Immune System

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Abstract

Immunity is the ability of an individual to protect against invading pathogens. The machineries utilized by the immune system are intended to recognize pathogens, their destruction, and a memory to remember previous exposure. The immunity is either an inborn reflex to eliminate pathogens (innate immunity) or developed slowly after the exposure of pathogens (acquired immunity). The components of innate immunity are anatomic and physiological barriers, immune effector cells, and soluble factors like antimicrobial peptides, complement system, cytokines, and interferons. The acquired immunity is either cell mediated associated with T lymphocytes or humoral, brought about by the antibodies produced from B lymphocytes. The pattern recognition receptors (PRRs) present in the immune effector cells recognize pathogens through pathogen-associated molecular pattern (PAMP) and damage-associated molecular pattern (DAMP). The toxins released by the pathogens are processed and presented by antigen-presenting cells (APC) with the help of major histocompatibility complex (MHC) as a mark of discrimination between self and nonself. After pathogen recognition, the effector responses such as phagocytosis, complement activation, and antibody-dependent cell-mediated cytotoxicity are initiated to eliminate the pathogens. The incoordination in the different immune components leads to immune pathology such as hypersensitivity, anaphylactic shock, and autoimmune disorders.

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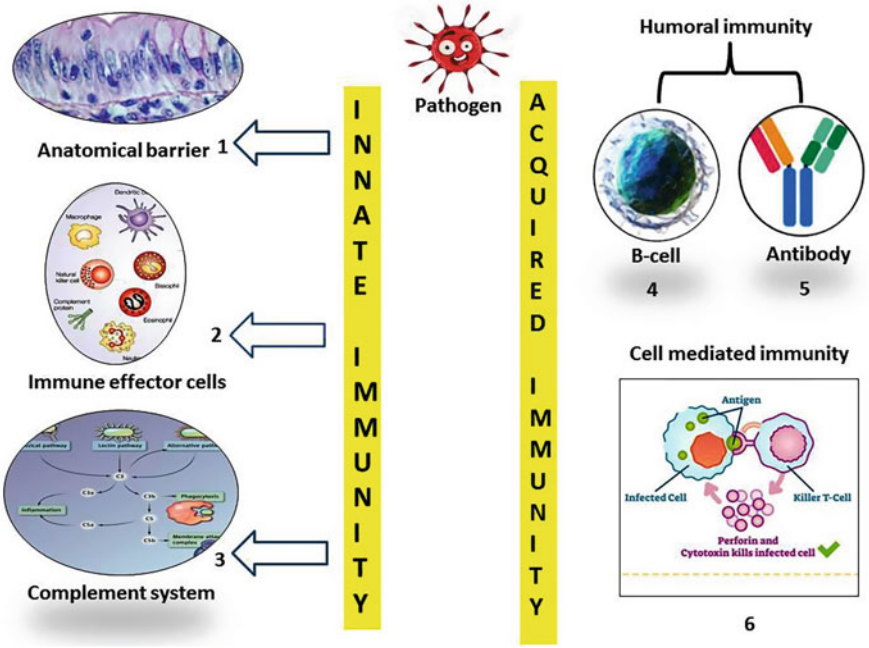
Graphical Abstract



Description of the graphic: Immunity is the ability of the body to protect against pathogens. It has two important components such as innate and adaptive immunity. The innate immune system is equipped with several components like anatomical and physiological barriers (1), immune effector cells and cell surface receptors (2), and complement system (3). The adaptive immunity is of two types, humoral and cell-mediated immunity. The humoral immune response is mediated through B lymphocytes (4) capable of producing antibodies (5), which bind with antigens and make them vulnerable for destruction. The cell-mediated immune response is mediated through T lymphocytes that produce signals to activate phagocytic cells to destroy them (6)

Keywords

Antigens · Innate immunity · Adaptive immunity · Antibodies · Autoimmune disorders

Learning Objectives

- The components of immunity
- Antigens and their types
- Components of innate and adaptive immune system
- Hypersensitivity and immunological disorders

5.1 Immunity

The word immunity was derived from Latin word "immunis," meaning "exempt." Immunity is the ability of an individual to protect against invading pathogenic microorganisms and cancer through interlinking networks of cellular and biochemical mechanisms collectively known as the immune system. Two key features of the immune system are the recognition and response. Immune recognition is a highly specific process to discriminate between foreign pathogens from the body's own cells and proteins and to identify different types of foreign pathogens through their specific chemical compositions. The responses are in two ways. Firstly, the immune system neutralizes or eliminates the pathogens through a series of cellular and biochemical reactions once the recognition is completed. This is called an effector response. The immune system has the ability to remember previous exposure of foreign organisms and rapidly eliminates the pathogen after its second exposure through a rapid and strong protection called memory response.

