating the serum. ATS is used for passive immunisation against tetanus. Similarly anti-diphtheric serum (ADS) and anti-gas gangrene serum (AGS) are also prepared.

Superantigens

Superantigens (SAgs) are a class of antigens that cause non-specific activation of T-cells resulting in polyclonal T cell activation and massive cytokine release. SAgs are produced by some pathogenic viruses and bacteria most likely as a defense mechanism against the immune system. Compared to a normal antigen-induced T-cell response where 0.0001-0.001% of the body's T-cells are activated, these SAgs are capable of activating up to 20% of the body's T-cells. Furthermore, Anti-CD3 and Anti-CD28 Antibodies (CD28-SuperMAB) have also shown to be highly potent superantigens (and can activate up to 100% of T cells).

The large number of activated T-cells generates a massive immune response which is not specific to any particular epitope on the SAg thus undermining one of the fundamental strengths of the adaptive immune system, that is, its ability to target antigens with high specificity. More importantly, the large number of activated T-cells secrete large amounts of cytokines, the most important of which is Interferon gamma. This excess amount of IFN-gamma in turn activates the macrophages. The activated macrophages, in turn, over-produce proinflammatory cytokines such as IL-1, IL-6 and TNF-alpha. TNF-alpha is particularly important as a part of the body's inflammatory response. In normal circumstances it is released locally in low levels and helps the immune system defeat pathogens. However, when it is systemically released in the blood and in high levels (due to mass T-cell activation resulting from the SAg binding), it can cause severe and life-threatening symptoms, including shock and multiple organ failure.

Superantigens are viral or bacterial proteins that bind simultaneously to the V_ domain of a T-cell receptor and to the chain of a class II MHC molecule. Both exogenous and endogenous superantigens have been identified. Crosslinkage of a T-cell receptor and class II MHC molecule by either type of superantigen produces an activating signal that induces T-cell activation and proliferation (Figure 10-16). *Exogenous* superantigens are soluble proteins secreted by bacteria. Among them are a variety of **exotoxins** secreted by gram-positive bacteria, such as staphylococcal enterotoxins, toxic-shock-syndrome toxin, and exfoliative-dermatitis toxin. Each of these exogenous superantigens binds particular V_ sequences in T-cell receptors (Table 10-3) and crosslinks the TCR to a class II MHC molecule. *Endogenous* superantigens are cell-membrane proteins encoded by certain viruses that infect mammalian cells. One group, encoded by mouse mammary tumor virus (MTV), can integrate into the DNA of certain inbred mouse strains; after integration, retroviral proteins are expressed on the membrane of the infected cells. These viral proteins, called **minor lymphocyte stimulating (Mls)** determinants, bind particular V_ sequences in T-cell receptors and crosslink the TCR to a class II MHC molecule. Four Mls superantigens, originating in different MTV strains, have been identified. Because superantigens bind outside of the TCR antigen binding cleft, any T cell expressing a particular V_ sequence will be activated by a corresponding superantigen. Hence, the activation is polyclonal and can affect a significant percentage (5% is not unusual) of the total TH population. The massive activations that follow crosslinkage by a superantigen results in overproduction of TH-cell cytokines, leading to systemic toxicity. The food poisoning induced by staphylococcal enterotoxins and the toxic shock induced by toxicshock-syndrome toxin are two examples of the consequences of cytokine overproduction induced by superantigens. Superantigens can also

influence T-cell maturation in the thymus. A superantigen present in the thymus during thymic processing will induce the negative selection of all thymocytes bearing a TCR V_ domain corresponding to the superantigen specificity. Such massive deletion can be caused by exogeneous or endogenous superantigens and is characterized by the absence of all T cells whose receptors possess V domains targeted by the superantigen.

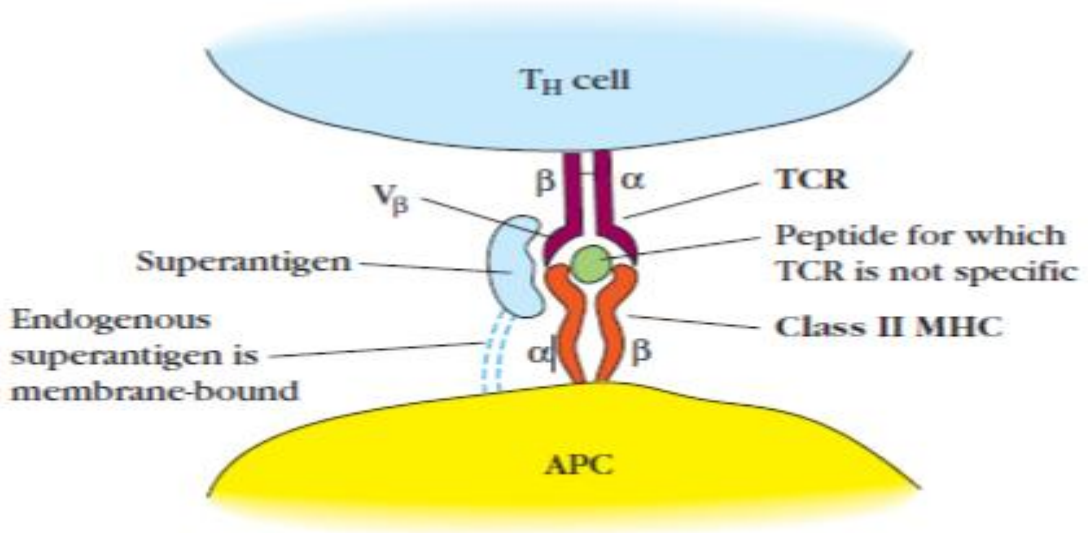


FIGURE 10-16 Superantigen-mediated crosslinkage of T-cell receptor and class II MHC molecules. A superantigen binds to all TCRs bearing a particular V_β sequence regardless of their antigenic specificity. Exogenous superantigens are soluble secreted bacterial proteins, including various exotoxins. Endogenous superantigens are membrane-embedded proteins produced by certain viruses; they include Mls antigens encoded by mouse mammary tumor virus.

Immune Response:

The immune response involves primary immune response and secondary immune response.

(a) The primary immune response:

After an initial contact with an antigen, no anti¬bodies are present for a period of several days. Then, a slow rise in the antibody titer o(arbitrary units) occurs, first IgM and then IgG followed by a gradual decline in antibody titer. This is called the primary immune response.

(b) The secondary immune response:

Memory cells may remain in the body for decades. Every new encounter with the same antigen results in a rapid proliferation of memory cells. This is also called "booster response". The antibody titer after subsequent encounters is far greater than during a primary response and consists mainly of IgG anti¬bodies. This accelerated, more intense response is called the secondary immune response. Antibodies produced during a secondary response have an even higher affinity for the antigen.

A person who had been suffering from diseases like measles, small pox or chicken pox becomes immune to subsequent attacks of these diseases. It includes spleen, lymph nodes, tonsils, Peyer's patches of small intestine and appendix.

The increased power and duration of the secondary immune response explain why immunization (method of providing immunity artificially, it is called vaccination) is usually accomplished by injecting antigen in multiple doses.

Differences between Primary and Secondary Immune Response

The primary immune response occurs when an antigen comes in contact to the immune system for the first time. During this time the immune system has to learn to recognize antigen and how to make antibody against it and eventually produce memory lymphocytes.

The secondary immune response occurs when the second time (3rd, 4th, etc.) the person is exposed to the same antigen. At this point immunological memory has been established and the immune system can start making antibodies immediately.

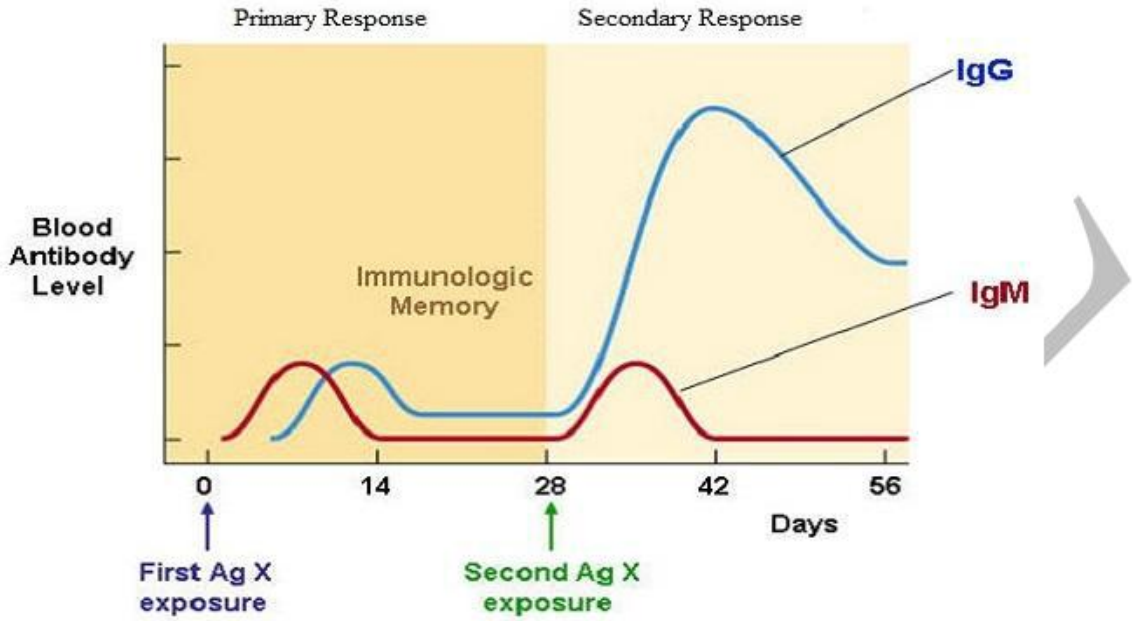


Fig. Immune Response and Secretion of antibodies

S.N.	Primary immune response	Secondary immune response
1.	This occurs as a result of primary contact with an antigen.	This occurs as a result of second and subsequent exposure of the same antigen
2	Responding cell is naïve B-cell and T-cell.	Responding cell is memory cell.
3	Lag phase is often longer (4-7 days), sometimes as long as weeks or months.	Lag phase is shorter (1-4 days) due to the presence of memory cell.
4	Level of antibody reaches peak in 7 to 10 days.	Level of antibody reaches peak in 3 to 5 days.
5	It takes longer time to establish immunity.	Takes shorter time to establish

		immunity.
6	First antibody produced is mainly IgM. Although small amount of IgG are also produced.	Mainly IgG antibody is produced. Although sometimes small amount of IgM are produced. Other immunoglobulins such as IgA and in the case of allergy IgE are produced.
7	Amount of antibody produced depends on nature of antigen. Usually produced in low amount.	Usually 100-1000 times more antibodies are produced.
8	Antibody level declines rapidly.	Antibody level remain high for longer period.
9.	Affinity of antibody is lower for its antigen.	Antibodies have greater affinity for antigen.
10	Primary response appears mainly in the lymph nodes and spleen.	Secondary response appears mainly in the bone marrow. followed by the spleen and lymph nodes.
11	Both Thymus dependent and Thymus independent antigen gives primary immune response.	Only Thymus-dependent antigen gives secondary immune response.

2 Marks

1. Define antigen
2. What is antibody
3. Define Agglutination
4. Explain Precipitation
5. What is super antigen
6. Explain primary immune response
7. Explain secondary immune response

8 marks

1. Elaborate History and scope of immunology
2. What are the types of immunity. Explain
3. What is immunoglobulin. Explain its type
4. Explain primary and secondary immune response and there difference
5. Elaborate Humoral and cell mediated immunity with suitable diagram.

IMMUNE RESPONSE

IMMUNE SYSTEM -- AN OVERVIEW

The immune system is composed of many interdependent cell types that collectively protect the body from bacterial, parasitic, fungal, viral infections and from the growth of tumor cells. Many of these cell types have specialized functions. The cells of the immune system can engulf bacteria, kill parasites or tumor cells, or kill viral-infected cells. Often, these cells depend on the T helper subset for activation signals in the form of secretions formally known as cytokines, lymphokines, or more specifically interleukins.

CELLS OF IMMUNE SYSTEM

Hematopoiesis

All blood cells arise from a type of cell called hematop[ietic stem cell(HSC). Stem cells that can differentiate into other cell types. They are self renewing, they maintain their population level by cell division. In human, hematopoiesis, the formation and development of red and white blood cells begins in the embryonic yolk sac during the first week of development.

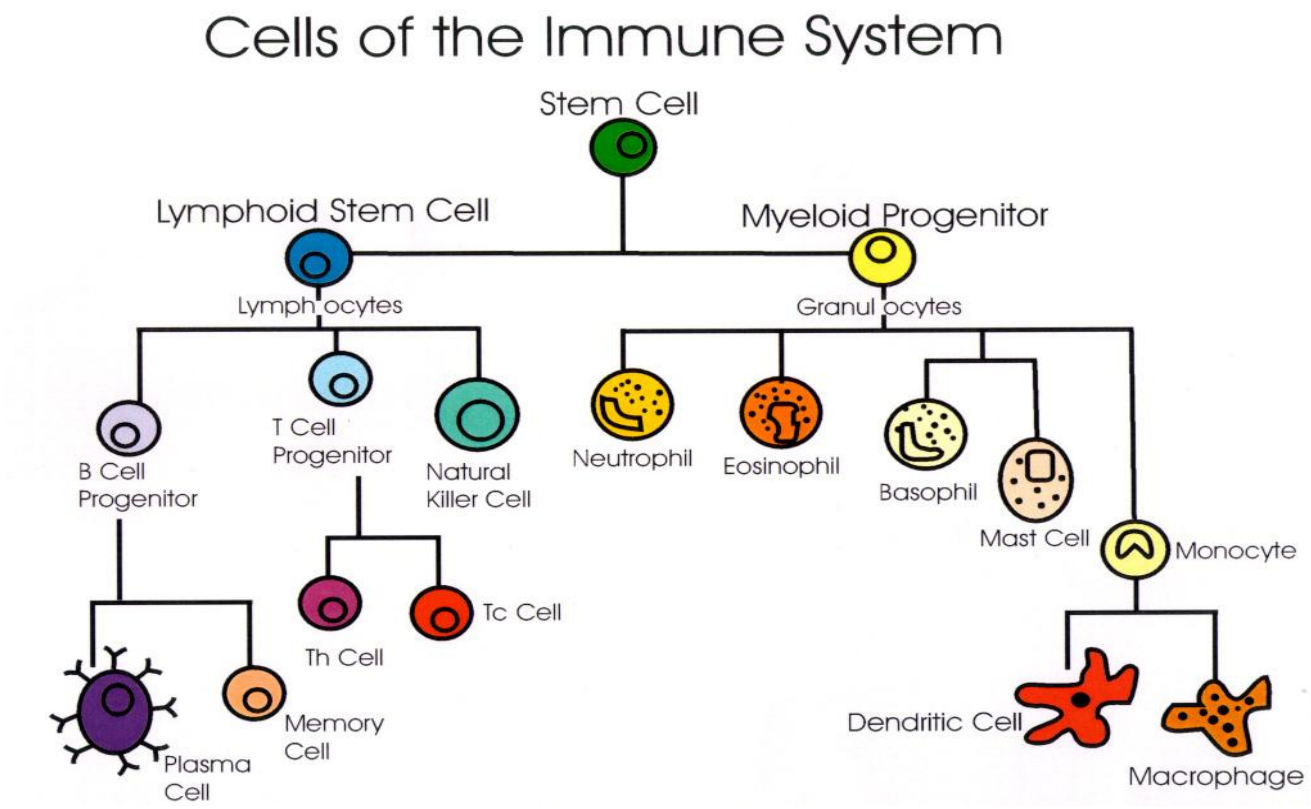


Fig: Cells of immune system

The Cells of the Immune System

Antigen-presenting cells	Cells which do not have antigen-specific receptors. Instead, they capture and process antigens, present them to T cell receptors. These cells include macrophages, dentritic cells and B cells.
B cells	Also known as *B cell lymphocytes*. B cells spend their entire early life in the bone marrow. Upon maturity, their job is to travel throughout the blood and lymph looking for antigens with which they can interlock. Once a B cell has identified an antigen, it starts replicating itself. These cloned cells mature into antibody-manufacturing *plasma cells*.
Basophils	Similar to mast cells, but distributed throughout the body. Like mast cells, basophils release histamine upon encountering certain antigens, thereby triggering an allergic reaction.
Cytotoxic T cells	Also called *cytotoxic T lymphocytes* or CTLs.
Dendritic cells	Mostly found in the skin and mucosal epithelium, where they are referred to as Langerhan's cells. Unlike macrophages, dendritic cells can also recognize viral particles as non-self. In addition, they can present antigens via **both** MHC I and MHC II, and can thus activate both CD8 and CD4 T cells, directly.
Granulocytes	Leukocytes (white blood cells) containing granules in the cytoplasm. Also known as a granular leukocyte. They seem to act as a first line of defense, as they rush toward an infected area and engulf the offending microbes. Granulocytes kill microbes by digesting them with killer enzymes contained in small units called lysosomes.
Helper T cells	These cells travel through the blood and lymph, looking for antigens (such as those captured by *antigen-presenting cells*). Upon locating an antigen, they notify other cells to assist in combating the invader. This is sometimes done through the use of cytokines (or specifically, lymphokines) which help destroy target cells and stimulate the production of healthy new tissue. Interferon is an example of such a cytokine.
Leukocytes	White blood cells. These are the cells which provide immunity, and they can be subdivided into three classes: lymphocytes, granulocytes and monocytes
Lymphocytes	Small white blood cells which are responsible for much of the work of the immune system. Lymphocytes can be divided into three classes: B cells, T cells and null cells.
Macrophages	Literally, "large eaters." These are large, long-lived phagocytes which

	capture foreign cells, digest them, and present protein fragments (peptides) from these cells and manifest them on their exterior. In this manner, they present the antigens to the T cells. Macrophages are strategically located in lymphoid tissues, connective tissues and body cavities, where they are likely to encounter antigens. They also act as effector cells in cell-mediated immunity.
Mast cells	Cells concentrated within the respiratory and gastrointestinal tracts, and within the deep layers of the skin. These cells release histamine upon encountering certain antigens, thereby triggering an allergic reaction.
Memory cells	Specialized B cells which grant the body the ability to manufacture more of a particular antibody as needed, in case a particular antigen is ever encountered again.
Monocytes	Large, agranular leukocytes with relatively small, eccentric, oval or kidney-shaped nuclei.
Plasma cells	Specialized B cells which churn out antibodies—more than two thousand per second. Most of these die after four to five days; however, a few survive to become *memory cells*.
T cells	Also known as *T cell lymphocytes.* Unlike B cells, these cells leave the marrow at an early age and travel to the thymus, where they mature. Here they are imprinted with critical information for recognizing "self" and "non-self" substances. Among the subclasses of T cells are *helper T cells* and *cytotoxic (or killer) T cells*.

ORGANS OF THE IMMUNE SYSTEM

A number of morphologically and functionally diverse or-gans and tissues have various functions in the development of immune responses. These can be distinguished by func-tion as the primary and secondary lymphoid organs (Figure). 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 addi-tion, 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.

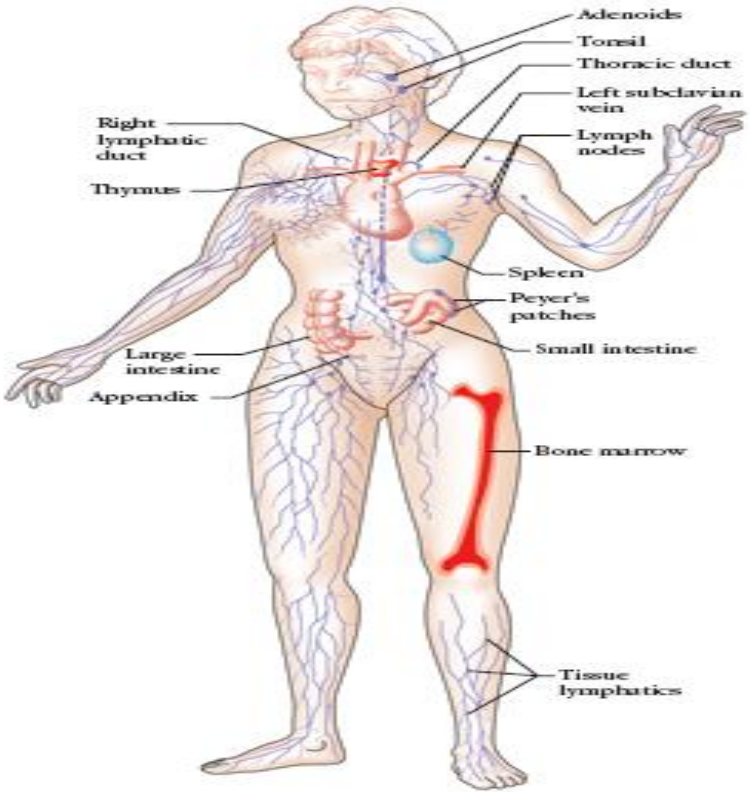


Figure: The human lymphoid system. The primary organs (bone marrow and thymus) are shown in red; secondary organs and tissues, in blue. These structurally and functionally diverse lymphoid organs and tissues are interconnected by the blood vessels (not shown) and lymphatic vessels (purple) through which lymphocytes circulate. Only one bone is shown, but all major bones contain ma-row and thus are part of the lymphoid system.

PRIMARY LYMPHOID ORGANS:

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

Also called central lymphoid organs, these are responsible for synthesis and maturation of immunocompetant cells. These include the bone marrow and the thymus.

(i) THYMUS:

The thymus is a gland located in the anterior mediastinum just above the heart, which reaches its greatest size just prior to birth, then atrophies with age.

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 compart-ments: the outer compartment, or *cortex,* is densely packed with immature T cells, called thymocytes, whereas the inner compartment, or *medulla,* is sparsely populated with thymo-cytes.

Both the cortex and medulla of the thymus are criss-crossed by a three-dimensional stromal-cell network com-posed 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 thymo-cytes (Figure). Some thymic epithelial cells in the outer cortex, called nurse cells, have long membrane extensions that surround as many as 50 thymocytes, forming large mul-ticellular 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 re-ceptors is generated by a random process that produces some T cells with receptors capable of recognizing antigen-MHC complexes. However, most of the T-cell recep-tors produced by this random process are incapable of recog-nizing 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 in-duces the death of those T cells that cannot recognize anti-gen-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 thy-mus without ever reaching maturity.

Children with no development of thymus suffer from DiGeorge syndrome that is characterized by deficiency in T cell development but normal numbers of B cells.

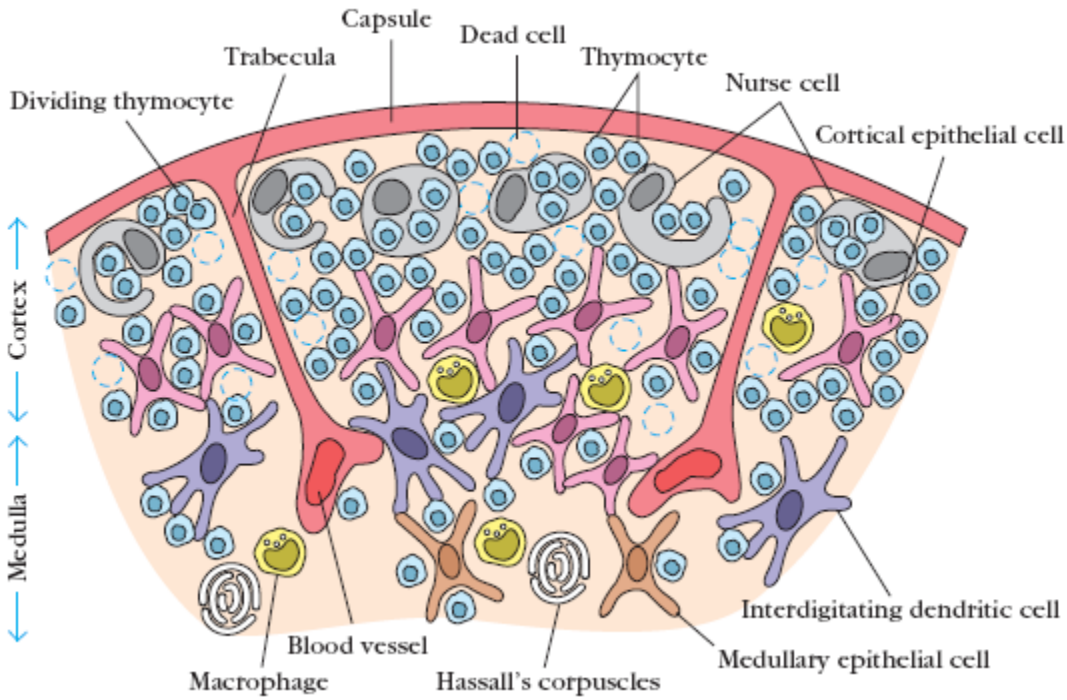


Fig: Diagrammatic cross section of a portion of the thymus, showing several lobules separated by connective tissue strands (trabeculae). The densely populated outer cortex is thought to con-tain many immature thymocytes (blue), which undergo rapid prolif-eration coupled with an enormous rate of cell death. Also present in the outer cortex are thymic nurse cells (gray), which are specialized epithelial cells with long membrane extensions that surround as many as 50 thymocytes. The medulla is sparsely populated and is thought to contain thymocytes that are more mature. During their stay within the thymus, thymocytes interact with various stromal cells, including cortical epithelial cells (light red), medullary epithelial cells (tan), interdigitating dendritic cells (purple), and macrophages (yellow). These cells produce thymic hormones and express high lev-els of class I and class II MHC molecules. Hassalls corpuscles, found in the medulla, contain concentric layers of degenerating ep-ithelial cells.

(ii) BONE MARROW:

In humans and mice, bone marrow is the site of B-cell origin and development. Arising from lymphoid progenitors, im-mature 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 T-cell maturation, a selection process within the bone marrow eliminates B cells with self-reactive antibody receptors.

Bone marrow is not the site of B-cell development in all species. In birds, a lymphoid organ called the bursa of Fabricius, In cattle and sheep, the primary lymphoid tis-sue hosting the

maturation, proliferation, and diversification of B cells early in gestation is the fetal spleen. Later in gesta-tion, this function is assumed by a patch of tissue embedded in the wall of the intestine called the ileal Peyer's patch The rabbit, too, uses gut-associated tissues such as the appendix as primary lym-phoid tissue

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 pro gressively larger collecting vessels called lymphatic vessels (Figure).

The largest lymphatic vessel, the thoracic duct, empties into the left subclavian vein near the heart. In this way, the lymphatic system captures fluid lost from the blood and returns it to the blood, thus ensuring steady-state levels of fluid within the circulatory system. The heart does not pump the lymph through the lymphatic system; instead the flow of lymph is achieved as the lymph vessels are squeezed by movements of the body's muscles. A series of one-way valves along the lymphatic vessels ensures that lymph flows only in one direction.

When a foreign antigen gains entrance to the tissues, it is picked up by the lymphatic system (which drains all the tissues of the body) and is carried to various organized lymphoid tissues such as lymph nodes, which trap the foreign antigen. As lymph passes from the tissues to lym-phatic vessels, it becomes progressively enriched in lympho-cytes. Thus, the lymphatic system also serves as a means of transporting lymphocytes and antigen from the connec-tive tissues to organized lymphoid tissues where the lympho-cytes may interact with the trapped antigen and undergo activation.