5.1.1 Classification of Immunity

The immunity can be classified on the basis of recognition of antigens or developmental and hereditary characteristics. On the basis of immune recognition, immunity can be classified into specific and nonspecific immunity. Nonspecific immunity responds equally to all pathogens to eliminate them. In

Table 5.1 Factors determining the antigenicity

Foreignness	To elicit an immune response, an antigen must be treated as a foreign substance to the individual.
Chemical nature	Proteins have good immunogenic response followed by polysaccharides and lipopolysaccharides. The nucleic acids are poor immunogenic. The lipids usually lack immunogenicity.
Molecular size	The molecules having a molecular mass of 100,000 Da have good immunogenic potential. Substances with a molecular mass less than 5000–10,000 Da have poor immunogenicity.
Physical form	Particulate and denatured antigens are more immunogenic compared to soluble and native form, respectively.
Genetic factor	Some antigens are able to induce immunogenicity in a particular species. Bovine serum albumin is nonimmunogenic to cow, poorly immunogenic to goat, but strongly immunogenic to chicken.
Dose of the antigen	All antigens require a particular dose to elicit immune response. The optimal immune response will not be achieved above or below this dose level.
Route of administration	Intravenous administration of antigens carried first to spleen but antigens administered carried first to local lymph nodes. Therefore, subcutaneous route is better compared to intravenous route.
Age	Very young or very poor individuals have diminished immunogenic response against an antigen.
Degradability	To elicit an immune response, the antigens must be processed by antigen-presenting cells. Therefore, antigens that can be easily phagocytosed are having good immunogenic response.

contrast, specific immunity uses different strategies for different microorganisms to neutralize them. On the basis of hereditary features, immunity can be classified as innate and adaptive immunity. The innate immunity is inborn and present before the onset of infection. Adaptive immunity comes into action after the pathogenic exposure. The innate immunity thus provides the first line of defense against invading pathogen and is less specific. In contrast, adaptive immunity is highly specific. The adaptive immune responses developed slowly after the exposure of pathogens or foreign materials, but a rapid response against pathogens was achieved in innate immune system that destroys the pathogens during the first critical hours of its exposure. One of the striking features of adaptive immunity is the memory, which means the initial exposure of any pathogen results in memory response and a quick and stronger response is achieved after the second exposure of the same pathogen.

5.1.2 Antigens

The molecules that induce humoral and cell-mediated immune response are called immunogens, and their ability to induce immune response is termed as immunogenicity. Immunogens after binding with B cells generate activated B cells to produce antibody. The binding of antigens with T-cell receptors leads to activation of T cells (cytotoxic T lymphocytes). The term antigens are not synonymous with immunogens. Antigens are the molecules that bind specifically with products of immune response induced by immunogens (i.e., antibodies or cytotoxic T lymphocytes), and this property is called antigenicity. An antigen may not be immunogenic if it fails to induce an immune response. Certain molecules like haptens (discussed later) can bind with T or B cells but fail to activate them. An antigen is said to be immunogen only when it can be able to induce an immune response (activation of humoral or cell-mediated immune response). Thus, it can be said that all immunogens are antigens, but not all antigens are immunogens. Some body cells like sperm and corneal cells may cause immunoreaction if injected in the same animal.

5.1.2.1 Determinants of Antigenicity/Immunogenicity

There are several factors that determine the antigenicity (Table 5.1).

5.1.2.2 Types of Antigens

The antigens can be classified on the basis of their source and immune response.

5.1.2.2.1 Classification Based on Source

Exogenous antigens: They enter inside the body from outside and are processed by antigen-presenting cells. Some antigens are exogenous at the beginning but later become endogenous, e.g., viral antigens.