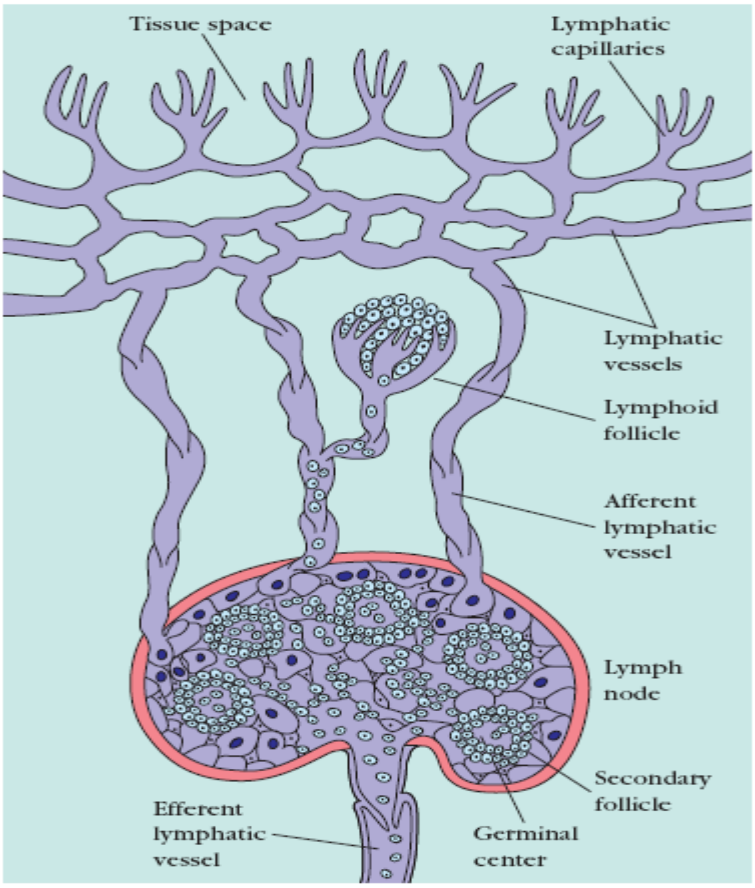


Fig: Lymphatic vessels. Small lymphatic capillaries open-ing into the tissue spaces pick up interstitial tissue fluid and carry it into progressively larger lymphatic vessels, which carry the fluid, now called lymph, into regional lymph nodes. As lymph leaves the nodes, it is carried through larger efferent lymphatic vessels, which eventu-ally drain into the circulatory system at the thoracic duct or right lymph duct

PERIPHERAL LYMPHOID ORGANS (SECONDARY):

While primary lymphoid organs are concerned with production and maturation of lymphoid cells, the secondary or peripheral lymphoid organs are sites where the lymphocytes localise, recognise foreign antigen and mount response against it. These include the lymph nodes, spleen, tonsils, adenoids, appendix, and clumps of lymphoid tissue in the small intestine known as Peyer's patches. They trap and concentrate foreign substances, and they are the main sites of production of antibodies. Some lymphoid organs are capsulated such as lymph node and spleen while others are non-capsulated, which include mostly mucosa-associated lymphoid tissue (MALT).

(i) LYMPH NODE:

Clusters of lymph nodes are strategically placed in the neck, axillae, groin, mediastinum and abdominal cavity, where they filter antigens from the interstitial tissue fluid and the lymph during its passage from the periphery to the thoracic duct. The key lymph nodes are the axillary lymph nodes, the inguinal lymph nodes, the mesenteric lymph nodes and the cervical lymph nodes.

Lymph nodes are encapsulated bean-shaped structures containing a reticular network packed with lymphocytes, macrophages, and dendritic cells. Clus-tered 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 mi-croenvironment 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 microenviron-ment (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 folli-cles, 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 popu-lated largely by T lymphocytes and also contains interdigitat-ing 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 pre-senting antigen to T_H cells. Lymph nodes taken from neona-tally thymectomized mice have unusually few cells in the paracortical region; the paracortex is therefore sometimes re-ferred to as a thymus-dependent area in contrast to the cor-tex, which is a thymus-independent area. The innermost layer of a lymph node, the medulla, is more sparsely popu-lated 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 para-cortex, resulting in the activation of T_H cells. The initial acti-vation of B cells is also thought to take place within the T-cell-rich paracortex. Once activated, T_H and B cells form small foci consisting largely of proliferating B cells at the edges of the paracortex. Some B cells within the foci differen-tiate 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 T_H 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 T_H cells take place, leading to development of a sec-ondary 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 (Figure b). 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 lympho-cytes than the afferent lymph.

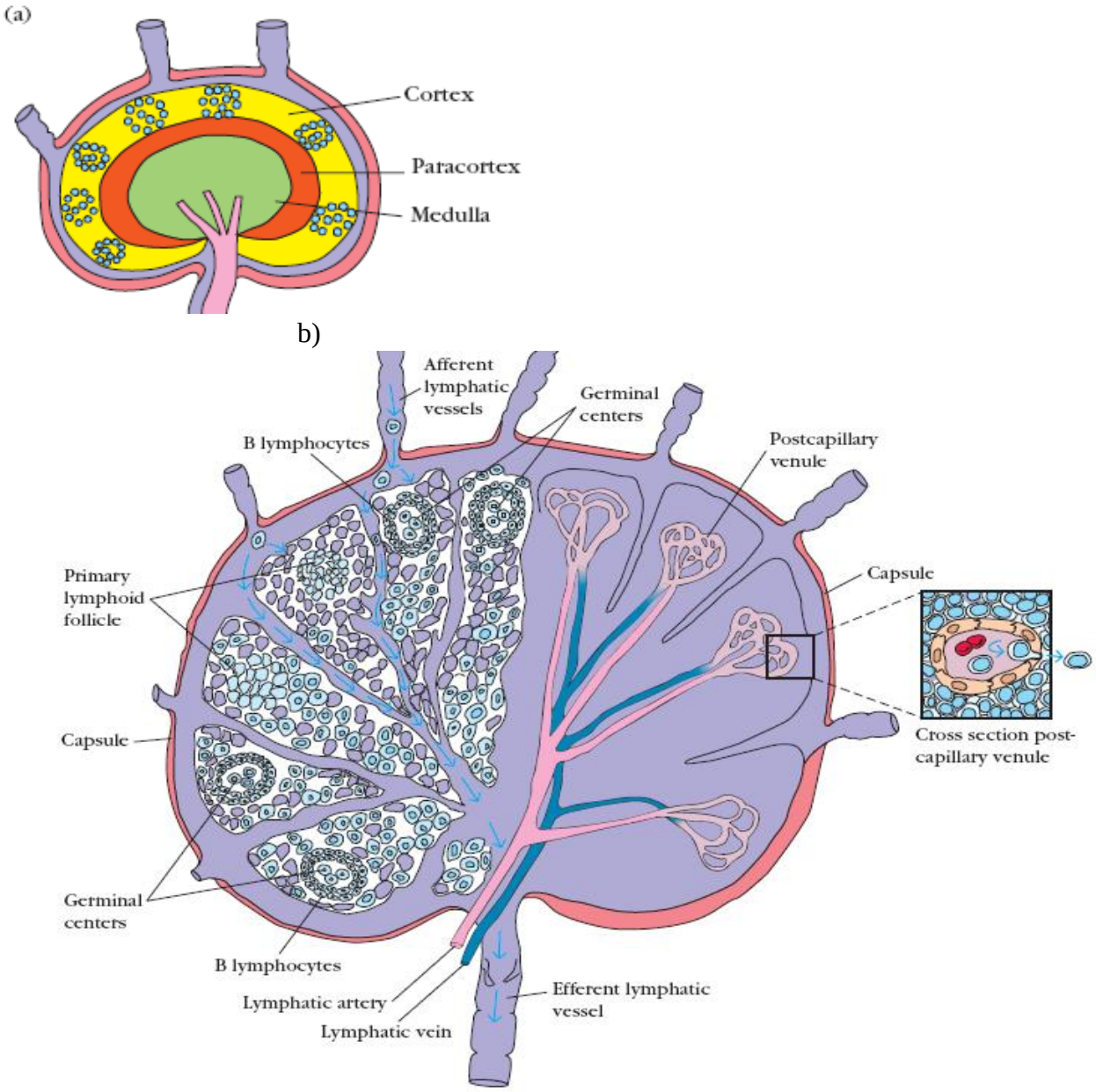


Fig: Structure of a lymph node. (a) The three layers of a lymph node support distinct microenvironments**. (b)** The left side depicts the arrangement of reticulum and lymphocytes within the various regions of a lymph node. Macrophages and dendritic cells, which trap antigen, are present in the cortex and paracortex. T_H cells are concentrated in the paracortex; B cells are

located primarily in the cortex, within follicles and germinal centers. The medulla is populated largely by antibody-producing plasma cells. Lymphocytes circu-lating in the lymph are carried into the node by afferent lymphatic vessels; they either enter the reticular matrix of the node or pass through it and leave by the efferent lymphatic vessel. The right side of (b) depicts the lymphatic artery and vein and the postcapillary venules. Lymphocytes in the circulation can pass into the node from the postcapillary venules by a process called extravasation

SPLEEN

The spleen plays a major role in mounting immune re-sponses to antigens in the blood stream. It is a large, ovoid secondary lymphoid organ situated high in the left abdomi-nal cavity and weighing about 150 grams. It is the largest single lymphoid organ in the body.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 re-spond to systemic infections. Unlike the lymph nodes, the spleen is not supplied by lymphatic vessels. Instead, blood-borne antigens and lymphocytes are carried into the spleen through the splenic artery. Experiments with radioactively labeled lymphocytes show that more recirculating lympho-cytes pass daily through the spleen than through all the lymph nodes combined.

The spleen is surrounded by a capsule that extends a num-ber 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 lym-phocytes; 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 sur-rounds the branches of the splenic artery, forming a periarte-riolar 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 con-tain 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 interdigi-tating dendritic cells, which carry it to the PALS. Lympho-cytes 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 T-cell-rich PALS. Here interdigitating dendritic cells capture antigen and present it combined with class II MHC mole-cules to T_H cells. Once activated, these T_H cells can then acti-vate B cells. The activated B cells, together with some T_H cells, then migrate to primary follicles in the marginal zone. Upon antigenic challenge, these primary follicles develop into char-acteristic 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.

In children, splenectomy often leads to an increased incidence of bacterial sepsis caused primarily by *Streptococcus pneumoniae, Neisse-ria meningitidis,* and *Haemophilus influenzae.*

Splenectomy in adults has less adverse effects, although it leads to some in-crease in blood-borne bacterial infections (bacteremia).

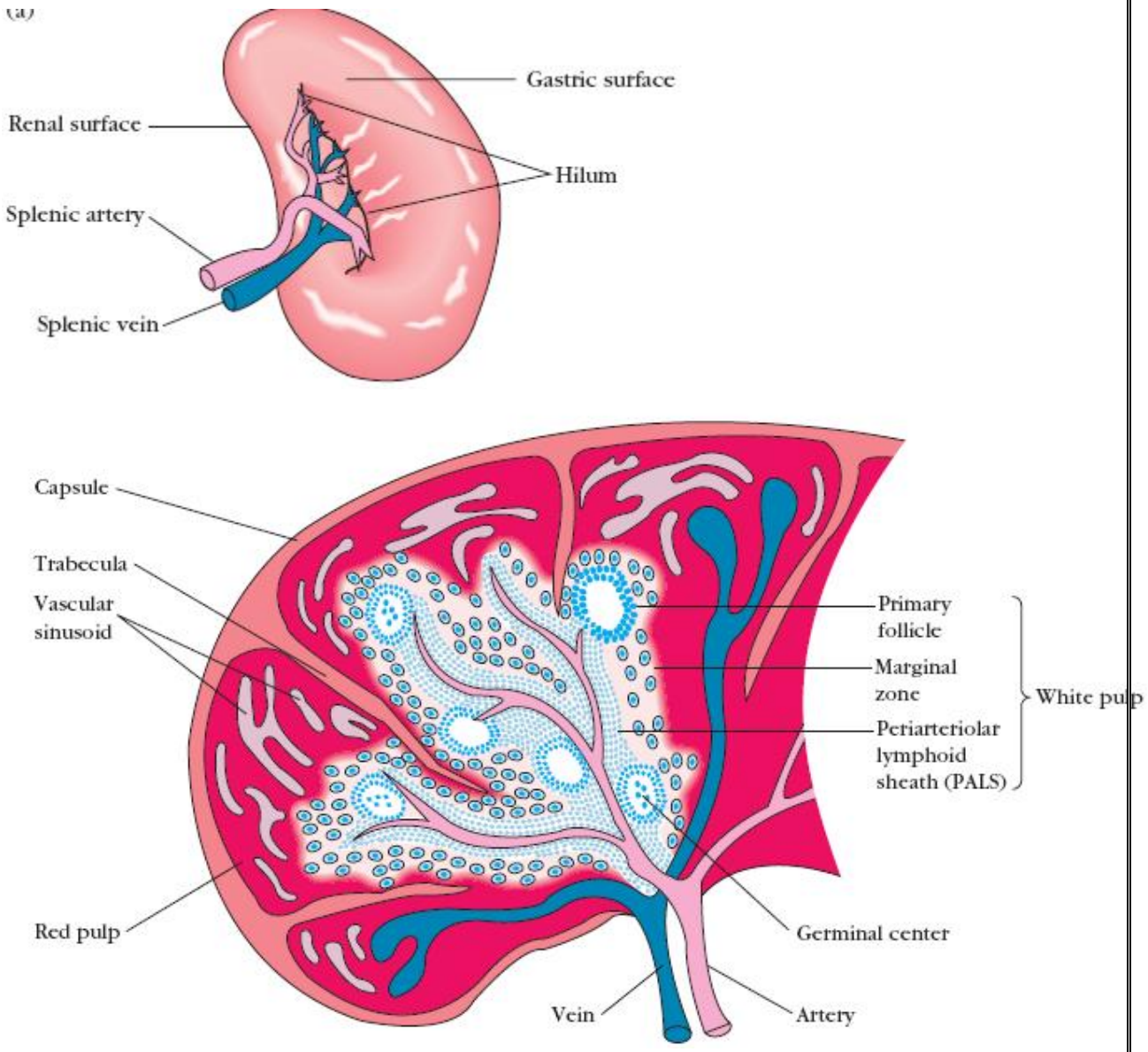


Fig: (a) The spleen, which is about 5 inches long in adults, is the largest secondary lymphoid organ. It is specialized for trapping blood-borne antigens**. (b) Diagrammatic cross section of the spleen**. The splenic artery pierces the capsule and divides into progressively smaller arterioles, ending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

MUCOSA ASSOCIATED LYMPHOID TISSUE (MALT):

Approximately >50% of lymphoid tissue in the body is found associated with the mucosal system. MALT is composed of gut-associated lymphoid tissues (GALT) lining the intestinal tract, bronchus-associated lymphoid tissue (BALT) lining the respiratory tract, and lymphoid tissue lining the genitourinary tract. The respiratory, alimentary and genitourinary tracts are guarded by subepithelial accumulations of lymphoid tissue that are not covered by connective tissue capsule. They may occur as diffuse collections of lymphocytes, plasma cells and phagocytes throughout the lung and lamina propria of intestine or as clearly organised tissue with well-formed lymphoid follicles. The well-formed follicles include the tonsils (lingual, palatine and pharyngeal), Peyer's patches in the intestine and appendix. The major function of these organs is to provide local immunity by way of sIgA (also IgE) production. Diffuse accumulations of lymphoid tissue are seen in the lamina propria of the intestinal wall. The intestinal epithelium overlying the Peyer's patches is specialized to allow the transport of antigens into the lymphoid tissue. This function is carried out by cuboidal absorptive epithelial cells termed "M" cells, so called because they have numerous microfolds on their luminal surface. M cells endocytosise, transport and present antigens to subepithelial lymphoid cells. Majority of intra-epithelial lymphocytes are T cells, and most often CD8+ lymphocytes. The intestinal lamina propria contains CD4+ lymphocytes, large number of B cells, plasma cells, macrophages, dendritic cells, eosinophils and mast cells. Peyer's patches contain both B cells and CD4+ T cells.

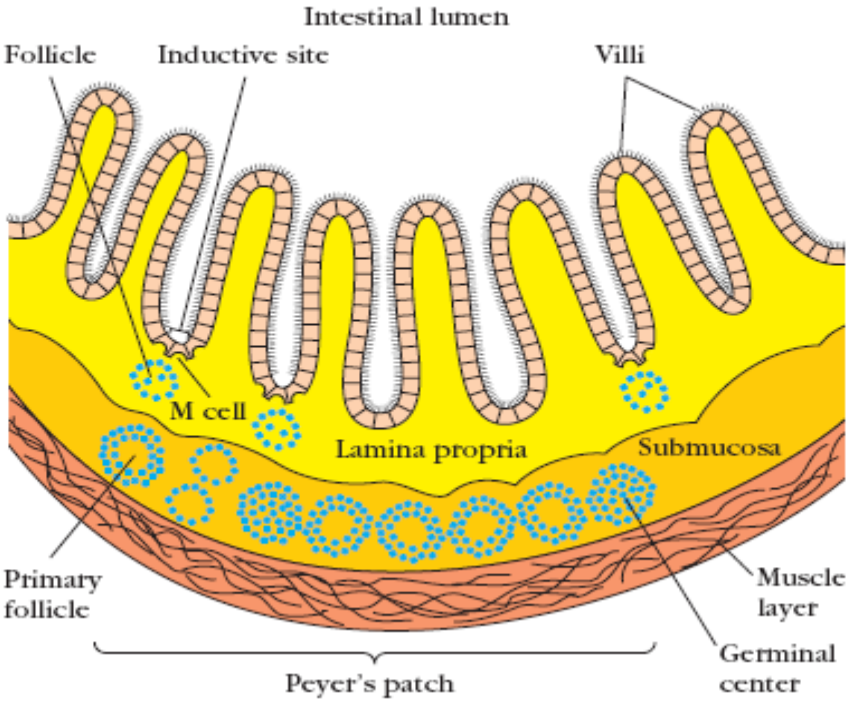


Fig: Cross-sectional diagram of the mucous membrane lining the intestine showing a nodule of lymphoid follicles that con-stitutes a Peyer's patch in the submucosa. The intestinal lamina pro-pria contains loose clusters of lymphoid cells and diffuse follicles.

B-Cell Activation and Proliferation

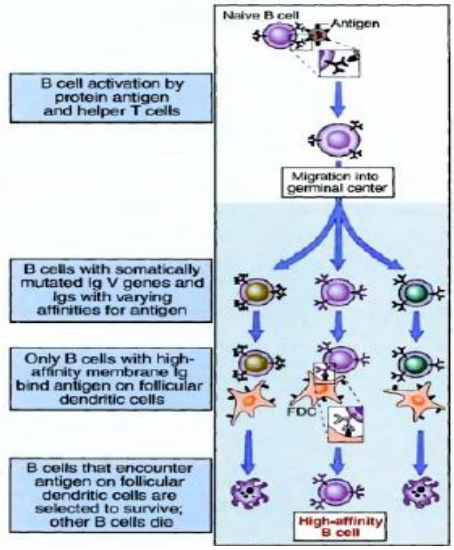
After export of B cells from the bone marrow, activation, proliferation, and differentiation occur in the periphery and require antigen.Antigen-driven activation and clonal selection of naive B cells leads to generation of plasma cells and memory B cells. In the absence of antigen-induced activation, 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.

Antibody affinity maturation class switching

Affinity maturation and class switching of antibodies are temporally, but not mechanistically, related processes. The basis of affinity maturation is the selection, in the germinal centers, of antibodies that bind the antigen better. Early in an immune response, the selection is from the primary repertoire; later, it is from mutants generated by hypermutation at the immunoglobulin loci. Recently, the door has been opened for the study of the molecular mechanism of hypermutation, which is expected to make a major contribution to general biology. Class switching has been studied in the past for its obvious clinical importance, but also at the basic level of DNA recombination. Progress in understanding class switching has been trailing the progress made in V(D)J recombination, but new in vitro systems and gene-targeted mice are closing the gap.

AFFINITY MATURATION

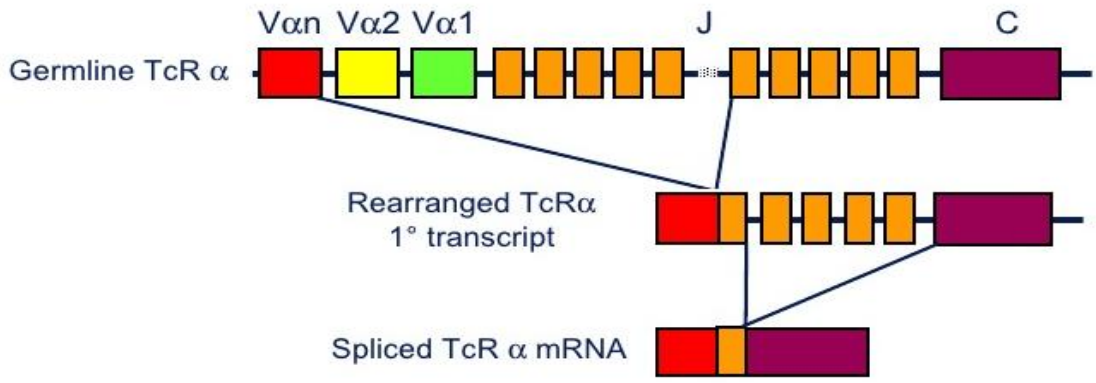


- process by which the affinity of antibodies produced in response to a protein antigen increases with prolonged or repeated exposure to that antigen
  - **advantage**: ability of antibodies to bind to a microbe or microbial antigen increases if the infection is persistent or recurrent
- occurs in the germinal centers of lymphoid follicles
- result of somatic hypermutation of Ig genes in dividing B-cells followed by the selection of high-affinity B-cells by antigen displayed by follicular dendritic cells (FDC)

Assembly of T cell Receptor Genes by Somatic Recombination

T-cell receptor genes are assembled by somatic recombination from sets of gene segments in the same way as are the immunoglobulin genes. This leads to a T-cell receptor in which the highest diversity is in the central part of the receptor, which contacts the bound peptide fragment of the ligand

TcR α gene rearrangement by SOMATIC RECOMBINATION



Rearrangement very similar to the IgL chains

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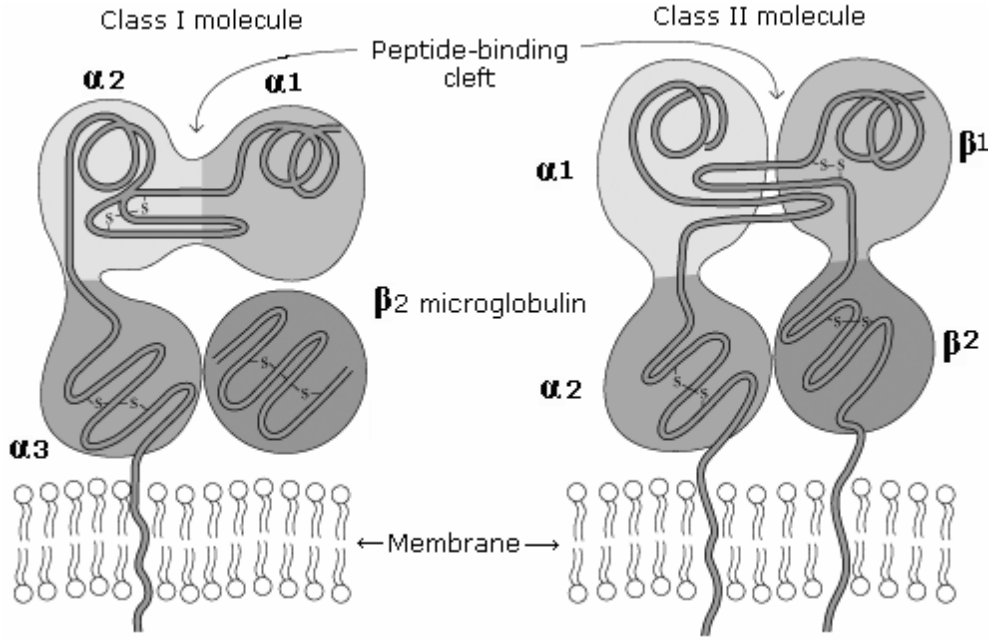
- resides inside the cell while the amino end projects on the surface of cell with a short intervening hydrophobic segment traverses the membrane.
- The α chain is coded by the MHC genes and has three globular domains α1, α2 and α3. β2-microglobulin is encoded by a gene on another chromosome.
- The α3 domain is non-covalently associated with the β2 microglobulin. Both α chain and β2-microglobulin are members of the Ig superfamily. Without the β2 microglobulin, the class I antigen will not be expressed on the cells surface.
- Individuals with defective β2 microglobulin gene do not express any class I antigen and hence they have a deficiency of cytotoxic T cells.
- A peptide-binding groove is formed between α1 and α2 helices with beta-pleated sheet as its floor.
- A peptide of 8- 10 amino acids long can be presented in this groove. The alloantigenic sites that carry determinants specific to each individual are found in the α1 and α2 domains.
- The greatest variability in amino acids (or polymorphism) occurs in the α1 and α2 sequences that line the wall and floor of the groove that binds the peptides.
- The polymorphism among class I MHC gene products creates variation in the chemical surface of the peptide-binding groove so that various peptide molecules can be accommodated.
- The specific binding of a peptide molecule in the peptide-binding groove of MHC requires the peptide to have one or more specific amino acid at a fixed position.
- Such sites are termed anchor sites. The other amino acids can be variable so that each MHC molecule can bind many different peptides.
- The α1 and α2 domains also bind T cell receptor (TCR) of CD8 T lymphocytes. The parts of these domains that are in contact with TCR also show polymorphism.
- The immunoglobulin-like region of α3 domain is constant (shows no variation) and is non-covalently bound β2 microglobulin.
- The importance of the highly conserved region of α3 is that CD8 molecules present on CD8 T lymphocytes binds to this region.

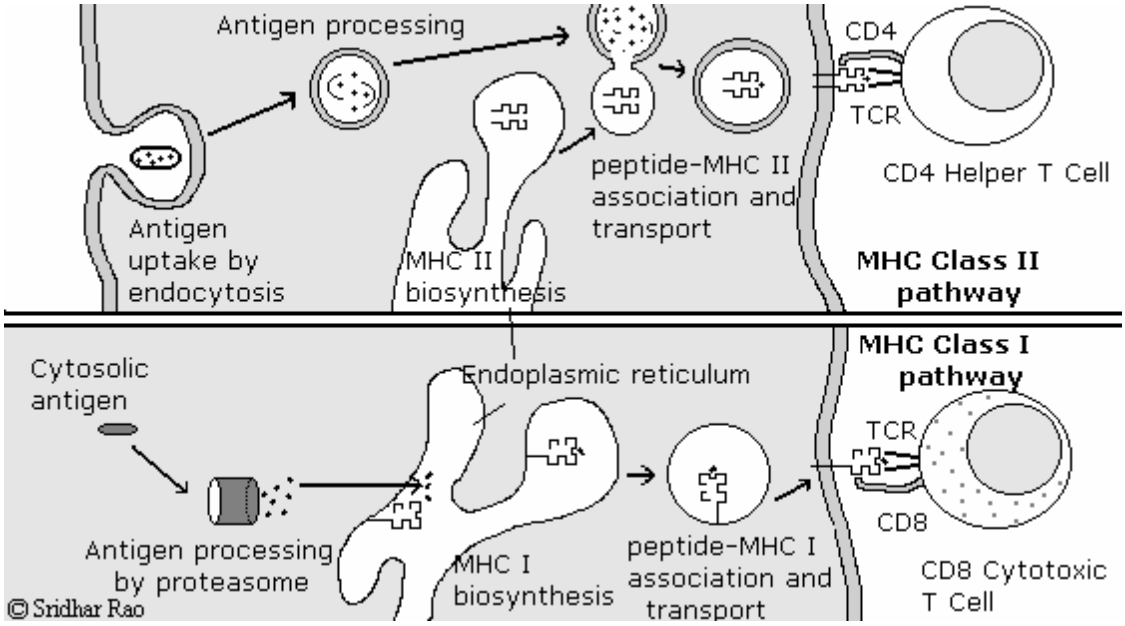


- CD8 T lymphocytes recognizes peptide antigen only when it is presented by the antigen presenting cell in the peptide binding groove of MHC I molecules.
- Class I molecules present peptide fragments in the cytosol (endogenous antigen, which could be fragments of viral or tumour proteins) to the CD8 lymphocytes.

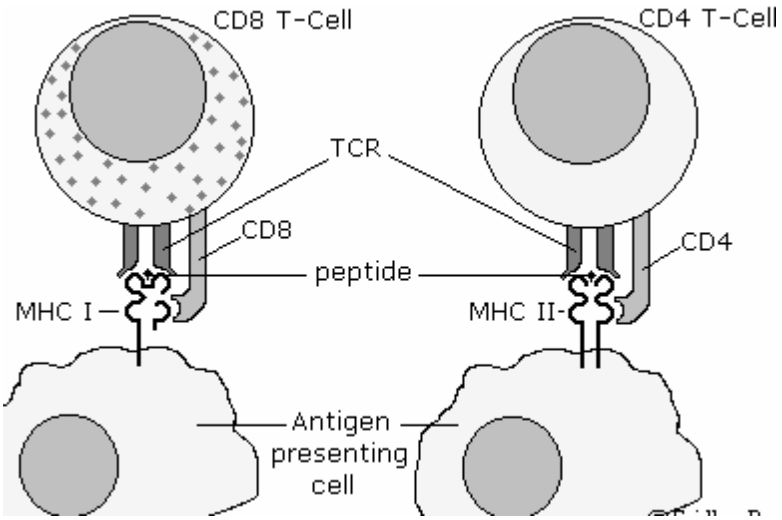
MHC Class II Molecule

- MHC class II molecules comprise two non-identical and non-covalently associated polypeptide chains (αand β).
- These two chains have amino ends on the surface, a short transmembrane stretch and intracytoplasmic carboxyl ends. Both α chain (34 kDa) and β chain (28 kDa) are MHC-encoded and polymorphic.
- The domains closest to the membrane in each chain are structurally related to immunoglobulins. With the exception of the α1 domain, all domains are stabilized by disulfide bridges.
- The β chain is shorter than the α chain and contains the alloantigenic sites. A peptide binding groove is formed in between α1 and β1 domains with a beta pleated floor.
- As in the case for class I MHC, the greatest polymorphic variability in the amino acids is in those facing the groove.

- This in turn determines the chemical structure of the groove and influences the specificity and affinity of peptide binding. Peptides associated with class II MHC are 13-25 amino acids long.
- As with class I MHC, anchor sites for one or more amino acids also exist in the groove of the class II MHC molecule. α2 and β2 are largely non-polymorphic.
- During antigen presentation, CD4 molecule of Helper T lymphocyte binds to β2 domain of the class II MHC molecules.
- Exogenous antigens (fragments of bacterial cells or viruses that are engulfed and processed by antigen presenting cell) are presented to helper T-cells along with MHC II molecules.



Because each MHC molecule (I and II) can bind many different peptides, the binding is said to be degenerate.



CD 4 Helper Tlymphocytes canrecognize peptideantigen only whenpresented along MHCII molecules.

CD8Cytotoxic T lymphocytes can recognize peptide antigen only when presented along MHC I molecules.

- Class I MHC molecules
- Class II MHC molecules

Each of these molecules plays a unique role in antigen recognition, ensuring that the immune system can recognize and respond to the different types of antigen that it encounters.

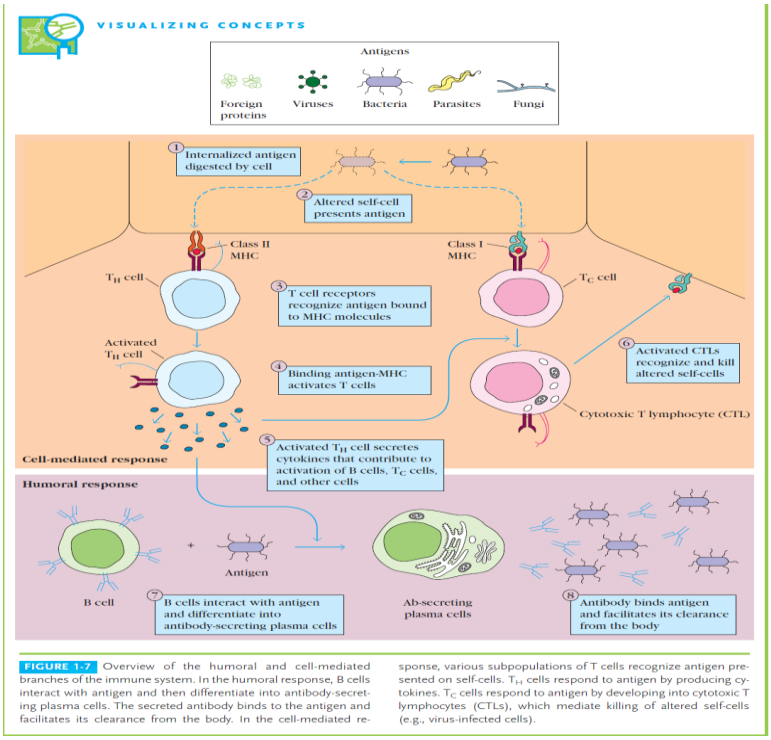


FIGURE 1-7 Overview of the humoral and cell-mediated branches of the immune system. In the humoral response, B cells interact with antigen and then differentiate into antibody-secreting plasma cells. The secreted antibody binds to the antigen and facilitates its clearance from the body. In the cell-mediated response, various subpopulations of T cells recognize antigen presented on self-cells. T_H cells respond to antigen by producing cytokines. T_C cells respond to antigen by developing into cytotoxic T lymphocytes (CTLs), which mediate killing of altered self-cells (e.g., virus-infected cells).

B and T Lymphocytes Utilize Similar Mechanisms to Generate Diversity in Antigen Receptors

The antigenic specificity of each B cell is determined by the membrane-bound antigen-binding receptor (i.e., antibody) expressed by the cell.As a B cell matures in the bone marrow, its specificity is created by random rearrangements of a series of gene segments that encode the antibody molecule. As a result of this process, each mature B cell possesses a single functional gene encoding the antibody heavychain and a single functional gene encoding the antibody light chain; the cell therefore synthesizes and displays antibody with one specificity on its membrane. All antibody molecules on a given B lymphocyte have identical specificity, giving each B lymphocyte, and the clone of daughter cells to which it gives rise, a distinct specificity for a single epitope on an antigen. The mature B lymphocyte is therefore said to be **antigenically committed.**

The random gene rearrangements during B-cell maturation in the bone marrow generate an enormous number of different antigenic specificities. The resulting B-cell population, which consists of individual B cells each expressing a

unique antibody, is estimated to exhibit collectively more than 1010 different antigenic specificities. The enormous diversity in the mature B-cell population is later reduced by a selection process in the bone marrow that eliminates any B cells with membrane-bound antibody that recognizes selfcomponents. The selection process helps to ensure that selfreactive antibodies (auto-antibodies) are not produced.

The attributes of specificity and diversity also characterize the antigen-binding T-cell receptor (TCR) on T cells.As in Bcell maturation, the process of T-cell maturation includes random rearrangements of a series of gene segments that encode the cell's antigen-binding receptor. Each T lymphocyte cell expresses about 105 receptors, and all of the receptors on the cell and its clonal progeny have identical specificity for antigen. The random rearrangement of the TCR genes is capable of generating on the order of 109 unique antigenic specificities. This enormous potential diversity is later diminished through a selection process in the thymus that eliminates any T cell with self-reactive receptors and ensures that only T cells with receptors capable of recognizing antigen associated with MHC molecules will be able to mature.

The Major Histocompatibility Molecules Bind Antigenic Peptides

The major histocompatibility complex (MHC) is a large genetic complex with multiple loci. The MHC loci encode two major classes of membrane-bound glycoproteins: **class I** and **class II MHC**

molecules. As noted above, TH cells generally recognize antigen combined with class II molecules, whereas TC cells generally recognize antigen combined with class I molecules (Figure 1-8).

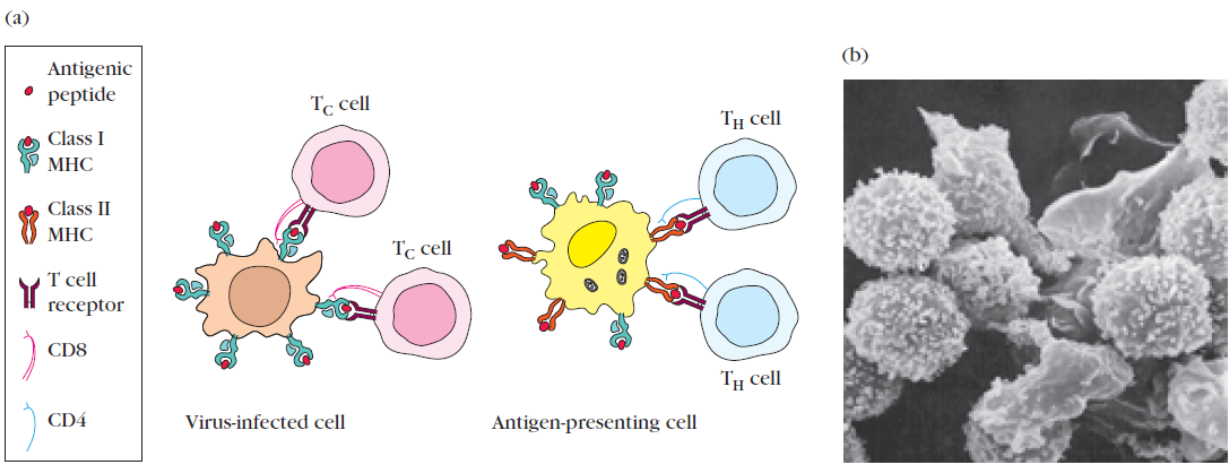


FIGURE 1-8 The role of MHC molecules in antigen recognition by T cells. (a) Class I MHC molecules are expressed on nearly all nucleated cells. Class II MHC molecules are expressed only on antigen-presenting cells. T cells that recognize only antigenic peptides displayed with a class II MHC molecule generally function as T helper (T_H) cells. T cells that recognize only antigenic peptides displayed with a class I MHC molecule generally function as T cytotoxic (T_C) cells. (b) This scanning electron micrograph reveals numerous T lymphocytes interacting with a single macrophage. The macrophage presents processed antigen combined with class II MHC molecules to the T cells. [*Photograph from W. E. Paul (ed.), 1991,* Immunology: Recognition and Response, *W. H. Freeman and Company, New York; micrograph courtesy of M. H. Nielsen and O. Werdelin.*]

MHC molecules function as antigen-recognition molecules, but they do not possess the fine specificity for antigen characteristic of antibodies and T-cell receptors. Rather, each MHC molecule can bind to a spectrum of **antigenic peptides** derived from the intracellular degradation of antigen molecules. In both class I and class II MHC molecules the distal regions (farthest from the membrane) of different alleles display wide variation in their amino acid sequences. These variable regions form a cleft within which the antigenic peptide sits and is presented to T lymphocytes (see Figure 1-8). Different allelic forms of the genes encoding class I and class II molecules confer different structures on the antigen-binding cleft with different specificity. Thus the ability to present an antigen to T lymphocytes is influenced by the particular set of alleles that an individual inherits.

UNIT-III

SYLLABUS

Transplantation: MLR, MHC and HLA typing, bone marrow transplantation, organ transplants, immunosuppressive therapy. Hybridoma technology and monoclonal antibodies, immuno-diagnosis and application of monoclonal antibodies in biomedical research, human monoclonal antibodies and catalytic antibodies, Xeno transplantation from various species.

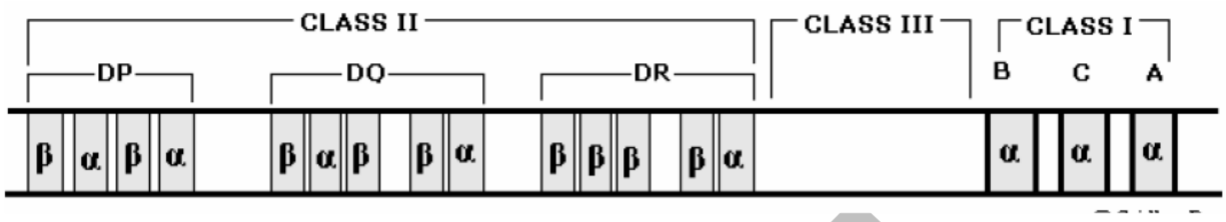
MHC (MAJOR HISTOCOMPATIBILITY COMPLEX)

MHC complex is group of genes on a single chromosome that codes the MHC antigens. Major as well as minor histocompatibility antigens (also called transplantation antigens) mediate rejection of grafts between two genetically different individuals. However, the role played by the major histocompatibility antigens supersedes the minor histocompatibility antigens. HLA (human leukocyte antigens) are the MHC antigens of humans, and called so because they were first detected on leukocytes. H-2 antigens are their equivalent MHC antigens of mouse. A set of MHC alleles present on each chromosome is called an MHC haplotype. Monozygotic human twins have the same histocompatibility molecules on their cells, and they can accept transplants of tissue from each other. Histocompatibility molecules of one individual act as antigens when introduced into a different individual. George Snell, Jean Dausset and Baruj Benacerraf received

the Nobel Prize in 1980 for their contributions to the discovery and understanding of the MHC in mice and humans MHC gene products were identified as responsible for graft rejection. MHC gene products that control immune responses are called the immune response (Ir) genes. Immune response genes influence responses to infections. The essential role of the HLA antigens lies in the induction and regulation of the immune response and defence against microorganisms. The physiologic function of MHC molecules is the presentation of peptide antigen to T lymphocytes. These antigens and their genes can be divided into three major classes: class I, class II and class III.

STRUCTURE:

The MHC complex resides in the short arm of chromosome 6 and overall size of the MHC is approximately 3.5 million base pairs. The complete three-dimensional structure for both class I and class II MHC molecules has been determined by x-ray crystallography. The class I gene complex contains three loci A, B and C, each of which codes of α chain polypeptides. The class II gene complex also contains at least three loci, DP, DQ and DR; each of these loci codes for one α and a variable number of β chain polypeptides. Class III region is not actually a part of the HLA complex, but is located within the HLA region, because its components are either related to the functions of HLA antigens or are under similar control mechanisms to the HLA genes. Class III antigens are associated with proteins in serum and other body fluids (e.g.C4, C2, factor B, TNF) and have no role in graft rejection.



NOMENCLATURE:

HLA specificities are identified by a letter for locus and a number (A1, B5, etc.), and the haplotypes are identified by individual specificities (e.g., A1, B7, Cw4, DP5, DQ10, DR8). Specificities which are defined by genomic analysis (PCR), are named with a letter for the locus and a four digit number (e.g. A0101, B0701, C0401, etc.)

INHERITANCE:

Histocompatibility genes are inherited as a group (haplotype), one from each parent. Thus, MHC genes are codominantly expressed in each individual. A heterozygous human inherits one paternal and one maternal haplotype, each containing three Class-I (B, C and A) and three Class II (DP, DQ and DR) loci. Each individual inherits a maximum of two alleles for each locus. The maximum number of class I MHC gene products expressed in an individual is six; that for class II MHC products can exceed six but is also limited. Thus, as each chromosome is found twice (diploid) in each individual, a normal tissue type of an individual will involve 12 HLA antigens. Haplotypes, normally, are inherited intact and hence antigens encoded by different loci are inherited together.

However, on occasions, there is crossing over between two parental chromosomes, thereby resulting in new recombinant haplotypes. There is no somatic DNA recombination that occurs for antibodies and for the TCR, so the MHC genes lack recombinational mechanisms for generating diversity. Many alleles of each locus permit thousands of possible assortments. There are at least 1000 officially recognized HLA alleles.

EXPRESSION:

Class I antigens are expressed on all nucleated cells (except those of the central nervous system) and platelets. The class II antigens are expressed on antigen presenting cells such as B lymphocytes, dendritic cells, macrophages, monocytes, Langerhans cells, endothelial cells and thymic epithelial cells. Cytokines, especially interferon gamma (IFN-γ), increase the level of expression of class I and class II MHC molecules.

MHC CLASS I MOLECULE:

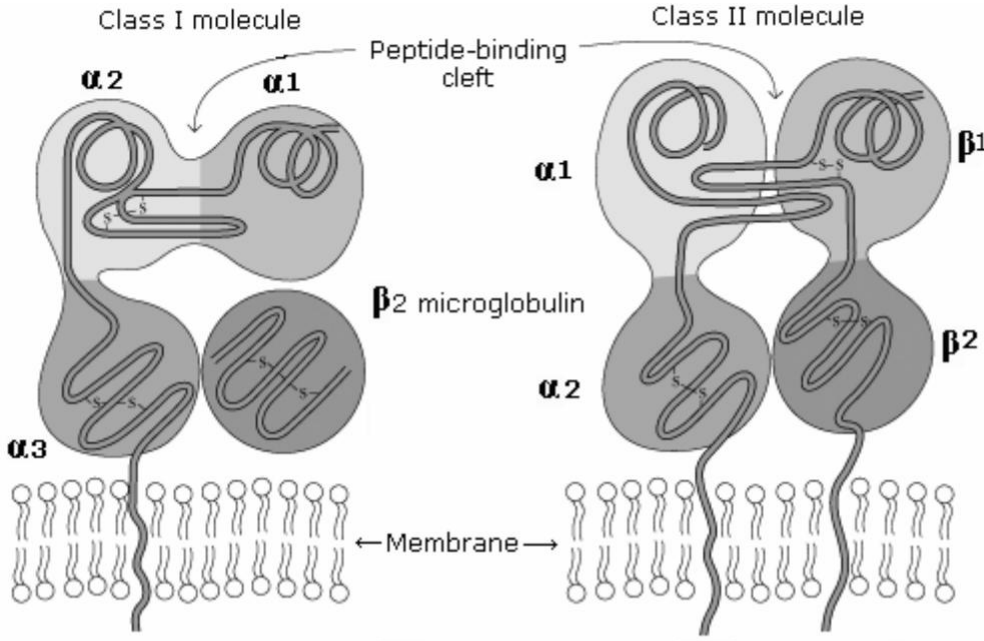
Class I MHC molecules contain two separate polypeptide chains, the heavier (44-47 KDa) alpha chain and the lighter (12 KDa) beta chain. The carboxyl end of α chain resides inside the cell while the amino end projects on the surface of cell with a short intervening hydrophobic segment traverses the membrane.

The α chain is coded by the MHC genes and has three globular domains α1, α2 and α3. β2-microglobulin is encoded by a gene on another chromosome. The α3 domain is non-covalently associated with the β2 microglobulin. Both α chain and β2-microglobulin are members of the Ig superfamily. Without the β2 microglobulin, the class I antigen will not be expressed on the cells

surface. Individuals with defective β2 microglobulin gene do not express any class I antigen and hence they have a deficiency of cytotoxic T cells.

A peptide-binding groove is formed between α1 and α2 helices with beta-pleated sheet as its floor. A peptide of 8- 10 amino acids long can be presented in this groove. The alloantigenic sites that carry determinants specific to each individual are found in the α1 and α2 domains. The greatest variability in amino acids (or polymorphism) occurs in the α1 and α2 sequences that line the wall and floor of the groove that binds the peptides. The polymorphism among class I MHC gene products creates variation in the chemical surface of the peptide-binding groove so that various peptide molecules can be accommodated. The specific binding of a peptide molecule in the peptide-binding groove of MHC requires the peptide to have one or more specific amino acid at a fixed position. Such sites are termed anchor sites. The other amino acids can be variable so that each MHC molecule can bind many different peptides.

The α1 and α2 domains also bind T cell receptor (TCR) of CD8 T lymphocytes. The parts of these domains that are in contact with TCR also show polymorphism. The immunoglobulin-like region of α3 domain is constant (shows no variation) and is non-covalently bound β2 microglobulin. The importance of the highly conserved region of α3 is that CD8 molecules present on CD8 T lymphocytes binds to this region. CD8 T lymphocytes recognizes peptide antigen only when it is presented by the antigen presenting cell in the peptide binding groove of MHC I molecules. Class I molecules present peptide fragments in the cytosol (endogenous antigen, which could be fragments of viral or tumour proteins) to the CD8 lymphocytes.



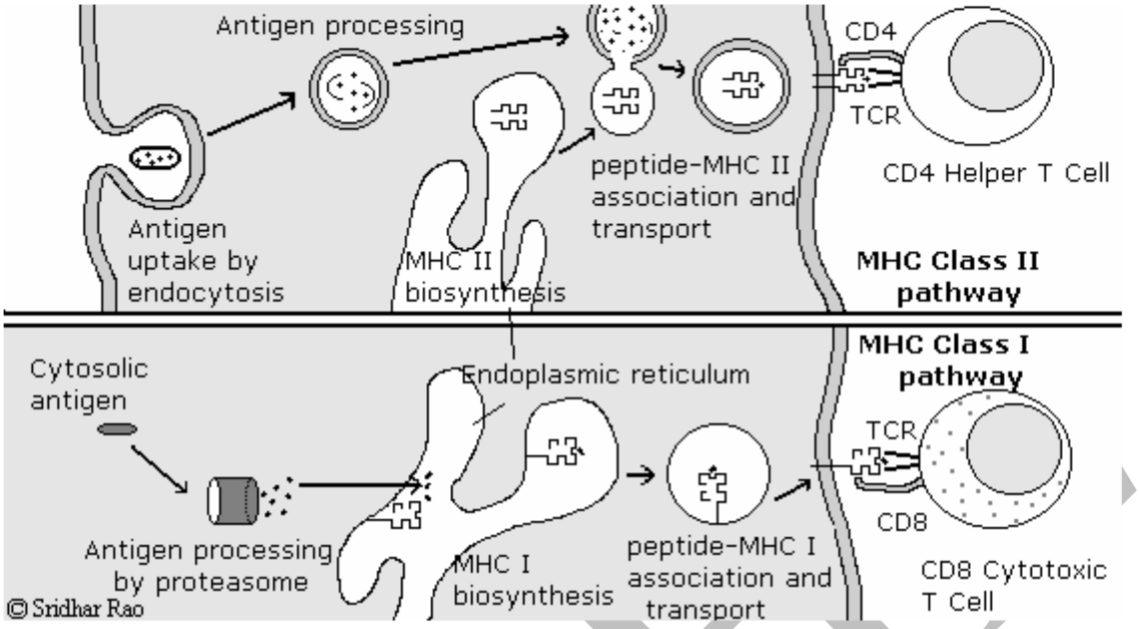
MHC CLASS II MOLECULE:

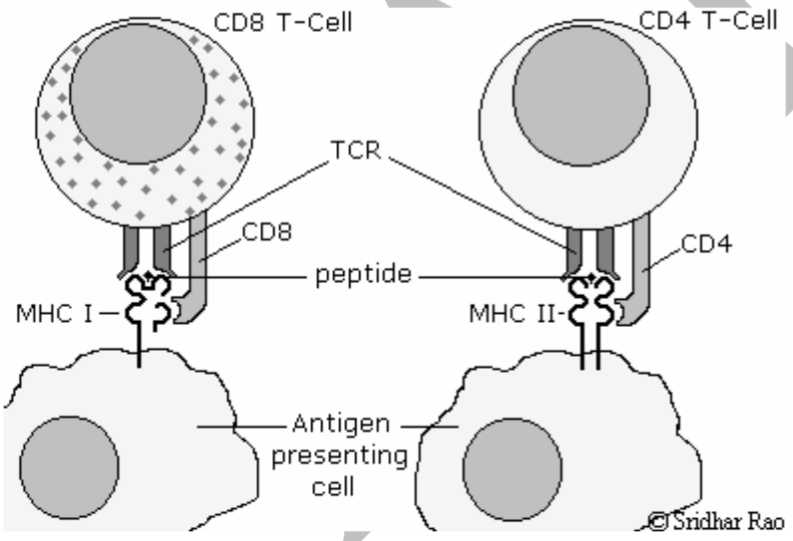
MHC class II molecules comprise two non-identical and non-covalently associated polypeptide chains (α and β). These two chains have amino ends on the surface, a short transmembrane stretch and intracytoplasmic carboxyl ends. Both α chain (34 kDa) and β chain (28 kDa) are MHC-encoded and polymorphic. The domains closest to the membrane in each chain are structurally related to immunoglobulins. With the exception of the α1 domain, all domains are stabilized by disulfide bridges. The β chain is shorter than the α chain and contains the alloantigenic sites.

A peptide binding groove is formed in between α1 and β1 domains with a beta pleated floor. As in the case for class I MHC, the greatest polymorphic variability in the amino acids is in those

facing the groove. This in turn determines the chemical structure of the groove and influences the specificity and affinity of peptide binding. Peptides associated with class II MHC are 13-25 amino acids long. As with class I MHC, anchor sites for one or more amino acids also exist in the groove of the class II MHC molecule. α2 and β2 are largely non-polymorphic. During antigen presentation, CD4 molecule of Helper T lymphocyte binds to β2 domain of the class II MHC molecules.

Exogenous antigens (fragments of bacterial cells or viruses that are engulfed and processed by antigen presenting cell) are presented to helper T-cells along with MHC II molecules. Because each MHC molecule (I and II) can bind many different peptides, the binding is said to be degenerate.





CD 4 Helper T lymphocytes can recognize peptide antigen only when presented along MHC II molecules.

CD8 Cytotoxic T lymphocytes can recognize peptide antigen only when presented along MHC I molecules.

HLA TYPING:

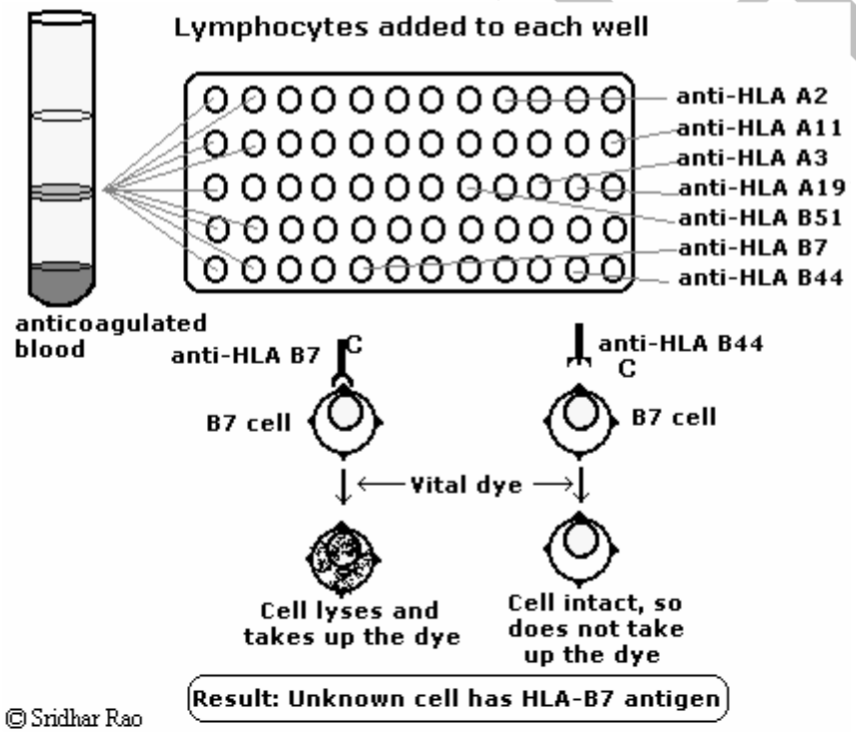
Because some HLA antigens are recognised on almost all of the tissues of the body (with few exceptions), the identification of HLA antigens is also described as "Tissue Typing". HLA matching between donor and recipient is desirable for allogenic transplantation. Class I typing methods include test such as microcytoxicity (for typing A, B, C loci) and cellular techniques such as CML (for HLA-DPw typing). Class II typing involves cellular techniques such as MLR/MLC (for DR typing) and molecular techniques such as PCR and direct sequencing (for DR, DQ typing).

Serologic methods:

Serologic techniques provide one of the most simplest and fastest methods for histocompatibility testing. These methods use sera that contain specific antibodies to HLA antigens. Tissue typing sera for the HLA were obtained in the past, from multiparous women who were exposed to the child's paternal antigens during the parturition and subsequently developed antibodies to these antigens. More recently they are being produced by the monoclonal antibody technology.

Microcytotoxicity assay: This is done by exposing the unknown lymphocyte to a battery of antisera of known HLA specificities. Lymphocytes are isolated from the peripheral blood (or from lymph node or spleen in cadavers) and separated from other cells by buoyant density gradient centrifugation. For HLA I antigens, T lymphocytes are chosen while for HLA II

antigens, B lymphocytes are chosen. An array of anti-HLA sera covering full range of HLA types are chosen. Individual serum is dispensed into microtitre wells. Approximately 2000 lymphocytes are dispensed per well and incubated. Complement is then added to each well and incubated. The duration of incubation is different for T and B lymphocytes. If the antibodies bind to lymphocytes, complement gets activated and results in lysis of that lymphocyte. The damaged cells are not completely lysed but suffer sufficient membrane damage to allow uptake of vital stains such as eosin Y, Trypan Blue or fluorescent stains such as Ethidium Bromide. Live cells don't stain but the dead cells take up the stain.



Antibody screening: This is used to detect the presence of HLA antibodies in the potential transplant recipients. A highly sensitive solid phase ELISA is used to detect antibodies in

recipient's serum. Purified preparations of HLA antigens are adsorbed on the solid phase of plastic plates. Recipient's serum is then added to different HLA antigen coated wells. After the removal of unbound antibodies by washing the wells are treated with enzyme-labelled antigamma globulin. The wells are washed and treated with colour generating substrate. If the recipient is positive for the HLA type of the donor (that means recipient has antibodies directed against donor's antigens), then transplantation is not possible.

Cellular Assays:

Lymphocytes from one donor, when cultured with lymphocytes from an unrelated donor, are stimulated to proliferate or become cytotoxic. This proliferation is due to a disparity in the class II MHC (DR) antigens.

Mixed Leukocyte Reaction (MLR) or Mixed Leukocyte Culture (MLC):

T cells of one individual interact with allogeneic class-II MHC antigen bearing cells (eg.B cells) of unrelated individual. When lymphocytes from individuals of different class II haplotypes are cultured together, blast cell transformation and mitosis occurs. The irradiated or mitomycin-C treated stimulator cells of recipient (usually containing B cells, macrophages, dendritic cells) are mixed with CD4 cells of responder (donor). The donor cells respond to different class II antigens on stimulator cells and undergo transformation (DNA synthesis and enlargement) and proliferation (mitogenesis). These changes were recorded by the addition of radioactive (tritiated, 3H) thymidine into the culture and monitoring its incorporation into DNA.

Cell mediated lympholysis (CML):

The responder cells not only undergoes blast transformation and proliferation on contact with different MHC II molecules, they also give rise to cytotoxic cells. These cytotoxic cells in turn identify the HLA I antigen on the stimulator cells and kill them.

Molecular techniques:

These methods involve detection of the genes coding for the antigens rather than detecting the antigen itself. These are Sequence-specific PCR, Restriction fragment length polymorphism and sequence specific oligonucleotide probe etc.

SIGNIFICANCE OF HLA TYPING:

Applications of Applications of Histocompatibility Testing

Anthropology: The fact that HLA types vary very widely among different ethnic populations has allowed

anthropologists to establish or confirm relationship among populations and migration pattern. HLA-A34,

which is present in 78% of Australian Aborigines, has a frequency of less than 1% in both Australian

Caucasoids and Chinese.

Paternity Testing: If a man and child share a HLA haplotype, then the possibility is there that the man may be the father but not proven. However, if they don't match or share a haplotype then it is agreed that he is not the father.

Transplantation: Because HLA plays such a dominant role in transplant immunity, pre-transplant histocompatibility testing is very important for organ transplantation. Results with closely related living donors matched with the recipient for one more both haplotypes are superior than those obtained with unrelated cadaveric donors.

Transfusion

Forensic science

Disease Correlation

A number of diseases have been found to occur at a higher frequency in individuals with certain MHC haplotypes. Most prominent among these are ankylosing spondylitis (B27), celiac disease (DR3), Reiter's syndrome (B27).

I. Disease associations with Class I HLA

Ankylosing spondylitis (B27), Reiter's disease (B27), Acute anterior Uvietis (B27), Psoriasis vulgaris (Cw6)

II. Disease associations with Class II HLA

Hashimoto's disease (DR5), Primary myxedema (DR3), Graves thyrotoxicosis (DR3), Insulin-dependent diabetes (DQ2/8), Addison's disease (adrenal) (DR3), Goodpasture's syndrome (DR2),

Rheumatoid arthritis (DR4), Juvenile rheumatoid arthritis (DR8), Sjogren's syndrome (DR3), Chronic active hepatitis (DR3), Multiple sclerosis (DR2, DR6), Celiac disease (DR3), Dermatitis herpetiformis (DR3)

No definite reason is known for this association. However, several hypotheses have been proposed: antigenic similarity between pathogens and MHC, antigenic hypo- and hyper-responsiveness controlled by the class II genes are included among them. Possible explanation for these associations is that the HLA antigen itself plays a role in disease, by a method similar to one of the following models:-

a) by being a poor presenter of a certain viral or bacterial antigen

b) by providing a binding site on the surface of the cell for a disease provoking virus or bacterium

c) by providing a transport piece for the virus to allow it to enter the cell by having a such a close molecular similarity to the pathogen that the immune system fails to recognise the pathogen as foreign and so fails to mount an immune response against it.

Transplantation

Transplantation as the term is used in 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.

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.

Clinical Transplantation

For a number of illnesses, a transplant is the only means of therapy. Figure 21-10 summarizes the major organ and cell transplants being performed at the present time. In addition, certain combinations of organs, such as heart and lung or kidney and pancreas, are being transplanted simultaneously with increasing frequency. Since the first kidney transplant was performed in the 1950s, approximately 400,000 kidneys have been transplanted worldwide. The next most frequently transplanted solid organ is the liver (52,000), followed by the heart (42,000) and, more distantly, by the lung (6,000) and pancreas (2,000). Bone-marrow transplants number around 80,000. Although the clinical results of transplantation of various cells, tissues, and organs in humans have improved considerably in the past few years, major obstacles to the use of this treatment exist. As explained above, the use of immunosuppressive drugs greatly increases the short-term survival of the transplant, but medical problems arise from use of these drugs, and chronic rejection is not prevented in most cases. The need for additional transplants after rejection exacerbates the shortage of organs which is a major obstacle to the widespread use of transplantation. Several of the organ systems for which transplantation is a common treatment are considered below. The frequency with which a given organ or tissue is transplanted depends on a number of factors:

_ Clinical situations in which transplantation is indicated

_ Availability of tissue or organs

_ Difficulty in performing transplantation and caring for post-transplantation patients

_ Specific factors that aid or hinder acceptance of the particular transplant

The urgency of the transplantation may depend on the affected organ. In the case of the heart, lung, and liver, few alternative procedures can keep the patient alive when these organs cease to function. Although dialysis may be used to maintain a patient awaiting a kidney transplant, there are no comparable measures for the heart or lungs if the allograft fails. Research on artificial organs is ongoing but there are no reports of long-term successes.

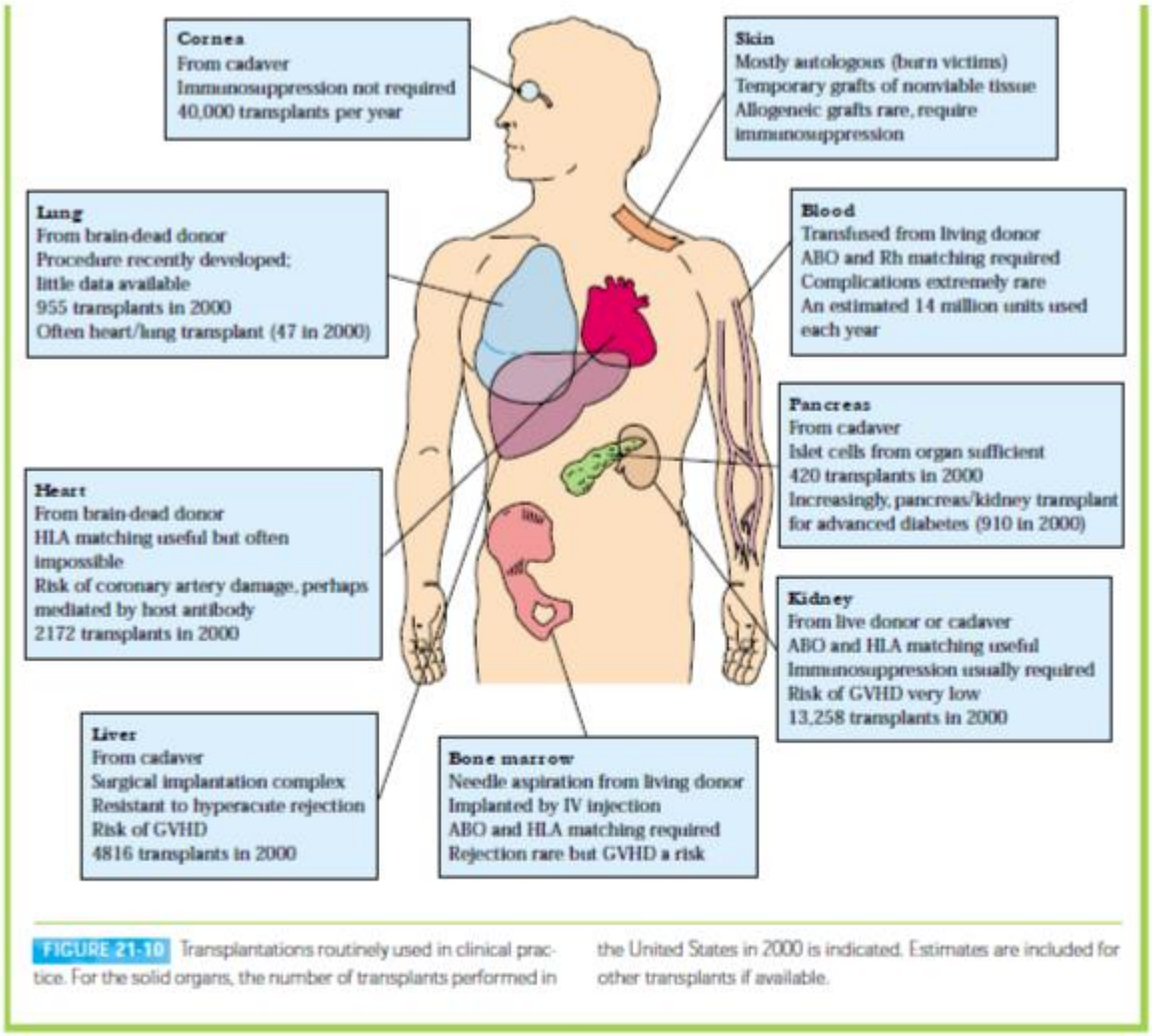


FIGURE 21-10 Transplantations routinely used in clinical practice. For the solid organs, the number of transplants performed in the United States in 2000 is indicated. Estimates are included for other transplants if available.

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 bonemarrow 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 as 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 reported at 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 allinclusive 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, that 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, such as SIV, may recombine with human variants to produce new agents of disease. The possibility of introducing new viruses into humans may be 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 in cells from unrelated species.

General Immunosuppressive Therapy

Allogeneic transplantation requires some degree of immunosuppression if the transplant is to survive. Most of the immunosuppressive treatments that have been developed have the disadvantage of being nonspecific; that is, they result in generalized immunosuppression of responses to all antigens, not just those of the allograft, which places the recipient at increased risk of infection. In addition, many immunosuppressive measures are aimed at slowing the proliferation of activated lymphocytes. However, because any rapidly dividing nonimmune cells (e.g., epithelial cells of the gut or bone-marrow hematopoietic stem cells) are also affected, serious or even life-threatening complications can occur. Patients on long-term immunosuppressive therapy are at increased risk of cancer, hypertension, and metabolic bone disease.

Mitotic Inhibitors Thwart T-Cell Proliferation

Azathioprine (Imuran), a potent mitotic inhibitor, is often given just before and after transplantation to diminish T-cell proliferation in response to the alloantigens of the graft. Azathioprine acts on cells in the S phase of the cell cycle to block synthesis of inosinic acid, which is a precursor of the purines adenylic and guanylic acid. Both B-cell and T-cell proliferation is diminished in the presence of azathioprine. Functional immune assays such as the MLR, CML, and skin test show a significant decline after azathioprine treatment, indicating an

overall decrease in T-cell numbers. Two other mitotic inhibitors that are sometimes used in conjunction with other immunosuppressive agents are cyclophosphamide and methotrexate. Cyclophosphamide is an alkylating agent that inserts into the DNA helix and becomes cross-linked, leading to disruption of the DNA chain. It is especially effective against rapidly dividing cells and therefore is sometimes given at the time of grafting to block T-cell proliferation. Methotrexate acts as a folic-acid antagonist to block purine biosynthesis. The fact that the mitotic inhibitors act on all rapidly dividing cells and not specifically on those involved in immune response against the allograft can lead to deleterious side reactions by thwarting division of other functional cells in the body.

Corticosteroids Suppress Inflammation

As corticosteroids, such as prednisone and dexamethasone, are potent anti-inflammatory agents that exert their effects at many levels of the immune response. These drugs are often given to transplant recipients together with a mitotic inhibitor such as azathioprine to prevent acute episodes of graft rejection.

Certain Fungal Metabolites Are Immunosuppressants

Cyclosporin A (CsA), FK506 (tacrolimus), and rapamycin (sirolimus) are fungal metabolites with immunosuppressive properties. Although chemically unrelated, CsA and FK506 have similar actions. Both drugs block activation of resting T cells by inhibiting the transcription of genes encoding IL-2 and the high-affinity IL-2 receptor (IL-2R), which are essential for activation. CsA and FK506 exert this effect by binding to cytoplasmic proteins called

immunophilins, forming a complex that blocks the phosphatase activity of calcineurin. This prevents the formation and nuclear translocation of the cytoplasmic subunit NFATc and its subsequent assembly into NFAT, a DNA-binding protein necessary for transcription of the genes encoding a number of molecules important to T-cell activation (see Figure 10-11). Rapamycin is structurally similar to FK506 and also binds to an immunophilin. However, the rapamycin-immunophilin complex does not inhibit calcineurin activity; instead, it blocks the proliferation and differentiation of activated TH cells in the G1 phase of the cell cycle. All three drugs, by inhibiting TH-cell proliferation and thus TH-cell cytokine expression, reduce the subsequent activation of various effector populations involved in graft rejection, including TH cells, TC cells, NK cells, macrophages, and B cells. The profound immunosuppressive properties of these three agents have made them a mainstay of heart, liver, kidney, and bone-marrow transplantation. Cyclosporin A has been shown to prolong graft survival in kidney, liver, heart, and heart-lung transplants. In one study of 209 kidney transplants from cadaver donors, the 1-year survival rate was 64% among recipients receiving other immunosuppressive treatments and 80% among those receiving cyclosporin A. Similar results have been obtained with liver transplants (Figure 21-8). Despite these impressive results, CsA does have some negative side effects, the most notable of which is toxicity to the kidneys. Acute nephrotoxicity is quite common, in some cases progressing to chronic nephrotoxicity and drug-induced kidney failure. FK506 and rapamycin are 10–100 times more potent as immune suppressants than CsA, and therefore can be administered at lower doses and with fewer side effects than CsA.

Specific Immunosuppressive Therapy

In addition to harmful side effects peculiar to the various immunosuppressive treatments described above, a major limitation common to all is that they lack specificity, thus producing a more-or-less generalized immunosuppression and increasing the recipient's risk for infection. What is needed ideally is an antigen-specific immunosuppressant that reduces the immune response to the alloantigens of the graft while preserving the recipient's ability to respond to other foreign antigens. Although this goal has not yet been achieved in human transplants, recent successes in animal experiments indicate that it may be possible. Specific immunosuppression to allografts has been achieved in animal experiments using antibodies or soluble ligands reactive with cell-surface molecules.

Monoclonal Antibodies

As 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 are heterogeneous, comprising a mixture of antibodies, each specific for one epitope (Figure 4-21). Such a **polyclonal antibody** response facilitates the localization, phagocytosis, and complement-mediated lysis of antigen; it thus has clear advantages for the organism in vivo. Unfortunately, the antibody heterogeneity that increases immune protection in vivo often 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 immortal growth properties of the myeloma cell and secreted the antibody produced by the B cell (see Figure 4-21). The resulting clones of hybridoma cells, which secrete large quantities of monoclonal antibody, can be cultured indefinitely. The development of techniques for producing monoclonal antibodies, gave immunologists a powerful and versatile research tool. The significance of the work by Köhler and Milstein was acknowledged when each was awarded a Nobel Prize.

Monoclonal Antibodies Have Important Clinical Uses

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 (Figure 4-22a). In theory, the attached monoclonal antibody will deliver the toxin chain specifically to tumor cells, where it will cause death by inhibiting protein synthesis (Figure 4-22b). 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 underway.

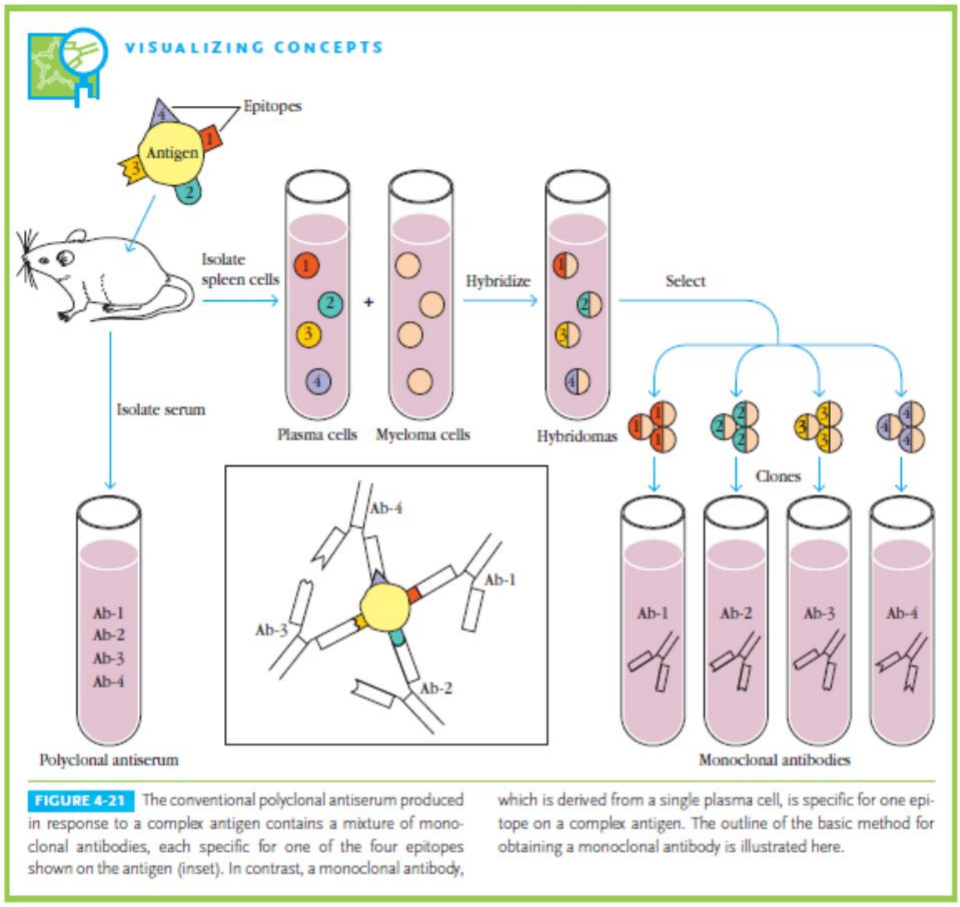


FIGURE 4-21 The conventional polyclonal antiserum produced in response to a complex antigen contains a mixture of monoclonal antibodies, each specific for one of the four epitopes shown on the antigen (inset). In contrast, a monoclonal antibody, which is derived from a single plasma cell, is specific for one epitope on a complex antigen. The outline of the basic method for obtaining a monoclonal antibody is illustrated here.

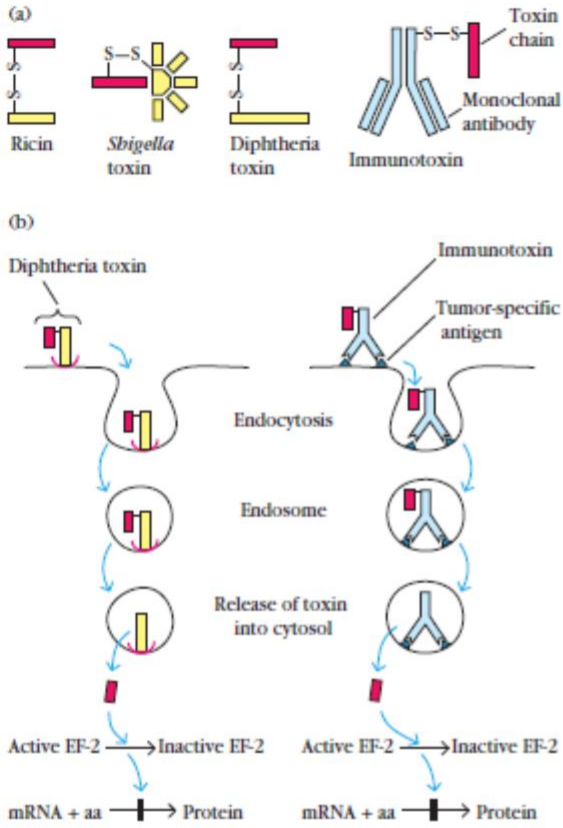


FIGURE 4-22 (a) Toxins used to prepare immunotoxins include ricin, *Shigella* toxin, and diphtheria toxin. Each toxin contains an inhibitory toxin chain (red) and a binding component (yellow). To make an immunotoxin, the binding component of the toxin is replaced with a monoclonal antibody (blue). (b) Diphtheria toxin binds to a cell-membrane receptor (*left*) and a diphtheria-immunotoxin binds to a tumor-associated antigen (*right*). In either case, the toxin is internalized in an endosome. The toxin chain is then released into the cytoplasm, where it inhibits protein synthesis by catalyzing the inactivation of elongation factor 2 (EF-2).

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 change in 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 to generate 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. Additionally, it may be possible to generate abzymes with the ability to dissolve blood clots or to cleave viral glycoproteins at specific sites, thus blocking viral infectivity.

Unfortunately, catalytic antibodies that cleave the peptide bonds of proteins have been exceedingly difficult to derive. Much of the research currently being pursued in this field is devoted to the solution of this important but difficult problem.

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 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 constructs (Figure 5-22).

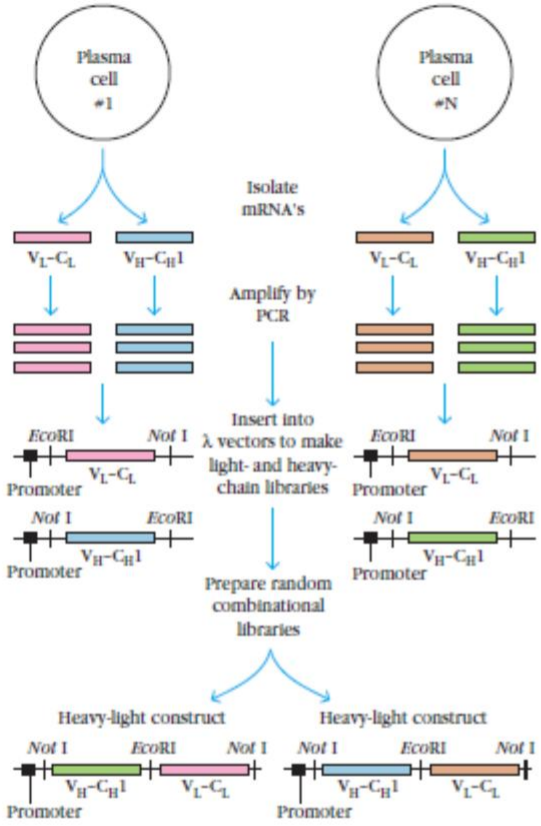


FIGURE 5-22 General procedure for producing gene libraries encoding Fab fragments. In this procedure, isolated mRNA that encodes heavy and light chains is amplified by the polymerase chain reaction (PCR) and cloned in λ vectors. Random combinations of heavy- and light-chain genes generate an enormous number of heavy-light constructs encoding Fab fragments with different antigenic specificity. *[Adapted from W. D. Huse et al., 1989,* Science **246:***1275.]*

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 immune response. The in vivo evolution of most humoral immune responses produces two desirable outcomes. One is class switching, in which a variety of antibody classes of the same specificity are produced. This is an important consideration because the class switching that occurs during an immune response produces antibodies that have the same specificity but different effector functions and hence, greater biological versatility. The other is the generation of antibodies of higher and higher affinity as the response progresses. A central goal of Ig-gene library approaches is the development of strategies to produce antibodies of appropriate affinity in vitro as readily as they are generated by an in vivo immune response. When the formidable technical obstacles to the achievement of these goals are overcome, combinatorial approaches based on phage 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.

Mice Have Been Engineered with Human Immunoglobulin Loci

It is possible to functionally knock out, or disable, the heavy and light-chain immunoglobulin loci in mouse embryonic stem (ES) cells. N. Lonberg and his colleagues followed this procedure and then introduced large DNA sequences (as much as 80 kb) containing human heavy- and light-chain gene segments. The DNA sequences contained constant-region gene segments, J

segments, many V-region segments, and, in the case of the heavy chain, DH segments. The ES cells containing these miniature human Ig gene loci (miniloci) are used to derive lines of transgenic mice that respond to antigenic challenge by producing antigen-specific *human* antibodies (Figure 5-23). Because the human heavy- and light-chain miniloci undergo rearrangement and all the other diversity-generating processes, such as N-addition, Paddition, and even somatic hypermutation after antigenic challenge, there is an opportunity for the generation of a great deal of diversity in these mice. The presence of human heavy-chain minilocus genes for more than one isotype and their accompanying switch sites allows class switching as well. A strength of this method is that these completely human antibodies are made in cells of the mouse B-cell lineage, from which antibody-secreting hybridomas are readily derived by cell fusion. This approach thus offers a solution to the problem of producing human monoclonal antibodies of any specificity desired.

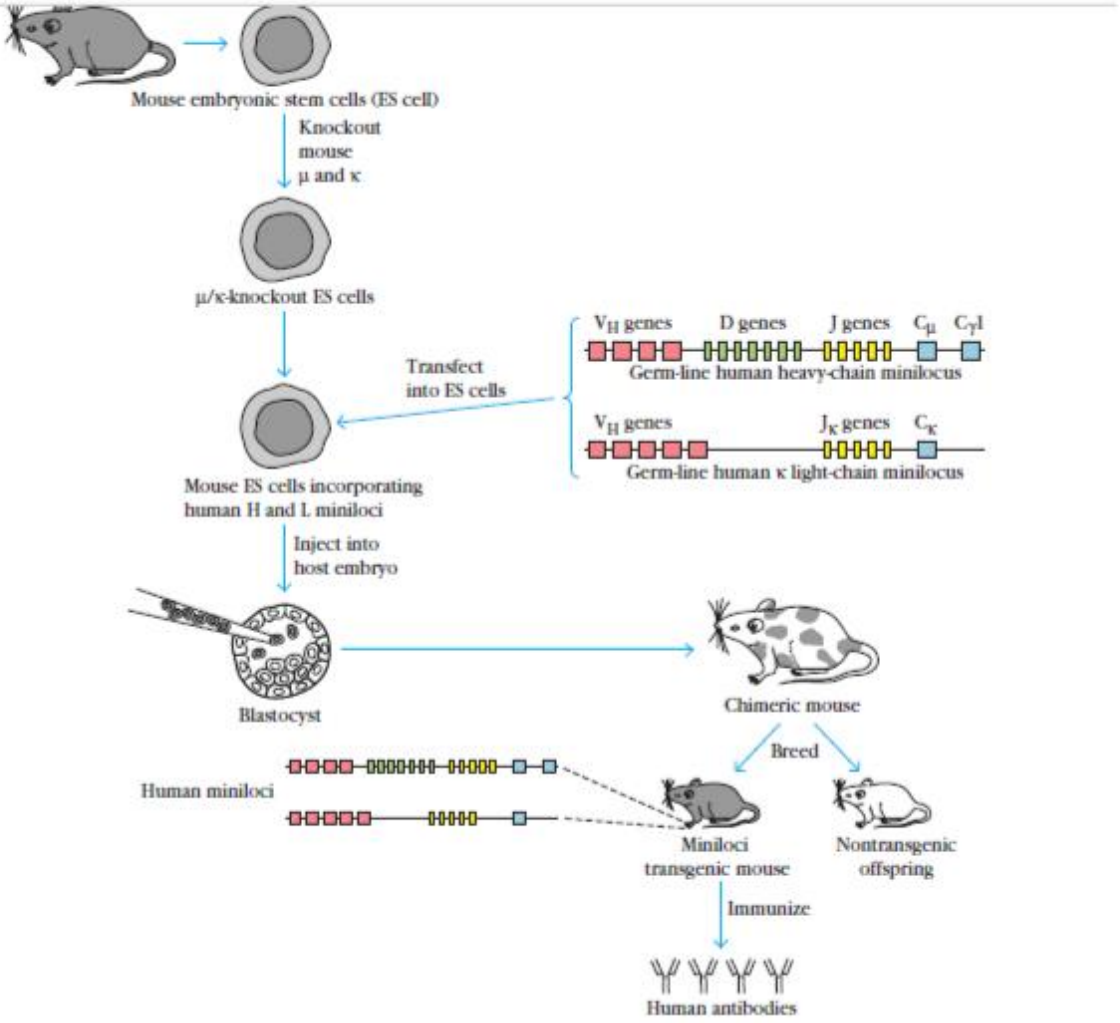


FIGURE 5-23 Grafting human heavy- and light-chain miniloci into mice. The capacity of mice to rearrange Ig heavy- and light-chain gene segments was disabled by knocking out the C_μ and C_κ loci. The antibody-producing capacity of these mice was reconstituted by introducing long stretches of DNA incorporating a large part of the human germ-line κ and heavy-chain loci (miniloci). Chimeric mice were then bred to establish a line of transgenic mice bearing both heavy- and light-chain human miniloci. Immunization of these mice results in the production of human antibody specific for the target antigen. *[N. Lonberg et al., 1994, Nature **368**:856.]*

Monoclonal Antibodies Can Suppress Graft-Rejection Responses

Monoclonal antibodies directed against various surface molecules on cells of the immune system have been used successfully to suppress T-cell activity in general or to suppress the activity of subpopulations of T cells. Results from studies with animal models suggest further that certain monoclonals may be used to suppress only T cells that are activated. Successes with animal models and trials with humans give reason to believe that two types of strategies involving antibodies to suppress rejection will find broad clinical use. Monoclonal antibodies may be used to deplete the recipient of a certain broad or specific cell population; alternatively, they may be used to block co-stimulatory signals. In the latter case, a state of anergy is induced in those T cells that react to antigens present on the allograft. A strategy to deplete immune cells involves use of a monoclonal antibody to the CD3 molecule of the TCR complex. Injection of such monoclonal antibodies results in a rapid depletion of mature T cells from the circulation. This depletion appears to be caused by binding of antibody-coated T cells to Fc receptors on phagocytic cells, which then phagocytose and clear the T cells from the circulation. In a further refinement of this strategy, a cytotoxic agent such as diphtheria toxin is coupled with the antibody. The cell with which the antibody reacts internalizes the toxin, causing its death. Another depletion strategy used to increase graft survival uses monoclonal antibodies specific for the high-affinity IL-2 receptor (anti-TAC). Since the high-affinity IL-2 receptor is expressed only on activated T cells, exposure to anti-TAC after the graft specifically blocks proliferation of T cells activated in response to the alloantigens of the graft. Monoclonal-antibody therapy, which

was initially employed to deplete T cells in graft recipients, also has been used to treat donors' bone marrow before it is transplanted. Such treatment is designed to deplete the immunocompetent T cells in the bone-marrow transplant; these are the cells that react with the recipient tissues, causing graft-versus-host disease (described below).Monoclonal antibodies with isotypes that activate the complement system are most effective in all cell-depletion strategies. The CD3 receptor and the high-affinity IL-2 receptor are targets present on all activated T cells; molecules present on particular T-cell subpopulations may also be targeted for immunosuppressive therapy. For example, a monoclonal antibody to CD4 has been shown to prolong graft survival. In one study, monkeys were given a single large dose of anti-CD4 just before they received a kidney transplant. Graft survival in the treated animals was markedly increased over that in untreated control animals. Interestingly, the anti-CD4 did not reduce the CD4+ T-cell count, but instead appeared to induce the T cells to enter an immunosuppressed state. This is an example of a nondepleting antibody. Other targets for monoclonal-antibody therapy are the cell-surface adhesion molecules. Simultaneous treatment with monoclonal antibodies to the adhesion molecules ICAM-1 and LFA-1 for 6 days after transplantation has permitted indefinite survival of cardiac grafts between allogeneic mice. However, when either monoclonal antibody was administered alone, the cardiac transplant was rejected. The requirement that both monoclonal antibodies be given at the same time probably reflects redundancy of the adhesion molecules: LFA-1 is known to bind to ICAM-2 in addition to ICAM-1; and ICAM-1 is known to bind to Mac-1 and CD43 in addition to LFA-1. Only when all

possible pairings among these adhesins are blocked at the same time is adhesion and signal transduction through this ligand pair blocked. A practical difficulty with using monoclonal antibodies to prolong graft survival in humans is that they are generally of mouse origin.Many recipients develop an antibody response to the mouse monoclonal antibody, rapidly clearing it from the body. This limitation has been overcome by the construction of human monoclonal antibodies and mouse-human chimeric antibodies Because cytokines appear to play an important role in allograft rejection, another strategy for prolonging graft survival is to inject animals with monoclonal antibodies specific for the implicated cytokines, particularly TNF-_, IFN-_, and IL-2. Monoclonal antibodies to TNF-_ have been shown to prolong bone-marrow transplants in mice and to reduce the incidence of graft-versus-host disease. Monoclonal antibodies to IFN-_ and to IL-2 have each been reported in some cases to prolong cardiac transplants in rats.

Possible Questions

1. Explain MLR, and HLA typing,
2. Elaborate bone marrow transplantation.
3. What is organ transplants? Explain.
4. What is immunosuppressive therapy? Explain its type.
5. What is hybridoma technology? Explain with suitable diagram
6. What is monoclonal antibodies? Explain application of monoclonal antibodies in biomedical research,
7. Elaborate various immuno-diagnosis technique.
8. Elaborate Xeno transplantation from various species

UNIT-IV

SYLLABUS

Hyper-sensitivity reactions, auto-immune disorders. Tumor immunology: Tumor antigens, immune response to tumours, cancer immunotherapy. Immunodeficiencies – primary and secondary.

Hypersensitive Reactions

An immune response mobilizes a battery of effector molecules that act to remove antigen by various mechanisms. Generally, these effector molecules induce a localized inflammatory response that eliminates antigen without extensively damaging the host's tissue. 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.

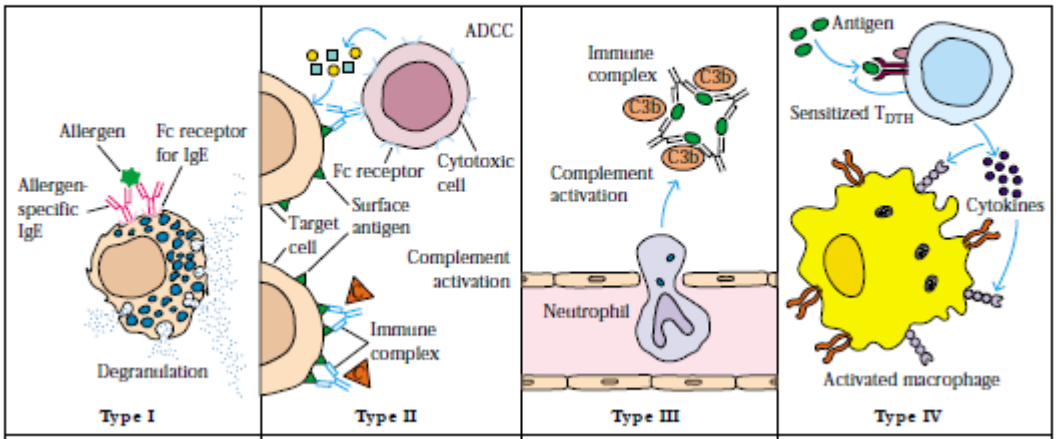
Gell and Coombs Classification

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. The effector molecules in the complement reactions are the membrane-attack complex and such complement split products as C3a, C4a, and C5a. In delayed-type hypersensitivity reactions, the effector molecules are various cytokines secreted by activated TH or TC cells. As it became clear that several different immune mechanisms give rise to hypersensitive reactions, P. G. H. Gell and R. R. A. Coombs proposed a classification scheme in which hypersensitive reactions are divided into four types. 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 (Figure 16-1). This classification scheme has served an important function in identifying the mechanistic differences among various hypersensitive reactions, but it is important to point out that secondary effects blur the boundaries between the

four categories.

VISUALIZING CONCEPTS



Type I	Type II	Type III	Type IV
IgE-Mediated Hypersensitivity	IgG-Mediated Cytotoxic Hypersensitivity	Immune Complex-Mediated Hypersensitivity	Cell-Mediated Hypersensitivity
Ag induces crosslinking of IgE bound to mast cells and basophils with release of vasoactive mediators	Ab directed against cell surface antigens meditates cell destruction via complement activation or ADCC	Ag-Ab complexes deposited in various tissues induce complement activation and an ensuing inflammatory response mediated by massive infiltration of neutrophils	Sensitized T_H1 cells release cytokines that activate macrophages or T_C cells which mediate direct cellular damage
Typical manifestations include systemic anaphylaxis and localized anaphylaxis such as hay fever, asthma, hives, food allergies, and eczema	Typical manifestations include blood transfusion reactions, erythroblastosis fetalis, and autoimmune hemolytic anemia	Typical manifestations include localized Arthus reaction and generalized reactions such as serum sickness, necrotizing vasculitis, glomerulnephritis, rheumatoid arthritis, and systemic lupus erythematosus	Typical manifestations include contact dermatitis, tubercular lesions and graft rejection

FIGURE 16-1 The four types of hypersensitive responses.

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 by the same mechanisms for other soluble antigens, resulting in the generation of antibody-secreting plasma cells and memory cells. 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 (Figure 16-2). The pharmacologically active mediators released from the granules act on the surrounding tissues. The principal effects—vasodilation and smooth-muscle contraction—may be either systemic or localized, depending on the extent of mediator release.

There Are Several Components of Type I Reactions

As depicted in Figure 16-2, several components are critical to development of type I hypersensitive reactions. This section will consider these components first and then describe the mechanism of degranulation.

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 tissue damaging type I hypersensitivity. The term

allergen refers specifically to nonparasitic antigens capable of stimulating type I hypersensitive responses in allergic individuals. The abnormal IgE response of atopic individuals is at least partly genetic—it often runs in families. Atopic individuals have abnormally high levels of circulating IgE and also more than normal numbers of circulating eosinophils. These individuals are more susceptible to allergies such as hay fever, eczema, and asthma. The genetic propensity to atopic responses has been mapped to several candidate loci. One locus, on chromosome 5q, is linked to a region that encodes a variety of cytokines, including IL-3, IL-4, IL-5, IL-9, IL-13, and GM-CSF. A second locus, on chromosome 11q,is linked to a region that encodes the _ chain of the high-affinity IgE receptor. It is known that inherited atopy is multigenic and that other loci probably also are involved. Indeed, as information from the Human Genome Project is analyzed,

other candidate genes may be revealed.

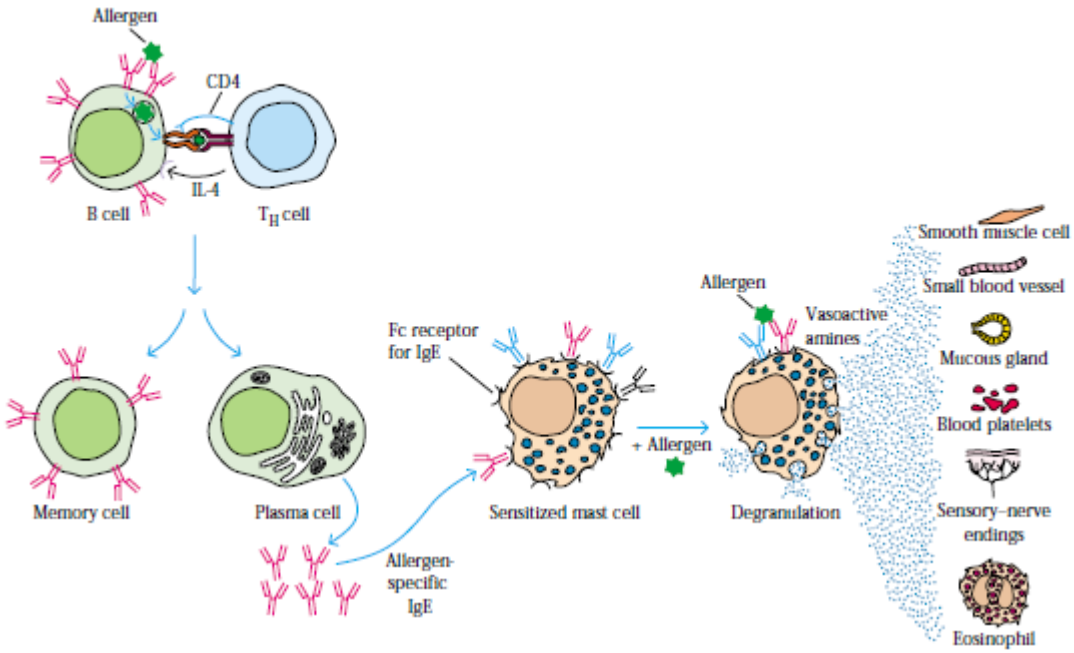


FIGURE 16-2 General mechanism underlying a type I hypersensitive reaction. Exposure to an allergen activates B cells to form IgE-secreting plasma cells. The secreted IgE molecules bind to IgE-specific Fc receptors on mast cells and blood basophils. (Many molecules of IgE with various specificities can bind to the IgE-Fc receptor.) Second exposure to the allergen leads to crosslinking of the bound IgE, triggering the release of pharmacologically active mediators, vasoactive amines, from mast cells and basophils. The mediators cause smooth-muscle contraction, increased vascular permeability, and vasodilation.

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 (see Figure 13-5), or it can mediate cell destruction by antibody dependent 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 (see Figure 14-12). 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 (see Figure 13-12). This section examines three examples

of type II hypersensitive reactions. Certain autoimmune diseases involve autoantibody– mediated cellular destruction by type II mechanisms.

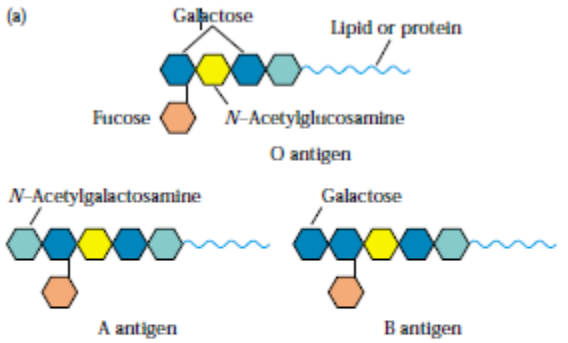
Transfusion Reactions Are Type II Reactions

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 (Figure 16-13a). 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 (Figure 16-13b). 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.

The clinical manifestations of transfusion reactions result from massive intravascular hemolysis of the transfused red blood cells by antibody plus complement. These manifestations may be

either immediate or delayed. Reactions that begin immediately are most commonly associated with ABO blood-group incompatibilities, which lead to complement mediated lysis triggered by the IgM isohemagglutinins. Within hours, free hemoglobin can be detected in the plasma; it is filtered through the kidneys, resulting in hemoglobinuria. Some of the hemoglobin gets converted to bilirubin, which at high levels is toxic. Typical symptoms include fever, chills, nausea, clotting within blood vessels, pain in the lower back, and hemoglobin in the urine. Treatment involves prompt termination of the transfusion and maintenance of urine flow with a diuretic, because the accumulation of hemoglobin in the kidney can cause acute tubular necrosis. Delayed hemolytic transfusion reactions generally occur in individuals who have received repeated transfusions of ABO-compatible blood that is incompatible for other bloodgroup antigens. The reactions develop between 2 and 6 days after transfusion, reflecting the secondary nature of these reactions. The transfused blood induces clonal selection and production of IgG against a variety of blood-group membrane antigens, most commonly Rh, Kidd, Kell, and Duffy. The predominant isotype involved in these reactions is IgG, which is less effective than IgM in activating complement. For this reason, complement-mediated lysis of the transfused red blood cells is incomplete, and many of the transfused cells are destroyed at extravascular sites by agglutination, opsonization, and subsequent phagocytosis by macrophages. Symptoms include fever, low hemoglobin, increased bilirubin, mild jaundice, and anemia. Free hemoglobin is usually not detected in the plasma or urine in these reactions because RBC destruction occurs in

extravascular sites.



(b)

Genotype	Blood-group phenotype	Antigens on erythrocytes (*agglutinins*)	Serum antibodies (*isohemagglutinins*)
AA or AO	A	A	Anti–B
BB or BO	B	B	Anti–A
AB	AB	A and B	None
OO	O	None	Anti–A and anti–B

FIGURE 16-13 ABO blood group. (a) Structure of terminal sugars, which constitute the distinguishing epitopes, in the A, B, and O blood antigens. (b) ABO genotypes and corresponding phenotypes, agglutinins, and isohemagglutinins.

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. During pregnancy, fetal red blood cells are separated from the mother's circulation by a layer of cells in the placenta called the trophoblast. During her first pregnancy with an Rh+ fetus, an Rh– woman is usually not exposed to enough fetal red blood cells to activate her Rh-specific B cells. At the time of delivery,

however, separation of the placenta from the uterine wall allows larger amounts of fetal umbilical-cord blood to enter the mother's circulation. These fetal red blood cells activate Rh-specific B cells, resulting in production of Rh-specific plasma cells and memory B cells in the mother. The secreted IgM antibody clears the Rh+ fetal red cells from the mother's circulation, but the memory cells remain, a threat to any subsequent pregnancy with an Rh+ fetus. Activation of these memory cells in a subsequent pregnancy results in the formation of IgG anti-Rh antibodies, which cross the placenta and damage the fetal red blood cells (Figure 16-14). Mild to severe anemia can develop in the fetus, sometimes with fatal consequences. In addition, conversion of hemoglobin to bilirubin can present an additional threat to the newborn because the lipid-soluble bilirubin may accumulate in the brain and cause brain damage. Hemolytic disease of the newborn caused by Rh incompatibility in a subsequent pregnancy can be almost entirely prevented by administering antibodies against the Rh antigen to the mother within 24–48 h after the first delivery. These antibodies, called **Rhogam,** bind to any fetal red blood cells that enter the mother's circulation at the time of delivery and facilitate their clearance before B-cell activation and ensuing memory-cell production can take place. In a subsequent pregnancy with an Rh+ fetus, a mother who has been treated with Rhogam is unlikely to produce IgG anti-Rh antibodies; thus, the fetus is protected from the damage that would occur when these antibodies crossed the placenta. The development of hemolytic disease of the newborn caused by Rh incompatibility can be detected by testing maternal serum at intervals during pregnancy for antibodies to the Rh antigen. A rise in the titer of these antibodies as pregnancy progresses indicates that the mother has been exposed to Rh antigens and is producing increasing amounts

of antibody. The presence of maternal IgG on the surface of fetal red blood cells can be detected by a Coombs test. Isolated fetal red blood cells are incubated with the Coombs reagent, goat antibody to human IgG antibody. If maternal IgG is bound to the fetal red blood cells, the cells agglutinate with the Coombs reagent. If hemolytic disease caused by Rh incompatibility is detected during pregnancy, the treatment depends on the severity of the reaction. For a severe reaction, the fetus can be given an intrauterine blood-exchange transfusion to replace fetal Rh+ red blood cells with Rh– cells. These transfusions are given every 10–21 days until delivery. In less severe cases, a blood-exchange transfusion is not given until after birth, primarily to remove bilirubin; the infant is also exposed to low levels of UV light to break down the bilirubin and prevent cerebral damage. The mother can also be treated during the pregnancy by **plasmapheresis.** In this procedure, a cellseparation machine is used to separate the mother's blood into two fractions, cells and plasma. The plasma containing the anti-Rh antibody is

discarded, and the cells are reinfused into the mother in an albumin or fresh-plasma solution.

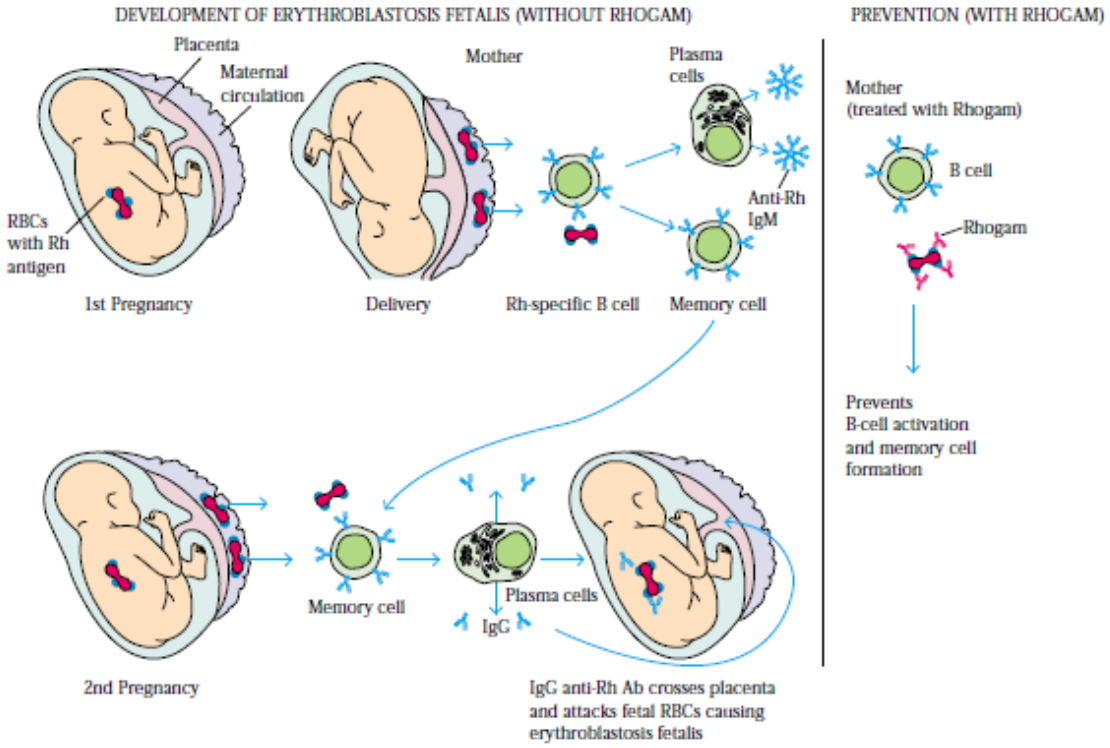


FIGURE 16-14 Development of erythroblastosis fetalis (hemolytic disease of the newborn) caused when an Rh⁻ mother carries an Rh⁺ fetus (*left*), and effect of treatment with anti-Rh antibody, or Rhogam (*right*).

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. In particular, complex deposition is frequently observed on blood-vessel walls, in the synovial membrane of joints, on the glomerular basement membrane of the kidney, and on the choroid plexus of the brain. The deposition of these complexes initiates a reaction that results in the recruitment of neutrophils to the site. The tissue there is injured as a consequence of granular release from the neutrophil. Type III hypersensitive reactions develop when immune complexes activate the complement system's array of immune effector molecules (see Figure 13-2). As the C3a, C4a, and C5a complement split products are anaphylatoxins that cause localized mast-cell degranulation and consequent increase in local vascular permeability. C3a, C5a, and C5b67 are also chemotactic factors for neutrophils, which can accumulate in large numbers at the site of immune-complex deposition. Larger immune complexes are deposited on the basement membrane of bloodvessel walls or kidney glomeruli, whereas smaller complexes may pass through the basement membrane and be deposited in the subepithelium. The type of lesion that results depends on the site of deposition of the complexes. 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 III Reactions Can Be Localized

Injection of an antigen intradermally or subcutaneously into an animal that has high levels of circulating antibody specific for that antigen leads to formation of localized immune complexes, which mediate an acute Arthus reaction within 4–8 h (Figure 16-15).Microscopic examination of the tissue reveals neutrophils adhering to the vascular endothelium and then migrating into the tissues at the site of immune complex deposition. As the reaction develops, localized tissue and vascular damage results in an accumulation of fluid (edema) and red blood cells (erythema) at the site. The severity of the reaction can vary from mild swelling and redness to tissue necrosis.

After an insect bite, a sensitive individual may have a rapid, localized type I reaction at the site. Often, some 4–8 h later, a typical Arthus reaction also develops at the site, with pronounced erythema and edema. Intrapulmonary Arthus-type reactions induced by bacterial spores, fungi, or dried fecal proteins can also cause pneumonitis or alveolitis. These reactions are known by a variety of common names reflecting the source of the antigen. For example, “farmer’s lung” develops after inhalation of thermophilic actinomycetes from moldy hay, and “pigeon fancier’s

disease" results from inhalation of a serum protein in dust derived from dried pigeon feces.

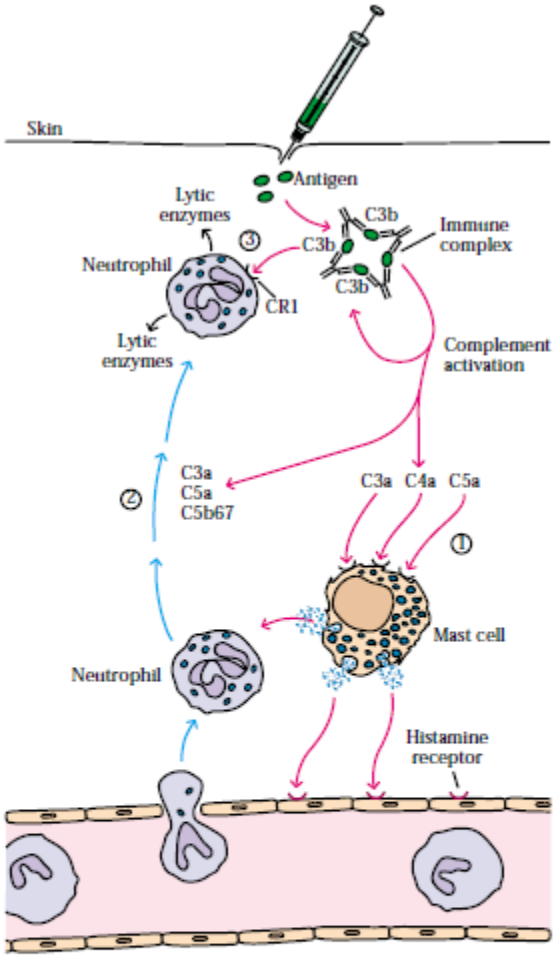


FIGURE 16-15 Development of a localized Arthus reaction (type III hypersensitive reaction). Complement activation initiated by immune complexes (classical pathway) produces complement intermediates that (1) mediate mast-cell degranulation, (2) chemotactically attract neutrophils, and (3) stimulate release of lytic enzymes from neutrophils trying to phagocytose C_3b-coated immune complexes.

Type III Reactions Can Also Be Generalized

When large amounts of antigen enter the bloodstream and bind to antibody, circulating immune complexes can form. If antigen is in excess, small complexes form; because these are not easily cleared by the phagocytic cells, they can cause tissue-damaging type III reactions at various sites.

Historically, generalized type III reactions were often observed after the administration of antitoxins containing foreign serum, such as horse antitetanus or antidiphtheria serum. In such cases, the recipient of a foreign antiserum develops antibodies specific for the foreign serum proteins; these antibodies then form circulating immune complexes with the foreign serum antigens. Typically, within days or weeks after exposure to foreign serum antigens, an individual begins to manifest a combination of symptoms that are called **serum sickness** (Figure 16-16). These symptoms include fever, weakness, generalized vasculitis (rashes) with edema and erythema, lymphadenopathy, arthritis, and sometimes glomerulonephritis. The precise manifestations of serum sickness depend on the quantity of immune complexes formed as well as the overall size of the complexes, which determine the site of their deposition. As mentioned above, the sites of deposition vary but, in general, complexes accumulate in tissues where filtration of plasma occurs. This explains the high incidence of glomerulonephritis (complex deposition in the kidney) and vasculitis (deposition in the arteries) and arthritis (deposition in the synovial joints) caused by serum sickness. Formation of circulating immune complexes contributes to the pathogenesis of a number of conditions other than serum sickness. These include the following:

_ *Autoimmune Diseases*

Systemic lupus erythematosus

Rheumatoid arthritis

Goodpasture's syndrome

_ *Drug Reactions*

Allergies to penicillin and sulfonamides

_ *Infectious Diseases*

Poststreptococcal glomerulonephritis

Meningitis

Hepatitis

Mononucleosis

Malaria

Trypanosomiasis

Complexes of antibody with various bacterial, viral, and parasitic antigens have been shown to induce a variety of type III hypersensitive reactions, including skin rashes, arthritic symptoms, and glomerulonephritis. Poststreptococcal glomerulonephritis, for example, develops when circulating complexes of antibody and streptococcal antigens are deposited in the kidney and damage the glomeruli. A number of autoimmune diseases stem from circulating complexes of antibody with self-proteins, with glycoproteins, or even with DNA. In systemic lupus erythematosus, complexes of DNA and anti-DNA antibodies accumulate in synovial membranes, causing arthritic symptoms, or accumulate on the basement membrane of the kidney, causing progressive kidney damage.

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 is always 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 develop and 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.

There Are Several Phases of the DTH Response

The development of the DTH response begins with an initial sensitization phase of 1–2 weeks after primary contact with an antigen. During this period, TH cells are activated and clonally expanded by antigen presented together with the requisite class II MHC molecule on an appropriate antigen presenting cell (Figure 16-17a).A variety of antigen-presenting cells have been shown to be involved in the activation of a DTH response, including Langerhans cells and

macrophages. Langerhans cells are dendritic cells found in the epidermis. These cells are thought to pick up antigen that enters through the skin and transport it to regional lymph nodes, where T cells are activated by the antigen. In some species, including humans, the vascular endothelial cells express class II MHC molecules and also function as antigen-presenting cells in the development of the DTH response. Generally, the T cells activated during the sensitization phase are CD4+, primarily of the TH1 subtype, but in a few cases CD8+ cells have also been shown to induce a DTH response. The activated T cells previously were called TDTH cells to denote their function in the DTH response, although in reality they are simply a subset of activated TH1 cells (or, in some cases, TC cells). A subsequent exposure to the antigen induces the effector phase of the DTH response (see Figure 16-17b). In the effector phase, TH1 cells secrete a variety of cytokines that recruit and activate macrophages and other nonspecific inflammatory cells. A DTH response normally does not become apparent until an average of 24 h after the second contact with the antigen; the response generally peaks 48–72 h after second contact. The delayed onset of this response reflects the time required for the cytokines to induce localized influxes of macrophages and their activation. Once a DTH response begins, a complex interplay of nonspecific cells and mediators is set in motion that can result in tremendous amplification. By the time the DTH response is fully developed, only about 5% of the participating cells are antigen-specific TH1 cells; the remainder are macrophages and other nonspecific cells. Macrophages are the principal effector cells of the DTH response. Cytokines elaborated by TH1 cells induce blood monocytes to adhere to vascular endothelial cells and migrate from the blood into the surrounding tissues. During this process the monocytes differentiate into activated

macrophages. Activated macrophages exhibit increased levels of phagocytosis and an increased ability to kill microorganisms through various cytotoxic mediators. In addition, activated macrophages express increased levels of class II MHC molecules and cell-adhesion molecules and therefore function more effectively as antigen-presenting cells. The influx and activation of macrophages in the DTH response is important in host defense against parasites and bacteria that live within cells, where circulating antibodies cannot reach them. The heightened phagocytic activity and the buildup of lytic enzymes from macrophages in the area of infection lead to nonspecific destruction of cells, and thus of the intracellular pathogen. Generally, the pathogen is cleared rapidly with little tissue damage. However, in some cases, especially if the antigen is not easily cleared, a prolonged DTH response can itself become destructive to the host as the intense inflammatory response develops into a visible granulomatous reaction. A granuloma develops when continuous activation of macrophages induces the macrophages to adhere closely to one another, assuming an epithelioid shape and sometimes fusing to form multinucleated giant cells (Figure 16-18). These giant cells displace the normal tissue cells, forming palpable nodules, and release high concentrations of lytic enzymes, which destroy surrounding tissue. In these cases, the response can damage blood vessels and lead to extensive tissue necrosis. The response to *Mycobacteriumtuberculosis* illustrates the double-edged nature of the DTH response. Immunity to this intracellular bacterium involves a DTH response in which activated macrophages wall off the organism in the lung and contain it within a granuloma-type lesion called a tubercle. Often, however, the concentrated release of lytic enzymes from the activated macrophages within

tubercles damages lung tissue.

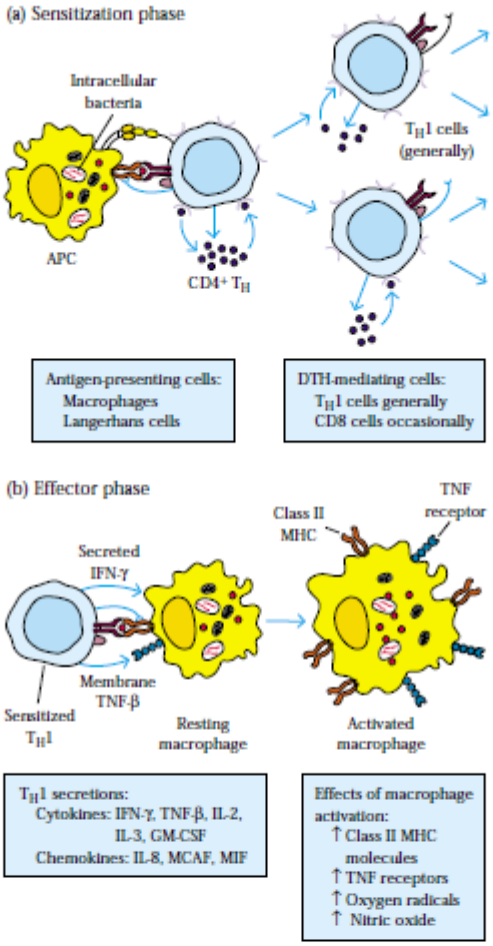


FIGURE 16-17 Overview of the DTH response. (a) In the sensitization phase after initial contact with antigen (e.g., peptides derived from intracellular bacteria), T_H cells proliferate and differentiate into T_H1 cells. Cytokines secreted by these T cells are indicated by the dark blue balls. (b) In the effector phase after subsequent exposure of sensitized T_H1 cells to antigen, the T_H1 cells secrete a variety of cytokines and chemokines. These factors attract and activate macrophages and other nonspecific inflammatory cells. Activated macrophages are more effective in presenting antigen, thus perpetuating the DTH response, and function as the primary effector cells in this reaction.

Cancer: Origin and Terminology

In most organs and tissues of a mature animal, a balance is usually maintained between cell renewal and cell death. The various types of mature cells in the body have a given life span; as these cells die, new cells are generated by the proliferation and differentiation of various types of

stem cells. Under normal circumstances, the production of new cells is regulated so that the number of any particular type of cell remains constant. Occasionally, though, cells arise that no longer respond to normal growth-control mechanisms. These cells give rise to clones of cells that can expand to a considerable size, producing a tumor, or **neoplasm.** A tumor that is not capable of indefinite growth and does not invade the healthy surrounding tissue extensively is **benign.** A tumor that continues to grow and becomes progressively invasive is **malignant;** the term *cancer* refers specifically to a malignant tumor. In addition to uncontrolled growth, malignant tumors exhibit **metastasis;** in this process, small clusters of cancerous cells dislodge from a tumor, invade the blood or lymphatic vessels, and are carried to other tissues, where they continue to proliferate. In this way a primary tumor at one site can give rise to a secondary tumor at another site (Figure 22-1).

Malignant tumors or cancers are classified according to the embryonic origin of the tissue from which the tumor is

derived. Most (>80%) are **carcinomas,** tumors that arise from endodermal or ectodermal tissues such as skin or the

epithelial lining of internal organs and glands. The majority of cancers of the colon, breast, prostate, and lung are carcinomas. The **leukemias** and **lymphomas** are malignant tumors of hematopoietic cells of the bone marrow and account for about 9% of cancer incidence in the United States. Leukemias proliferate as single cells, whereas lymphomas tend to grow as tumor masses. **Sarcomas,** which arise less frequently (around 1% of the incidence in the United States), are derived from mesodermal connective tissues such as bone, fat, and cartilage.

Malignant Transformation of Cells

Treatment of normal cultured cells with chemical carcinogens, irradiation, and certain viruses can alter their morphology and growth properties. In some cases this process, referred to as **transformation,** makes the cells able to produce tumors when they are injected into animals. Such cells are said to have undergone malignant transformation, and they often exhibit properties in vitro similar to those of cancer cells. For example, they have decreased requirements for growth factors and serum,are no longer anchorage-dependent, and grow in a density-independent fashion. Moreover, both cancer cells and transformed cells can be subcultured indefinitely; that is, for all practical purposes, they are immortal. Because of the similar properties of cancer and transformed cells, the process of malignant transformation has been studied extensively as a model of cancer induction. Various chemical agents (e.g.,DNA-alkylating reagents) and physical agents (e.g., ultraviolet light and ionizing radiation) that cause mutations have been shown to induce transformation. Induction of malignant transformation with chemical or physical carcinogens appears to involve multiple steps and at least two distinct phases: initiation and promotion. Initiation involves changes in the genome but does not, in itself, lead to malignant transformation. After initiation, promoters stimulate cell division and lead to malignant transformation. The importance of mutagenesis in the induction of cancer is illustrated by diseases such as xeroderma pigmentosum. This rare disorder is caused by a defect in the gene that encodes a DNA-repair enzyme called UV-specific endonuclease. Individuals

with this disease are unable to repair UV-induced mutations and consequently develop skin cancers. A number of DNA and RNA viruses have been shown to induce malignant transformation. Two of the best-studied are SV40 and polyoma. In both cases the viral genomes, which integrate randomly into the host chromosomal DNA, include several genes that are expressed early in the course of viral replication. SV40 encodes two early proteins called large T and little T, and polyoma encodes three early proteins called large T, middle T, and little T. Each of these proteins plays a role in the malignant transformation of virus-infected cells. Most RNA viruses replicate in the cytosol and do not induce malignant transformation. The exceptions are retroviruses, which transcribe their RNA into DNA by means of a reverse-transcriptase enzyme and then integrate the transcript into the host's DNA. This process is similar in the cytopathic retroviruses such as HIV-1 and HIV-2 and in the transforming retroviruses, which induce changes in the host cell that lead to malignant transformation. In some cases, retrovirus-induced transformation is related to the presence of **oncogenes,** or "cancer genes," carried by the retrovirus. One of the best-studied transforming retroviruses is the **Rous sarcoma virus.** This virus carries an oncogene called v-*src*, which encodes a 60-kDa protein kinase (v-Src) that catalyzes the addition of phosphate to tyrosine residues on proteins. The first evidence that oncogenes alone could induce malignant transformation came from studies of the v-*src* oncogene from Rous sarcoma virus. When this oncogene was cloned and transfected into normal cells in culture, the cells underwent malignant transformation.

Oncogenes and Cancer Induction

In 1971, Howard Temin suggested that oncogenes might not be unique to transforming viruses but might also be found in normal cells; indeed, he proposed that a virus might acquire oncogenes from the genome of an infected cell. He called these cellular genes **proto-oncogenes,** or **cellular oncogenes** (c-*onc*), to distinguish them from their viral counterparts (v-*onc*). In the mid-1970s, J. M. Bishop and H. E. Varmus identified a DNA sequence in normal chicken cells that is homologous to v-*src* from Rous sarcoma virus. This cellular oncogene was designated c-*src.* Since these early discoveries, numerous cellular oncogenes have been identified. Sequence comparisons of viral and cellular oncogenes reveal that they are highly conserved in evolution. Although most cellular oncogenes consist of a series of exons and introns, their viral counterparts consist of uninterrupted coding sequences, suggesting that the virus might have acquired the oncogene through an intermediate RNA transcript from which the intron sequences had been removed during RNA processing. The actual coding sequences of viral oncogenes and the corresponding proto-oncogenes exhibit a high degree of homology; in some cases, a single point mutation is all that distinguishes a viral oncogene from the corresponding proto-oncogene. It has now become apparent that most, if not all, oncogenes (both viral and cellular) are derived from cellular genes that encode various growth-controlling proteins. In addition, the proteins encoded by a particular oncogene and its corresponding proto-oncogene appear to have very similar functions. As described below, the conversion of a proto-oncogene into an oncogene appears in many cases to accompany a change in the level of expression of a normal growth-controlling protein.

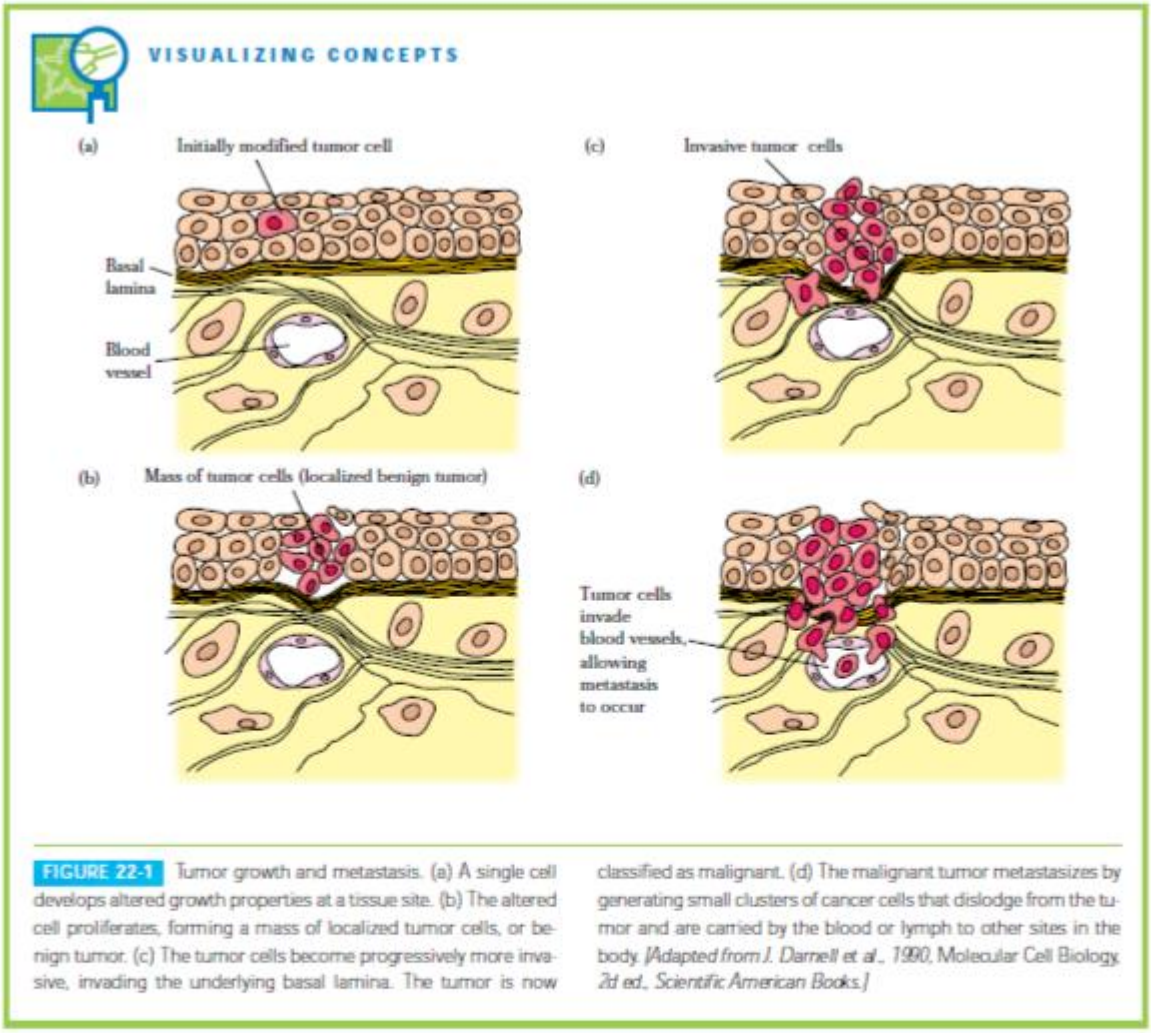


FIGURE 22-1 Tumor growth and metastasis. (a) A single cell develops altered growth properties at a tissue site. (b) The altered cell proliferates, forming a mass of localized tumor cells, or benign tumor. (c) The tumor cells become progressively more invasive, invading the underlying basal lamina. The tumor is now classified as malignant. (d) The malignant tumor metastasizes by generating small clusters of cancer cells that dislodge from the tumor and are carried by the blood or lymph to other sites in the body. *[Adapted from J. Darnell et al., 1990, Molecular Cell Biology, 2d ed., Scientific American Books.]*

Cancer-Associated Genes Have Many Functions

Homeostasis in normal tissue is maintained by a highly regulated process of cellular proliferation balanced by cell death. If there is an imbalance, either at the stage of cellular proliferation or at the stage of cell death, then a cancerous state will develop. Oncogenes and tumor suppressor genes have been shown to play an important role in this process, by regulating either cellular

proliferation or cell death. Cancer-associated genes can be divided into three categories that reflect these different activities, summarized in Table 22-1.

INDUCTION OF CELLULAR PROLIFERATION

One category of proto-oncogenes and their oncogenic counterparts encodes proteins that induce cellular proliferation. Some of these proteins function as growth factors or growthfactor receptors. Included among these are *sis,* which encodes a form of platelet-derived growth factor, and *fms, erbB,* and *neu,* which encode growth-factor receptors. In normal cells, the expression of growth factors and their receptors is carefully regulated. Usually, one population of cells secretes a growth factor that acts on another population of cells that carries the receptor for the factor, thus stimulating proliferation of the second population. Inappropriate expression of either a growth factor or its receptor can result in uncontrolled proliferation. Other oncogenes in this category encode products that function in signal-transduction pathways or as transcription factors. The *src* and *abl* oncogenes encode tyrosine kinases, and the *ras* oncogene encodes a GTP-binding protein. The products of these genes act as signal transducers. The *myc,*

jun, and *fos* oncogenes encode transcription factors. Overactivity of any of these oncogenes may result in unregulated proliferation.

INHIBITION OF CELLULAR PROLIFERATION

A second category of cancer-associated genes—called **tumorsuppressor genes,** or anti-oncogenes—encodes proteins that inhibit excessive cell proliferation. Inactivation of these results in unregulated proliferation. The prototype of this category of oncogenes is *Rb,* the retinoblastoma gene. Hereditary retinoblastoma is a rare childhood cancer, in which tumors

develop from neural precursor cells in the immature retina. The affected child has inherited a mutated *Rb* allele; somatic inactivation of the remaining *Rb* allele leads to tumor growth. Probably the single most frequent genetic abnormality in human cancer is mutation in *p53*, which encodes a nuclear phosphoprotein. Over 90% of small-cell lung cancers and over 50% of breast and colon cancers have been shown to be associated with mutations in *p53*.

REGULATION OF PROGRAMMED CELL DEATH

A third category of cancer-associated genes regulates programmed cell death. These genes encode proteins that either block or induce apoptosis. Included in this category of oncogenes is *bcl-2*, an anti-apoptosis gene. This oncogene was originally discovered because of its association with B-cell follicular lymphoma. Since its discovery, *bcl-2* has been shown to play an important role in regulating cell survival during hematopoiesis and in the survival of selected B cells and T cells during maturation. Interestingly, the Epstein-Barr virus contains a gene that has sequence homology to *bcl-2* and may act in a similar manner to suppress apoptosis.

Proto-Oncogenes Can Be Converted to Oncogenes

In 1972, R. J. Huebner and G. J. Todaro suggested that mutations or genetic rearrangements of proto-oncogenes by carcinogens or viruses might alter the normally regulated function of these genes, converting them into potent cancer-causing oncogenes (Figure 22-2). Considerable evidence supporting this hypothesis accumulated in subsequent years. For example, some malignantly transformed cells contain multiple copies of cellular oncogenes, resulting in increased production of oncogene products. Such amplification of cellular oncogenes has been observed in cells from various types of human cancers. Several groups have identified c-*myc*

oncogenes in homogeneously staining regions (HSRs) of chromosomes from can-cer cells; these HSRs represent long tandem arrays of amplified

genes. In addition, some cancer cells exhibit chromosomal translocations, usually the movement of a proto-oncogene from one chromosomal site to another (Figure 22-3). In many cases of Burkitt's lymphoma, for example, c-*myc* is moved from its normal position on chromosome 8 to a position near the immunoglobulin heavy-chain enhancer on chromosome 14. As a result of this translocation, synthesis of the c-Myc protein, which functions as a transcription factor, increases. Mutation in proto-oncogenes also has been associated with cellular transformation, and it may be a major mechanism by which chemical carcinogens or x-irradiation convert a proto-oncogene into a cancer-inducing oncogene. For instance, single-point mutations in c-*ras* have been detected in a significant fraction of several human cancers, including carcinomas of the bladder, colon, and lung. Some of these mutations appear to reduce the ability of Ras to associate with GTPase-stimulating proteins, thus prolonging the growth activated state of Ras. Viral integration into the host-cell genome may in itself serve to convert a proto-oncogene into a transforming oncogene oncogene. For example, avian leukosis virus (ALV) is a retrovirus that does not carry any viral oncogenes and yet is able to transform B cells into lymphomas. This particular retrovirus has been shown to integrate within the c-*myc* proto-oncogene, which contains three exons. Exon 1 of c-*myc* has an unknown function; exons 2 and 3 encode the Myc protein. Insertion of AVL between exon 1 and exon 2 has been shown in some cases to allow the provirus promoter to increase transcription of exons 2 and 3, resulting in increased synthesis of c-Myc. A variety of tumors have been shown to express significantly increased levels of growth factors or

growth-factor receptors. Expression of the receptor for epidermal growth factor, which is encoded by c-*erbB*, has been shown to be amplified in many cancer cells.And in breast cancer, increased synthesis of the growth-factor receptor encoded by c-*neu* has been linked with a poor prognosis.

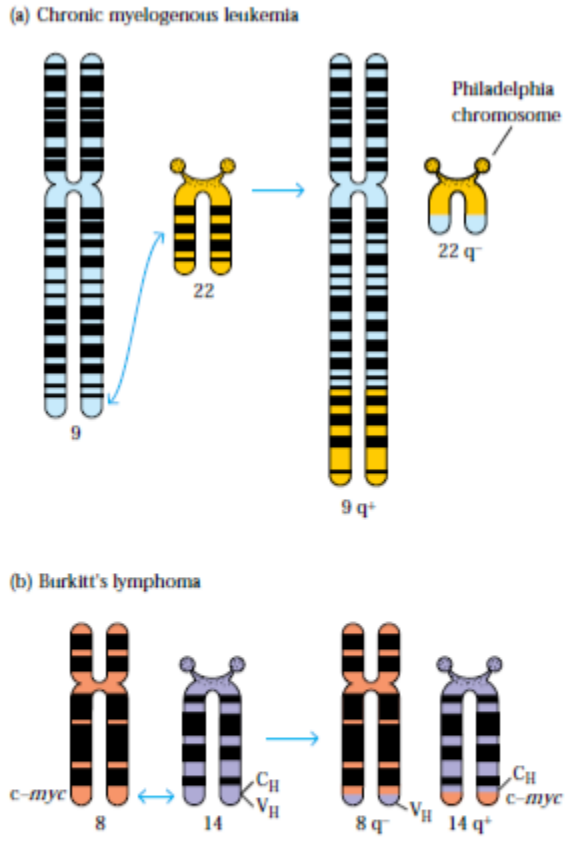


FIGURE 22-3 Chromosomal translocations in (a) chronic myelogenous leukemia (CML) and (b) Burkitt's lymphoma. Leukemic cells from all patients with CML contain the so-called Philadelphia chromosome, which results from a translocation between chromosomes 9 and 22. Cancer cells from some patients with Burkitt's lymphoma exhibit a translocation that moves part of chromosome 8 to chromosome 14. It is now known that this translocation involves c-*myc*, a cellular oncogene. Abnormalities such as these are detected by banding analysis of metaphase chromosomes. Normal chromosomes are shown on the left, and translocated chromosomes on the right.

Primary Immunodeficiencies

A primary immunodeficiency may affect either adaptive or innate immune functions. Deficiencies involving components of adaptive immunity, such as T or B cells, are thus differentiated from immunodeficiencies in which the nonspecific mediators of innate immunity, such as phagocytes or complement, are impaired. immunodeficiencies are conveniently categorized by the type or the developmental stage of the cells involved. Figure 19-1 reviews the overall cellular development in the immune system, showing the locations of defects that give rise to primary immunodeficiencies. The two main cell lineages important to immune function are lymphoid and myeloid. Most defects that lead to immunodeficiencies affect either one or the other. The lymphoid cell disorders may affect T cells, B cells, or, in combined immunodeficiencies, both B and T cells. The myeloid cell disorders affect phagocytic function.Most of the primary immunodeficiencies are inherited, and the precise molecular variations and the genetic defects that lead to many of these dysfunctions have been determined (Table 19-1 and Figure 19-2). In addition, there are immunodeficiencies that stem from developmental defects that impair proper function of an organ of the immune system. The consequences of primary immunodeficiency depend on the number and type of immune system components involved. Defects in components early in the hematopoietic developmental scheme affect the entire immune system. In this category is reticular dysgenesis, a stem-cell defect that affects the maturation of all leukocytes; the resulting general failure of immunity leads to susceptibility to infection by a variety of microorganisms. Without aggressive treatment, the affected individual usually dies young from severe infection. In the more restricted case of

defective phagocytic function, the major consequence is susceptibility to bacterial infection. Defects in more highly differentiated compartments of the immune system have consequences that are more specific and usually less severe. For example, an individual with selective IgA deficiency may enjoy a full life span, troubled only by a greater than normal susceptibility to infections of the respiratory and genitourinary tracts.

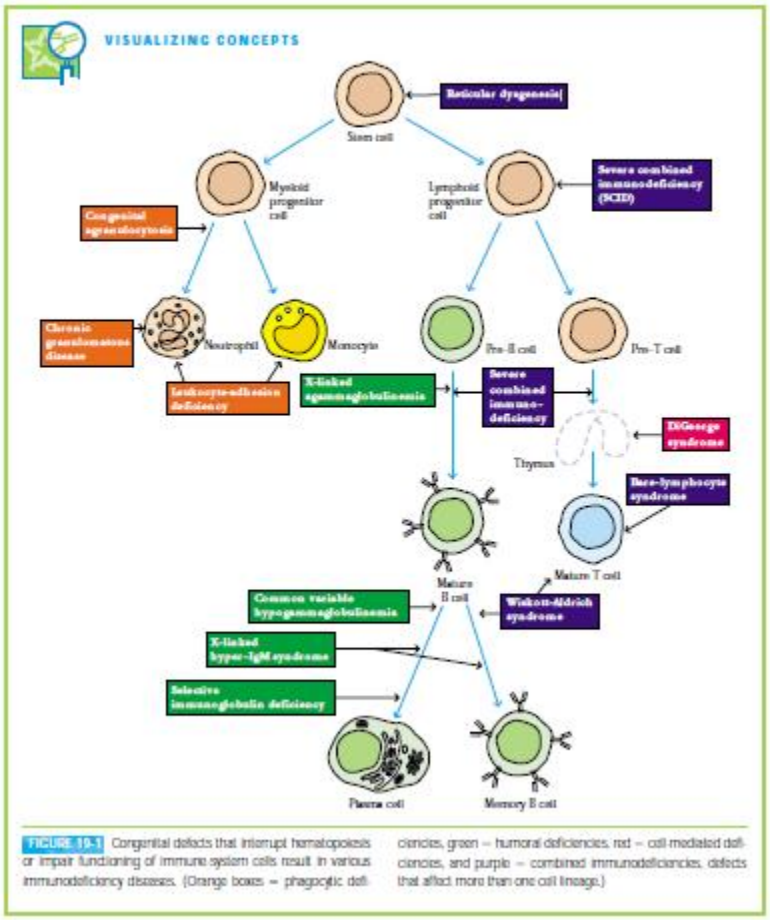


FIGURE 19-1 Congenital defects that interrupt hematopoiesis or impair functioning of immune system cells result in various immunodeficiency diseases. (Orange boxes = phagocytic deficiencies, green = humoral deficiencies, red = cell mediated deficiencies, and purple = combined immunodeficiencies, defects that affect more than one cell lineage.)

TABLE 19-1 Some primary human immunodeficiency diseases and underlying genetic defects

Immunodeficiency disease	Specific defect	Impaired function	Inheritance mode*	Chromosomal defect
Severe combined immunodeficiency (SCID)	RAG-1/RAG-2 deficiency	No TCR or Ig gene rearrangement	AR	11p13
	ADA deficiency	Toxic metabolite in T and B cells	AR	20q13
	PNP deficiency		AR	14q13
	JAK-3 deficiency	Defective signals from IL-2, 4, 7, 9, 15,	AR	19p13
	IL-2Rγ-deficiency		XL	Xq13
	ZAP-70 deficiency	Defective signal from TCR	AR	2q12
Bare lymphocyte syndrome	Defect in MHC class II gene promoter	No class II MHC molecules	AR	16p13
Wiskott-Aldrich syndrome (WAS)	Cytoskeletal protein (CD43)	Defective T cells and platelets	XL	Xp11
Interferon gamma receptor	IFN-γ–receptor defect	Impaired immunity to mycobacteria	AR	6q23
DiGeorge syndrome	Thymic aplasia	T- and B-cell development	AD	22q11
Ataxia telangiectasia	Defective cell-cycle kinase	Low IgA, IgE	AR	11q22
Gammaglobulinemias	X-linked agammaglobulinemia	Bruton's tyrosine kinase (Btk); no mature B cells	XL	Xq21
	X-linked hyper-IgM syndrome	Defective CD40 ligand	XL	Xq26
	Common variable immunodeficiency	Low IgG, IgA; variable IgM	Complex	
	Selective IgA deficiency	Low or no IgA	Complex	
Chronic granulomatous disease	Cyt $p91^{phox}$	No oxidative burst for bacterial killing	XL	Xp21
	Cyt $p67^{phox}$		AR	1q25
	Cyt $p22^{phox}$		AR	16q24
Chediak-Higashi syndrome	Defective intracellular transport protein (LYST)	Inability to lyse bacteria	AR	1q42
Leukocyte-adhesion defect	Defective integrin β2 (CD18)	Leukocyte extravasation	AR	21q22

*AR = autosomal recessive; AD = autosomal dominant; XL = X linked; "Complex" indicates conditions for which precise genetic data are not available and that may involve several interacting loci.

AIDS and Other Acquired or Secondary Immunodeficiencies

As described above, a variety of defects in the immune system give rise to immunodeficiency. In addition to the primary immunodeficiencies, there are also acquired, or secondary, immunodeficiencies. One that has been known for some time is called acquired hypogammaglobulinemia. (As mentioned above, this condition is sometimes confused with

common variable immunodeficiency, a condition that shows genetic predisposition.) The origin of acquired hypogammaglobulinemia is unknown, and its major symptom, recurrent infection, manifests itself in young adults. The patients generally have very low but detectable levels of total immunoglobulin. T-cell numbers and function may be normal, but there are some cases with T-cell defects and these may grow more severe as the disease progresses. The disease is generally treated by immunoglobulin therapy, allowing patients to survive into their seventh and eighth decades. Unlike similar deficiencies described above, there is no evidence for genetic transmission of this disease. Mothers with acquired hypogammaglobulinemia deliver normal infants. However, at birth the infants will be deficient in circulating immunoglobulin, because the deficiency in maternal circulation is reflected in the infant. Another form of secondary immunodeficiency, known as agent-induced immunodeficiency, results from exposure to any of a number of chemical and biological agents that induce an immunodeficient state. Certain of these are drugs used to combat autoimmune diseases such as rheumatoid arthritis or lupus erythematosis. Corticosteroids, which are commonly used for autoimmune disorders, interfere with the immune response in order to relieve disease symptoms. Similarly, a state of immunodeficiency is deliberately induced in transplantation patients who are given immunosuppressive drugs, such as cyclosporin A, in order to blunt the attack of the immune system on transplanted organs. The recent efforts to use more specific means of inducing tolerance to allografts to circumvent the unwanted side effects of general immunosuppression. The mechanism of action of the immunosuppressive agents varies, although T cells are a common target. In addition, cytotoxic drugs or radiation treatments given to treat various forms

of cancer frequently damage the dividing cells in the body, including those of the immune system, and induce a state of immunodeficiency as an unwanted consequence. Patients undergoing such therapy must be monitored closely and treated with antibiotics or immunoglobulin if infection appears.

Possible Questions:

1. Explain the types of hyper-sensitivity reactions.
2. What is auto-immune disorders. Explain with suitable example.
3. Elaborate immune response to tumours.
4. What are the various cancer immunotherapy.
5. Define Immunodeficiencies. Elaborate primary and secondary.

UNIT-V

SYLLABUS

Vaccines: Vaccine technology including DNA vaccines, identification of B and T epitopes for vaccine development. Immunodiagnosis of infectious diseases, immune screening of recombinant library.

Vaccines

The great scientist of early vaccination trials were Edward Jenner and Louis Pasteur. Since those pioneering efforts, vaccines have been developed for many diseases that were once major afflictions of mankind. The incidence of diseases such as diphtheria,measles,mumps, pertussis (whooping cough), rubella (German measles), poliomyelitis, and tetanus has declined dramatically as vaccination has become more common. Clearly, vaccination is a cost-effective weapon for disease prevention. Perhaps in no other case have the benefits of vaccination been as dramatically evident as in the eradication of smallpox, one of mankind's long-standing and most terrible scourges. Since October 1977, not a single naturally acquired smallpox case has been reported anywhere in the world. Equally encouraging is the predicted eradication of polio. The last recorded case of naturally acquired polio in the Western Hemisphere occurred in Peru in 1991, and the World Health Organization (WHO) predicts that paralytic polio will be eradicated throughout the world within the next few years. A new addition to the weapons against

childhood disease is a vaccine against bacterial pneumonia, a major cause of infant death. A crying need remains for vaccines against other diseases. Every year, millions throughout the world die from malaria, tuberculosis, and AIDS, diseases for which there are no effective vaccines. It is estimated by the World Health Organization that 16,000 individuals a day, or 5.8 million a year, become infected with HIV-1, the virus that causes AIDS. An effective vaccine could have an immense impact on the control of this tragic spread of death and disaster. In addition to the challenges presented by diseases for which no vaccines exist, there remains the need to improve the safety and efficacy of present vaccines and to find ways to lower their cost and deliver them efficiently to all who need them, especially in developing countries of the world. The WHO estimates that millions of infant deaths in the world are due to diseases that could be prevented by existing vaccines (see Clinical Focus). The road to successful development of a vaccine that can be approved for human use, manufactured at reasonable cost, and efficiently delivered to at-risk populations is costly, long, and tedious. Procedures for manufacture of materials that can be tested in humans and the ways they are tested in clinical trials are regulated closely. Even those candidate vaccines that survive initial scrutiny and are approved for use in human trials are not guaranteed to find their way into common usage. Experience has shown that not every vaccine candidate that was successful in laboratory and animal studies prevents disease in humans. Some potential vaccines cause unacceptable side effects, and some may even worsen the disease they were meant to prevent. Live virus vaccines pose a special threat to those with primary or acquired immunodeficiency. Stringent testing is an absolute necessity, because vaccines will be given to large numbers of well persons. Adverse

side effects, even those that occur at very low frequency, must be balanced against the potential benefit of protection by the vaccine. Vaccine development begins with basic research. Recent advances in immunology and molecular biology have led to effective new vaccines and to promising strategies for finding new vaccine candidates. Knowledge of the differences in epitopes recognized by T cells and B cells has enabled immunologists to begin to design vaccine candidates to maximize activation of both arms of the immune system. As differences in antigen-processing pathways became evident, scientists began to design vaccines and to use adjuvants that maximize antigen presentation with class I or class II MHC molecules. Genetic engineering techniques can be used to develop vaccines to maximize the immune response to selected epitopes and to simplify delivery of the vaccines. This chapter describes the vaccines now in use and describes vaccine strategies, including experimental designs that may lead to the vaccines of the future.

Active and Passive Immunization

Immunity to infectious microorganisms can be achieved by active or passive **immunization**. In each case, immunity can be acquired either by natural processes (usually by transfer from mother to fetus or by previous infection by the organism) or by artificial means such as injection of antibodies or vaccines (Table 18-1). The agents used for inducing passive immunity include antibodies from humans or animals, whereas active immunization is achieved by inoculation with microbial pathogens that induce immunity but do not cause disease or with antigenic components from the pathogens. This section describes current usage of passive and active immunization techniques.

TABLE 18-1 Acquisition of passive and active immunity

Type	Acquired through
Passive immunity	Natural maternal antibody
	Immune globulin*
	Humanized monoclonal antibody
	Antitoxin†
Active immunity	Natural infection
	Vaccines‡
	Attenuated organisms
	Inactivated organisms
	Purified microbial macromolecules
	Cloned microbial antigens
	Expressed as recombinant protein
	As cloned DNA alone or in virus vectors
	Multivalent complexes
	Toxoid§

*An antibody-containing solution derived from human blood, obtained by cold ethanol fractionation of large pools of plasma; available in intramuscular and intravenous preparations.

†An antibody derived from the serum of animals that have been stimulated with specific antigens.

‡A suspension of attenuated live or killed microorganisms, or antigenic portions of them, presented to a potential host to induce immunity and prevent disease.

§A bacterial toxin that has been modified to be nontoxic but retains the capacity to stimulate the formation of antitoxin.

Passive Immunization Involves Transfer of Preformed Antibodies

Jenner and Pasteur are recognized as the pioneers of vaccination, or induction of active immunity, but similar recognition is due to Emil von Behring and Hidesaburo Kitasato for their contributions to passive immunity. These investigators were the first to show that immunity elicited in one animal can be transferred to another by injecting it with serum from the first. Passive immunization, in which preformed antibodies are transferred to a recipient, occurs naturally by transfer of maternal antibodies across the placenta to the developing fetus.

Maternal antibodies to diphtheria, tetanus, streptococci, rubeola, rubella, mumps, and poliovirus all afford passively acquired protection to the developing fetus. Maternal antibodies present in colostrum and milk also provide passive immunity to the infant.

Passive immunization can also be achieved by injecting a recipient with preformed antibodies. In the past, before vaccines and antibiotics became available, passive immunization provided a major defense against various infectious diseases. Despite the risks incurred by injecting animal sera, usually horse serum, this was the only effective therapy for otherwise fatal diseases.

Currently, there are several conditions that warrant the use of passive immunization. These include:

_ Deficiency in synthesis of antibody as a result of congenital or acquired B-cell defects, alone or together with other immunodeficiencies.

_ Exposure or likely exposure to a disease that will cause complications (e.g., a child with leukemia exposed to varicella or measles), or when time does not permit adequate protection by active immunization.

_ Infection by pathogens whose effects may be ameliorated by antibody. For example, if individuals who have not received up-to-date active immunization against tetanus suffer a puncture wound, they are given an injection of horse antiserum to tetanus toxin. The preformed horse antibody neutralizes any tetanus toxin produced by *Clostridium tetani* in the wound.

Passive immunization is routinely administered to individuals exposed to botulism, tetanus, diphtheria, hepatitis, measles, and rabies (Table 18-2). Passively administered antiserum

is also used to provide protection from poisonous snake and insect bites. Passive immunization can provide immediate protection to travelers or health-care workers who will soon be exposed to an infectious organism and lack active immunity to it. Because passive immunization does not activate the immune system, it generates no memory response and the protection provided is transient. For certain diseases such as the acute respiratory failure in children caused by respiratory syncytial virus (RSV), passive immunization is the best preventative currently available. A monoclonal antibody or a combination of two monoclonal antibodies may be administered to children at risk for RSV disease. These monoclonal antibodies are prepared in mice but have been "humanized" by splicing the constant regions of human IgG to the mouse variable regions.

This modification prevents many of the complications that may follow a second injection of the complete mouse antibody, which is a highly immunogenic foreign protein. Although passive immunization may be an effective treatment, it should be used with caution because certain risks are associated with the injection of preformed antibody. If the antibody was produced in another species, such as a horse, the recipient can mount a strong response to the isotypic determinants of the foreign antibody. This anti-isotype response can cause serious complications. Some individuals, for example, produce IgE antibody specific for determinants on the injected antibody. Immune complexes of this IgE bound to the passively administered antibody can mediate systemic mast cell degranulation, leading to systemic anaphylaxis. Other individuals produce IgG or IgM antibodies specific for the foreign antibody, which form complement-activating immune complexes. The deposition of these complexes in the tissues can lead to

type III hypersensitive reactions. Even when human gamma globulin is administered passively, the recipient can generate an anti-allotype response to the human immunoglobulin, although its intensity is usually much less than that of an anti-isotype response.

TABLE 18-2 Common agents used for passive immunization

Disease	Agent
Black widow spider bite	Horse antivenin
Botulism	Horse antitoxin
Diphtheria	Horse antitoxin
Hepatitis A and B	Pooled human immune gamma globulin
Measles	Pooled human immune gamma globulin
Rabies	Pooled human immune gamma globulin
Respiratory disease	Monoclonal anti-RSV*
Snake bite	Horse antivenin
Tetanus	Pooled human immune gamma globulin or horse antitoxin

*Respiratory syncytial virus

Active Immunization Elicits Long-Term Protection

Whereas the aim of passive immunization is transient protection or alleviation of an existing condition, the goal of active immunization is to elicit protective immunity and immunologic memory. When active immunization is successful, a subsequent exposure to the pathogenic agent elicits a heightened immune response that successfully eliminates the pathogen or prevents disease mediated by its products. Active immunization can be achieved by natural infection with a microorganism, or it can be acquired artificially by administration of a **vaccine** (see Table 18-1). In active immunization, as the name implies, the immune system plays an active role—proliferation of antigen-reactive T and B cells results in the formation of memory cells. Active

immunization with various types of vaccines has played an important role in the reduction of deaths from infectious diseases, especially among children. Vaccination of children is begun at about 2 months of age. The recommended program of childhood immunizations in this country, updated in 2002 by the American Academy of Pediatrics, is outlined in Table 18-3. The program includes the following vaccines:

_ Hepatitis B vaccine

_ Diphtheria-pertussis (acellular)-tetanus (DPaT) combined vaccine

_ Inactivated (Salk) polio vaccine (IPV); the oral (Sabin) vaccine is no longer recommended for use in the United States

_ Measles-mumps-rubella (MMR) combined

vaccine _ *Haemophilus influenzae* (Hib) vaccine

_ Varicella zoster (Var) vaccine for chickenpox

_ Pneumococcal conjugate vaccine (PCV); a new addition to the list.

In addition, hepatitis A vaccine at 18 months and influenza vaccines after 6 months are recommended for infants in high-risk populations.

TABLE 18-3 Recommended childhood immunization schedule in the United States, 2002

Vaccine*	AGE: Birth	1 mo	2 mos	4 mos	6 mos	12 mos	15 mos	18 mos	4–6 yrs
Hepatitis B[†]		†							
			†			†			
Diphtheria, tetanus, pertussis[‡]			†	†	†		†		†
H. influenzae type b			†	†	†	†			
Inactivated polio[§]			†	†	†				†
Pneumococcal conjugate			†	†	†	†			
Measles, mumps, rubella						†			†
Varicella[¶]						†			

*This schedule indicates the recommended ages for routine administration of currently licensed childhood vaccines. Bars indicate ranges of recommended ages. Any dose not given at the recommended age should be given as a "catch-up" immunization at any subsequent visit when indicated and feasible.

†Different schedules exist depending upon the HBsAg status of the mother. A first vaccination after the first month is recommended only if the mother is HBsAg negative.

‡"DTaP" (diphtheria and tetanus toxoids and acellular pertussis vaccine) is the preferred vaccine for all doses in the immunization series. Td (tetanus and diphtheria toxoids) is recommended at 11–12 years of age if at least 5 years have elapsed since the last dose.

§Only inactivated poliovirus (IPV) vaccine is now recommended for use in the United States. However, OPV remains the vaccine of choice for mass immunization campaigns to control outbreaks due to wild poliovirus.

¶Varicella (Var) vaccine is recommended at any visit on or after the first birthday for susceptible children, i.e., those who lack a reliable history of chickenpox (as judged by a health-care provider) and who have not been immunized. Susceptible persons 13 years of age or older should receive 2 doses, given at least 4 weeks apart.

SOURCE: Adapted from the ECIIT Web site (see references); approved by the American Academy of Pediatrics.

Designing Vaccines for Active Immunization

Several factors must be kept in mind in developing a successful vaccine. First and foremost, the development of an immune response does not necessarily mean that a state of protective immunity has been achieved. What is often critical is which branch of the immune system is activated, and therefore vaccine designers must recognize the important differences between activation of the humoral and the cell mediated branches. A second factor is the development of immunologic memory. For example, a vaccine that induces a protective primary response may fail to induce the formation of memory cells, leaving the host unprotected after the primary response to the vaccine subsides. The role of memory cells in immunity depends, in part, on the incubation period of the pathogen. In the case of influenza virus, which has a very short incubation period (1 or 2 days), disease symptoms are already under way by the time memory cells are activated. Effective protection against influenza therefore depends on maintaining high

levels of neutralizing antibody by repeated immunizations; those at highest risk are immunized each year. For pathogens with a longer incubation period, maintaining detectable neutralizing antibody at the time of infection is not necessary. The poliovirus, for example, requires more than 3 days to begin to infect the central nervous system. An incubation period of this length gives the memory B cells time to respond by producing high levels of serum antibody. Thus, the vaccine for polio is designed to induce high levels of immunologic memory. After immunization with the Salk vaccine, serum antibody levels peak within 2 weeks and then decline, but the memory response continues to climb, reaching maximal levels at 6 months and persisting for years (Figure 18-3). If an immunized individual is later exposed to the poliovirus, these memory cells will respond by differentiating into plasma cells that produce high levels of serum antibody, which defend the individual from the infection. In the remainder of this chapter, various approaches to the design of vaccines—both currently used vaccines and experimental ones—are described, with an examination of their ability to induce humoral and cell-mediated immunity and the production of memory cells.

Whole-Organism Vaccines

As Table 18-4 indicates, many of the common vaccines currently in use consist of inactivated (killed) or live but attenuated (avirulent) bacterial cells or viral particles.

TABLE 18-4 Classification of common vaccines for humans

Disease or pathogen	Type of vaccine
WHOLE ORGANISMS	
Bacterial cells	
Anthrax	Inactivated
Cholera	Inactivated
Pertussis*	Inactivated
Plague	Inactivated
Tuberculosis	Live attenuated BCG†
Typhoid	Live attenuated
Viral particles	
Hepatitis A	Inactivated
Influenza	Inactivated
Measles	Live attenuated
Mumps	Live attenuated
Polio (Sabin)	Live attenuated
Polio (Salk)	Inactivated
Rabies	Inactivated
Rotavirus	Live attenuated
Rubella	Inactivated
Varicella zoster (chickenpox)	Live attenuated
Yellow fever	Live attenuated
PURIFIED MACROMOLECULES	
Toxoids	
Diphtheria	Inactivated exotoxin
Tetanus	Inactivated exotoxin
Capsular polysaccharides	
Haemophilus influenzae type b	Polysaccharide + protein carrier
Neisseria meningitidis	Polysaccharide
Streptococcus pneumoniae	23 distinct capsular polysaccharides
Surface antigen	
Hepatitis B	Recombinant surface antigen (HBsAg)

*There is an new also an acellular pertussis vaccine consisting of toxoids and inactivated bacteria components.

†Bacillus Calmette-Guerin (BCG) is an avirulent strain of *Mycobacterium bovis*.

The primary characteristics of these two types of vaccines are compared in Table 18-5 to one another and to DNA vaccines that are currently being tested for use in humans.

TABLE 18-5 Comparison of attenuated (live), inactivated (killed), and DNA vaccines

Characteristic	Attenuated vaccine	Inactivated vaccine	DNA vaccine
Production	Selection for avirulent organisms: virulent pathogen is grown under adverse culture conditions or prolonged passage of a virulent human pathogen through different hosts	Virulent pathogen is inactivated by chemicals or irradiation with γ-rays	Easily manufactured and purified
Booster requirement	Generally requires only a single booster	Requires multiple boosters	Single injection may suffice
Relative stability	Less stable	More stable	Highly stable
Type of immunity induced	Humoral and cell-mediated	Mainly humoral	Humoral and cell-mediated
Reversion tendency	May revert to virulent form	Cannot revert to virulent form	Cannot revert

Attenuated Viruses and Bacteria Cause Immunity Without Disease

In some cases, microorganisms can be attenuated so that they lose their ability to cause significant disease (pathogenicity) but retain their capacity for transient growth within an inoculated host. Attenuation often can be achieved by growing a pathogenic bacterium or virus for prolonged periods under abnormal culture conditions. This procedure selects mutants that are better suited to growth in the abnormal culture conditions and are therefore less capable of growth in the natural host. For example, an attenuated strain of*Mycobacterium bovis* called **Bacillus Calmette-Guerin (BCG)** was developed by growing *M. bovis* on a medium containing increasing concentrations of bile. After 13 years, this strain had adapted to growth in strong bile and had become sufficiently attenuated that it was suitable as a vaccine for tuberculosis. The Sabin polio vaccine and the measles vaccine both consist of attenuated viral strains. The poliovirus used in the Sabin vaccine was attenuated by growth in monkey kidney epithelial cells. The measles vaccine contains a strain of rubella virus that was grown in duck embryo cells and later in human cell lines. Attenuated vaccines have advantages and disadvantages. Because of their capacity for transient growth, such vaccines provide prolonged immune-system exposure to the individual epitopes on the attenuated organisms, resulting in increased immunogenicity and production of memory cells. As a consequence, these vaccines often require only a single immunization, eliminating the need for repeated boosters. This property is a major advantage in Third World countries, where epidemiologic studies have shown that roughly 20% of individuals fail to return for each subsequent booster. The ability of

many attenuated vaccines to replicate within host cells makes them particularly suitable for inducing a cellmediated response.

The Sabin polio vaccine, consisting of three attenuated strains of poliovirus, is administered orally to children on a sugar cube or in sugar liquid. The attenuated viruses colonize the intestine and induce protective immunity to all three strains of virulent poliovirus. Sabin vaccine in the intestines induces production of secretory IgA, which serves as an important defense against naturally acquired poliovirus. The vaccine also induces IgM and IgG classes of antibody. Unlike most other attenuated vaccines, which require a single immunizing dose, the Sabin polio vaccine requires boosters, because the three strains of attenuated poliovirus in the vaccine interfere with each other's replication in the intestine. With the first immunization, one strain will predominate in its growth, inducing immunity to that strain. With the second immunization, the immunity generated by the previous immunization will limit the growth of the previously predominant strain in the vaccine, enabling one of the two remaining strains to predominate and induce immunity. Finally, with the third immunization, immunity to all three strains is achieved.

A major disadvantage of attenuated vaccines is the possibility that they will revert to a virulent form. The rate of reversion of the Sabin polio vaccine (OPV) leading to subsequent paralytic disease is about one case in 2.4 million doses of vaccine. This reversion implies that pathogenic forms of the virus are being passed by a few immunized individuals and can find their way into the water supply, especially in areas where sanitation standards are not rigorous or where waste water must be recycled. This possibility has led to the exclusive use of the inactivated polio vaccine in this country (see Table 18-3). The projected eradication of paralytic polio (Figure 18-

4) will be impossible as long as OPV is used anywhere in the world. The alternative inactivated Salk vaccine should be substituted as the number of cases decrease, although there are problems in delivering this vaccine in developing countries. Attenuated vaccines also may be associated with complications similar to those seen in the natural disease. A small percentage of recipients of the measles vaccine, for example, develop post-vaccine encephalitis or other complications. However, the risk of vaccine related complications is much lower than risks from infection. An independent study showed that 75 million doses of measles vaccine were given between 1970 and 1993, with an incidence of 48 cases of vaccine-related encephalopathy. The low in-cidence of this side effect compared with the rate of encephalopathy associated with infection argues for the efficacy of the vaccine. A more convincing argument for vaccination is the high death rate associated with measles infection even in developed countries.

Genetic engineering techniques provide a way to attenuate a virus irreversibly by selectively removing genes that are necessary for virulence. This has been done with a herpesvirus vaccine for pigs, in which the thymidine kinase gene was removed. Because thymidine kinase is required for the virus to grow in certain types of cells (e.g., neurons), removal of this gene rendered the virus incapable of causing disease. It is possible that similar genetic engineering techniques could eliminate the risk of reversion of the attenuated polio vaccine. More recently, a vaccine against rotavirus, a major cause of infant diarrhea, was developed using genetic engineering techniques to modify an animal rotavirus to contain antigens present on the human viruses.

Pathogenic Organisms Are Inactivated by Heat or Chemical Treatment

Another common approach in vaccine production is inactivation of the pathogen by heat or by chemical means so that it is no longer capable of replication in the host. It is critically important to maintain the structure of epitopes on surface antigens during inactivation. Heat inactivation is generally unsatisfactory because it causes extensive denaturation of proteins; thus, any epitopes that depend on higher orders of protein structure are likely to be altered significantly. Chemical inactivation with formaldehyde or various alkylating agents has been successful. The Salk polio vaccine is produced by formaldehyde inactivation. Attenuated vaccines generally require only one dose to induce long-lasting immunity. Killed vaccines, on the other hand, often require repeated boosters to maintain the immune status of the host. In addition, killed vaccines induce a predominantly humoral antibody response; they are less effective than attenuated vaccines in inducing cell-mediated immunity and in eliciting a secretory IgA response.

Even though they contain killed pathogens, inactivated whole-organism vaccines are still associated with certain risks. A serious complication with the first Salk vaccines arose when formaldehyde failed to kill all the virus in two vaccine lots, which caused paralytic polio in a high percentage of recipients.

DNA Vaccines

In a recently developed vaccination strategy, plasmid DNA encoding antigenic proteins is injected directly into the muscle of the recipient. Muscle cells take up the DNA and the encoded protein antigen is expressed, leading to both a humoral antibody response and a cell-mediated response. What is most surprising about this finding is that the injected DNA is taken up and expressed by the muscle cells with much greater efficiency than in tissue culture. The DNA

appears either to integrate into the chromosomal DNA or to be maintained for long periods in an episomal form. The viral antigen is expressed not only by the muscle cells but also by dendritic cells in the area that take up the plasmid DNA and express the viral antigen. The fact that muscle cells express low levels of class I MHC molecules and do not express costimulatory molecules suggests that local dendritic cells may be crucial to the development of antigenic responses to DNA vaccines (Figure 18-6). DNA vaccines offer advantages over many of the existing vaccines. For example, the encoded protein is expressed in the host in its natural form—there is no denaturation or modification. The immune response is therefore directed to the antigen exactly as it is expressed by the pathogen. DNA vaccines also induce both humoral and cell-mediated immunity; to stimulate both arms of the immune response with non-DNA vaccines normally requires immunization with a live attenuated preparation, which introduces additional elements of risk. Finally, DNA vaccines cause prolonged expression of the antigen, which generates significant immunological memory. The practical aspects of DNA vaccines are also very promising (Table 18-5). Refrigeration is not required for the handling and storage of the plasmid DNA, a feature that greatly lowers the cost and complexity of delivery. The same plasmid vector can be custom tailored to make a variety of proteins, so that the same manufacturing techniques can be used for different DNA vaccines, each encoding an antigen from a different pathogen. An improved method for administering these vaccines entails coating microscopic gold beads with the plasmid DNA and then delivering the coated particles through the skin into the underlying muscle with an air gun (called a *gene gun*). This will allow rapid delivery of avaccine to large populations without the requirement for huge supplies of needles

and syringes. Tests of DNA vaccines in animal models have shown that these vaccines are able to induce protective immunity against a number of pathogens, including the influenza virus. It has been further shown that the inclusion of certain DNA sequences in the vector leads to enhanced immune response. At present, there are human trials underway with several different DNA vaccines, including those for malaria, AIDS, influenza, and herpesvirus. Future experimental trials of DNA vaccines will mix genes for antigenic proteins with those for cytokines or chemokines that direct the immune response to the optimum pathway. For example, the IL-12 gene may be included in a DNA vaccine; expression of IL-12 at the site of immunization will stimulate TH1-type immunity induced by the vaccine. DNA vaccines will likely be used for human immunization within the next few years. However, they are not a universal solution to the problems of vaccination; for example, only protein antigens can be encoded—certain vaccines, such as those for pneumococcal and meningococcal infections, use protective polysaccharide antigens.

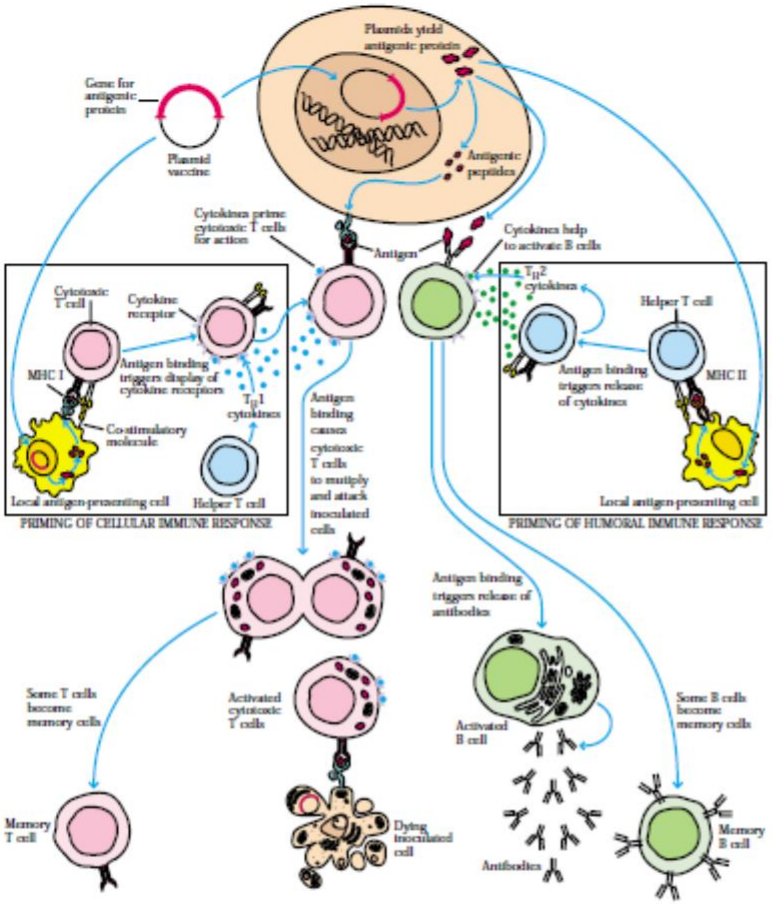


FIGURE 18-6 Use of DNA vaccines raises both humoral and cellular immunity. The injected gene is expressed in the injected muscle cell and in nearby APCs. The peptides from the protein encoded by the DNA are expressed on the surface of both cell types after processing as an endogenous antigen by the MHC class I pathway. Cells that present the antigen in the context of class I MHC molecules stimulate development of cytotoxic T cells. The protein encoded by the injected DNA is also expressed as a soluble, secreted protein, which is taken up, processed, and presented in the context of class II MHC molecules. This pathway stimulates B-cell immunity and generates antibodies and B-cell memory against the protein. *(Adapted from D. B. Weiner and R. C. Kennedy, 1999, Sci. Am. 281:50.)*

Multivalent Subunit Vaccines

One of the limitations of synthetic peptide vaccines and recombinant protein vaccines is that they tend to be poorly

immunogenic; in addition, they tend to induce a humoral antibody response but are less likely to induce a cell-mediated response. What is needed is a method for constructing synthetic peptide vaccines that contain both immunodominant B-cell and T-cell epitopes. Furthermore, if a CTL

response is desired, the vaccine must be delivered intra-cellularly so that the peptides can be processed and presented together with class I MHC molecules. A number of innovative techniques are being applied to develop multivalent vaccines that can present multiple copies of a given peptide or a mixture of peptides to the immune system (Figure 18-7). One approach is to prepare solid matrix–antibodyantigen (SMAA) complexes by attaching monoclonal antibodies to particulate solid matrices and then saturating the antibody with the desired antigen. The resulting complexes are then used as vaccines. By attaching different monoclonal antibodies to the solid matrix, it is possible to bind a mixture of peptides or proteins, composing immunodominant epitopes for both T cells and B cells, to the solid matrix (see Figure 18-7a). These multivalent complexes have been shown to induce vigorous humoral and cell-mediated responses. Their particulate nature contributes to their increased immunogenicity by facilitating phagocytosis by phagocytic cells. Another means of producing a multivalent vaccine is to use detergent to incorporate protein antigens into protein micelles,

lipid vesicles (called liposomes), or immunostimulating complexes (see Figure 18-7b). Mixing proteins in detergent and then removing the detergent forms micelles. The individual proteins orient themselves with their hydrophilic

residues toward the aqueous environment and the hydrophobic residues at the center so as to exclude their interaction with the aqueous environment. Liposomes containing protein antigens are prepared by mixing the proteins with a suspension of phospholipids under conditions that form vesicles bounded by a bilayer. The proteins are incorporated into the bilayer with the hydrophilic residues exposed. Immunostimulating complexes (ISCOMs) are lipid carriers

prepared by mixing protein with detergent and a glycoside called Quil A. Membrane proteins from various pathogens, including influenza virus, measles virus, hepatitis B virus, and HIV have been incorporated into micelles, liposomes, and ISCOMs and are currently being assessed as potential vaccines. In addition to their increased immunogenicity, liposomes and ISCOMs appear to fuse with the plasma membrane to deliver the antigen intracellularly, where it can be processed by the cytosolic pathway and thus induce a cell-mediated response (see Figure 18-7c).

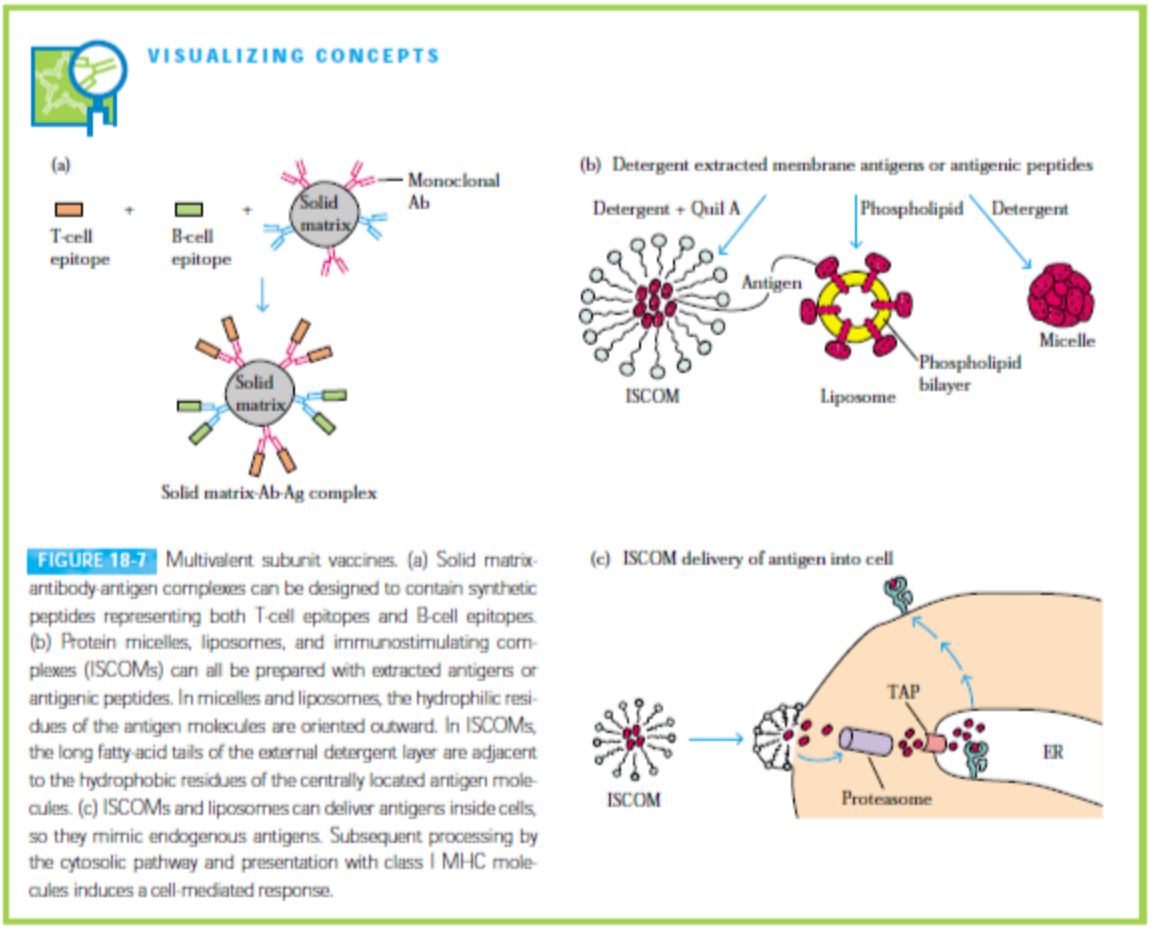


FIGURE 18-7 Multivalent subunit vaccines. (a) Solid matrix-antibody-antigen complexes can be designed to contain synthetic peptides representing both T-cell epitopes and B-cell epitopes. (b) Protein micelles, liposomes, and immunostimulating complexes (ISCOMs) can all be prepared with extracted antigens or antigenic peptides. In micelles and liposomes, the hydrophilic residues of the antigen molecules are oriented outward. In ISCOMs, the long fatty-acid tails of the external detergent layer are adjacent to the hydrophobic residues of the centrally located antigen molecules. (c) ISCOMs and liposomes can deliver antigens inside cells, so they mimic endogenous antigens. Subsequent processing by the cytosolic pathway and presentation with class I MHC molecules induces a cell-mediated response.

Enzyme-Linked Immunosorbent Assay

Enzyme-linked immunosorbent assay, commonly known as **ELISA** (or EIA), is similar in principle to RIA but depends on an enzyme rather than a radioactive label. An enzyme conjugated with an antibody reacts with a colorless substrate to generate a colored reaction product. Such a substrate is called **a chromogenic substrate.** A number of enzymes have been employed for ELISA, including alkaline phosphatase, horseradish peroxidase, and _-galactosidase. These assays approach the sensitivity of RIAs and have the advantage of being safer and less costly.

There Are Numerous Variants of ELISA

A number of variations of ELISA have been developed, allowing qualitative detection or quantitative measurement of either antigen or antibody. Each type of ELISA can be used qualitatively to detect the presence of antibody or antigen. Alternatively, a standard curve based on known concentrations of antibody or antigen is prepared, from which the unknown concentration of a sample can be determined.

INDIRECT ELISA

Antibody can be detected or quantitatively determined with an indirect ELISA (Figure 6-10a). Serum or some other sample containing primary antibody (Ab1) is added to an antigen-coated microtiter well and allowed to react with the antigen attached to the well. After any free Ab1 is washed away, the presence of antibody bound to the antigen is detected by adding an enzyme-conjugated secondary anti-isotype antibody (Ab2), which binds to the primary antibody. Any free Ab2 then is washed away, and a substrate for the enzyme is added. The amount of colored reaction product that forms is measured by specialized spectrophotometric plate readers,

which can measure the absorbance of all of the wells of a 96-well plate in seconds. Indirect ELISA is the method of choice to detect the presence of serum antibodies against human immunodeficiency virus (HIV), the causative agent of AIDS. In this assay, recombinant envelope and core proteins of HIV are adsorbed as solid-phase antigens to microtiter wells. Individuals infected with HIV will produce serum antibodies to epitopes on these viral proteins. Generally, serum antibodies to HIV can be detected by indirect ELISA within 6 weeks of infection.

SANDWICH ELISA

Antigen can be detected or measured by a sandwich ELISA (Figure 6-10b). In this technique, the antibody (rather than the antigen) is immobilized on a microtiter well. A sample containing antigen is added and allowed to react with the immobilized antibody.After the well is washed, a second enzyme- linked antibody specific for a different epitope on the antigen is added and allowed to react with the bound antigen. After any free second antibody is removed by washing, substrate is added, and the colored reaction product is measured. COMPETITIVE ELISA

Another variation for measuring amounts of antigen is competitive ELISA (Figure 6-10c). In this technique, antibody is first incubated in solution with a sample containing antigen. The antigen-antibody mixture is then added to an antigencoated microtiter well. The more antigen present in the sample, the less free antibody will be available to bind to the antigen-coated well. Addition of an enzyme-conjugated secondary antibody (Ab2) specific for the isotype of the primary antibody can be used to determine the amount of primary antibody bound to the well as in an indirect

ELISA. In the competitive assay, however, the higher the concentration of antigen in the original sample, the lower the absorbance.

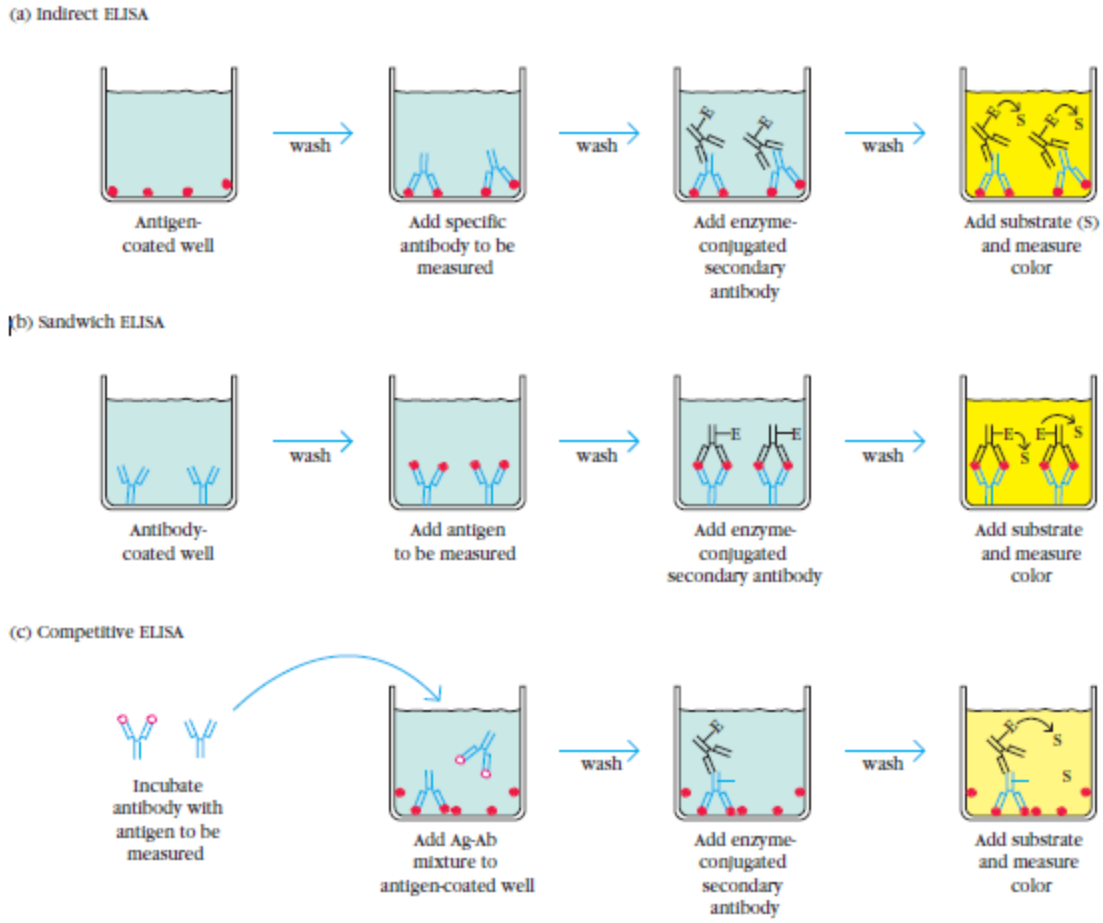


FIGURE 6-10 Variations in the enzyme-linked immunosorbent assay (ELISA) technique allow determination of antibody or antigen. Each assay can be used qualitatively, or quantitatively by comparison with standard curves prepared with known concentrations of antibody or antigen. Antibody can be determined with an indirect ELISA (a), whereas antigen can be determined with a sandwich ELISA (b) or competitive ELISA (c). In the competitive ELISA, which is an inhibition-type assay, the concentration of antigen is inversely proportional to the color produced.

CHEMILUMINESCENCE

Measurement of light produced by chemiluminescence during certain chemical reactions provides a convenient and highly sensitive alternative to absorbance measurements in ELISA

assays. In versions of the ELISA using chemiluminescence, a luxogenic (light-generating) substrate takes the place of the chromogenic substrate in conventional ELISA reactions.

For example, oxidation of the compound luminol by H2O2 and the enzyme horseradish peroxidase (HRP) produces light:

luminol _ H2O2 Ab-HRP _ Ag □→Ab-HRP-Ag □□□□□→light

The advantage of chemiluminescence assays over chromogenic ones is enhanced sensitivity. In general, the detection limit can be increased at least ten-fold by switching from a chromogenic to a luxogenic substrate, and with the addition of enhancing agents, more than 200-fold. In fact, under ideal conditions, as little as 5 _ 10_18 moles (5 attomoles) of target antigen have been detected.

ELISPOT ASSAY

A modification of the ELISA assay called the ELISPOT assay allows the quantitative determination of the number of cells in a population that are producing antibodies specific for a given antigen or an antigen for which one has a specific antibody (Figure 6-11). In this approach, the plates are coated with the antigen (capture antigen) recognized by the antibody of interest or with the antibody (capture antibody) specific for the antigen whose production is being assayed. A suspension of the cell population under investigation is then added to the coated plates and incubated. The cells settle onto the surface of the plate, and secreted molecules reactive with the capture molecules are bound by the capture molecules in the vicinity of the secreting cells, producing a ring of antigen-antibody complexes around each cell that is producing the molecule of interest. The plate is then washed and an enzyme-linked antibody specific for the secreted

antigen or specific for the species (e.g., goat anti-rabbit) of the secreted antibody is added and allowed to bind. Subsequent development of the assay by addition of a suitable chromogenic or chemiluminescence-producing substrate reveals the position of each antibody- or antigen-producing cell as a point of color or light.

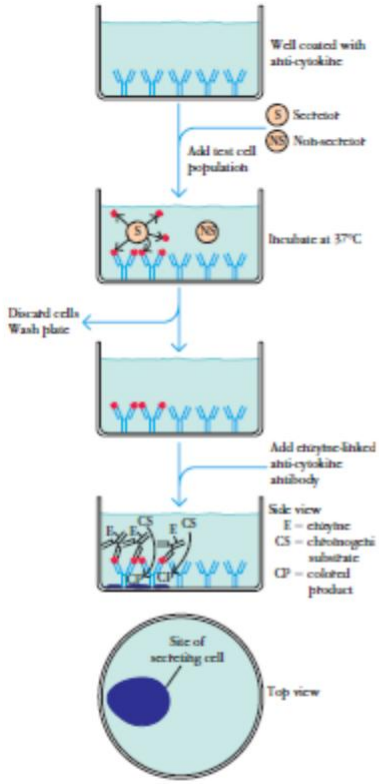


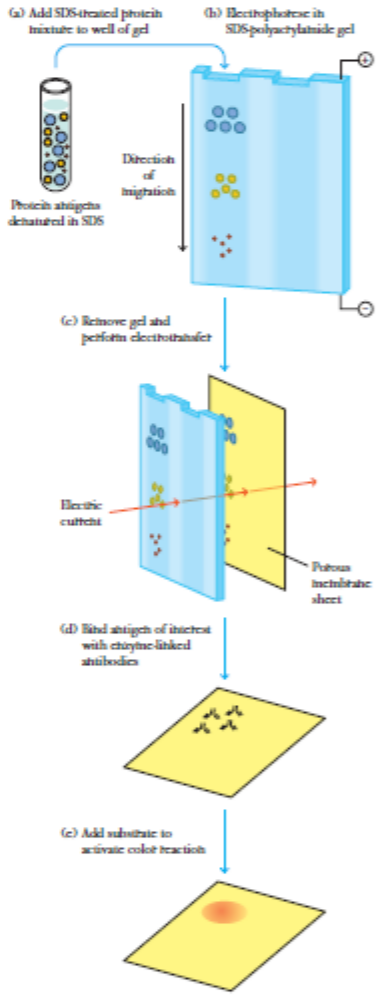
FIGURE 6-11 In the ELISPOT assay, a well is coated with antibody against the antigen of interest, a cytokine in this example, and then a suspension of a cell population thought to contain some members synthesizing and secreting the cytokine are layered onto the bottom of the well and incubated. Most of the cytokine molecules secreted by a particular cell react with nearby well-bound antibodies. After the incubation period, the well is washed and an enzyme-labeled anti-cytokine antibody is added. After washing away unbound antibody, a chromogenic substrate that forms an insoluble colored product is added. The colored product (purple) precipitates and forms a spot only on the areas of the well where cytokine-secreting cells had been deposited. By counting the number of colored spots, it is possible to determine how many cytokine-secreting cells were present in the added cell suspension.

Western Blotting

Identification of a specific protein in a complex mixture of proteins can be accomplished by a technique known as **Western blotting,** named for its similarity to Southern blotting, which detects DNA fragments, and Northern blotting, which detects mRNAs. In Western blotting, a protein mixture is electrophoretically separated on an **SDS-polyacrylamide gel (SDS-PAGE),** a slab gel infused with sodium dodecyl sulfate (SDS), a dissociating agent (Figure 6-12). The protein bands are transferred to a nylon membrane by electrophoresis and the individual protein bands are identified by flooding the nitrocellulose membrane with radiolabeled or enzymelinked polyclonal or monoclonal antibody specific for the protein of interest. The Ag-Ab complexes that form on the band containing the protein recognized by the antibody can be visualized in a variety of ways. If the protein of interest was bound by a radioactive antibody, its position on the blot can be determined by exposing the membrane to a sheet of x-ray film, a procedure called autoradiography. However, the most generally used detection procedures employ enzyme-linked antibodies against the protein. After binding of the enzyme antibody conjugate, addition of a chromogenic substrate that produces a highly colored and insoluble product causes the appearance of a colored band at the site of the target antigen. The site of the protein of interest can be determined with much higher sensitivity if a chemiluminescent compound along with suitable enhancing agents is used to produce light at the antigen site.

Western blotting can also identify a specific antibody in a mixture. In this case, known antigens of well-defined molecular weight are separated by SDS-PAGE and blotted onto nitrocellulose.

The separated bands of known antigens are then probed with the sample suspected of containing antibody specific for one or more of these antigens. Reaction of an antibody with a band is detected by using either radiolabeled or enzyme-linked secondary antibody that is specific for the species of the antibodies in the test sample. The most widely used application of this procedure is in confirmatory testing for HIV, where Western blotting is used to determine whether the patient has antibodies that react with one or more viral proteins.



Immunoprecipitation

The immunoprecipitation technique has the advantage of allowing the isolation of the antigen of interest for further analysis. It also provides a sensitive assay for the presence of a particular antigen in a given cell or tissue type. An extract produced by disruption of cells or tissues is mixed with an antibody against the antigen of interest in order to form an antigen-antibody complex that will precipitate. However, if the antigen concentration is low (often the case in cell and tissue extracts), the assembly of antigen-antibody complexes into precipitates can take hours, even days, and it is difficult to isolate the small amount of immunoprecipitate that forms.

Fortunately, there are a number of ways to avoid these limitations. One is to attach the antibody to a solid support, such as a synthetic bead, which allows the antigen-antibody complex to be collected by centrifugation. Another is to add a secondary antibody specific for the primary antibody to bind the antigen-antibody complexes. If the secondary antibody is attached to a bead, the immune complexes can be collected by centrifugation. A particularly ingenious version of this procedure involves the coupling of the secondary antibody to magnetic beads. After the secondary antibody binds to the primary antibody, immunoprecipitates are collected by placing a magnet against the side of the tube (Figure 6-13). When used in conjunction with biosynthetic radioisotope labeling, immunoprecipitation can also be used to determine whether a particular antigen is actually synthesized by a cell or tissue. Radiolabeling of proteins synthesized by cells of interest can be done by growing the cells in cell-culture medium containing one or more radiolabeled amino acids. Generally, the amino acids used for this application are those most resistant to metabolic modification, such as leucine, cysteine, or methionine. After growth in the

radioactive medium, the cells are lysed and subjected to a primary antibody specific for the antigen of interest. The Ag-Ab complex is collected by immunoprecipitation, washed free of unincorporated radiolabeled amino acid and other impurities, and then analyzed. The complex can be counted in a scintillation counter to obtain a quantitative determination of the amount of the protein synthesized. Further analysis often involves disruption of the complex, usually by use of SDS and heat, so that the identity of the immunoprecipitated antigen can be confirmed by checking that its molecular weight is that expected for the antigen of interest. This is done by separation of the disrupted complex by SDS-PAGE and subsequent autoradiography to determine the position of the radiolabeled antigen on the gel.

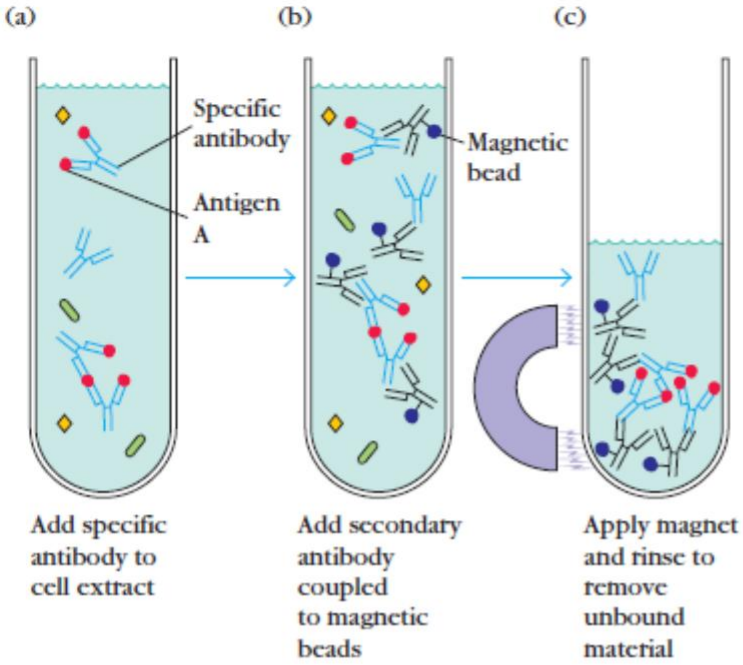


FIGURE 6-13 Immunoprecipitates can be collected using magnetic beads coupled to a secondary antibody. (a) Treatment of a cell extract containing antigen A (red) with a mouse anti-A antibody (blue) results in the formation of antigen-antibody complexes. (b) Addition of magnetic beads to which a rabbit anti-mouse antibody is linked binds the antigen-antibody complexes (and any unreacted mouse Ig). (c) Placing a magnet against the side of the tube

Possible Questions:

1. Elaborate DNA vaccines
2. Write a detailed account on epitopes for vaccine development.
3. What are the various immunodiagnosis of infectious diseases
4. Explain immuno screening of recombinant library