Endogenous antigens: These antigens are produced within the body. They are usually body's own cells or cell products such as corneal tissue, blood group antigens, and HLA (histocompatibility leukocyte antigens). There are several types of endogenous antigens like autoantigens (person's own self antigens, e.g., thyroglobulin, corneal tissue, DNA) and alloantigens (found in different members of the same species, e.g., red blood cell antigens A and B).

5.1.2.2.2 Classification Based on Immune Response

Complete Antigen or Immunogen: They are capable to induce immune response by their own. They are usually proteins or polysaccharide in nature having a molecular weight above 10,000 Da.

Incomplete Antigen or Hapten: They are low-molecular-weight (below 10,000 Da) substances that react with its corresponding antibody but unable to induce immune

Table 5.2 Different components of innate and adaptive immunity

Immunity	Anatomical barrier	Physiological barrier	Cellular	Humoral
Innate immunity	Skin and mucous membrane	Body temperature, pH	Dendritic cells, granulocytes, mononuclear phagocytic system (MPS), dendritic cells (DCs), innate lymphoid cells (ILCs), natural killer (NK) cells, epithelial and endothelial cells, platelets, pattern recognition receptors	Inflammatory serum proteins/acute-phase proteins (APPs), antimicrobial peptides (AMPs), complement system, cytokines, interferons
Adaptive immunity			B lymphocytes, T lymphocytes	Antibodies

response by their own. Their immunogenic property can by augmented by carrier molecules (albumin or globulin), e.g., pneumococcal capsular polysaccharide, polysaccharide C of *Streptococci*, and cardiolipin antigens.

Superantigens: These antigens are able to activate a large proportion of T cells (up to 25%) in comparison to conventional antigens that are able to induce only 1–2% T cells. The superantigens cause hyperactivation of immune system. Pyrogenic exotoxins (lead to shock) and enterotoxins (lead to food poisoning) of *Staphylococci* are the examples of superantigens.

5.1.2.3 Epitopes

Epitopes are the antigenic determinants, a small site of an antigen that can activate immune response by activating T or B cells. Based on the specificity of binding, epitopes can either be B-cell epitopes or T-cell epitopes. B-cell epitopes bind with antibody, and T-cell epitopes bind with T-cell receptor after presented with MHC molecules by antigen-presenting cells. The properties of an epitope are required to design vaccines.

5.1.2.4 Adjutants

Adjuvants are the substances that enhance the immunogenicity of an antigen by one of the following mechanisms:

- Acting as a depot and leading to sustained release of an antigen from the site of delivery
- Stimulating the production of cytokines and chemokines
- Facilitating the recruitment of immune effector cells at the site of delivery
- Helping in antigen uptake and further processing and presentation by antigen-presenting cells
- Activating inflammatory mediators

The common examples of adjuvants are Freund's complete adjuvant (containing inactivated *Mycobacterium tuberculosis* in oil, alum nonionic surfactants, and muramyl peptides). There are adjuvants licensed for human use such as MF59, AS04, and virosomes.

5.1.3 Components of Immune System

The immune system is equipped with several components like anatomical and physiological barriers, immune effector cells and cell surface receptors, inflammatory serum proteins, antimicrobial peptides, and antibodies to inhibit the entry pathogens, which resist the establishment of infection along with clearing of host and microbial debris from the site of infection. Some of the components are cellular, and some components circulate freely in the body fluids called humoral factors. Table 5.2 summarizes different components of innate and adaptive immunity.

5.2 Innate Immunity

Innate immunity is the evolutionary defensive reflex against foreign materials owned by birth. The response is nonspecific in nature. It serves as the first line of defense to present infection. Therefore, the dysfunction of the innate immune system leads to life-threatening infections or development of autoimmune disorders. Innate immunity also helps to develop adaptive immune responses. There are several components of innate immunity.

5.2.1 Anatomical Barriers

The skin and mucosal membrane restrict the entry of pathogens. The epidermis of the skin contains tightly packed epithelial cells inside and dead cells and keratin in the outside. The keratin is bacteriostatic due to the presence of esterified and nonesterified fatty acids like myristic acid, palmitoleic acid, and linoleic acid. It also contains cationic proteins that make alterations in the cell wall of pathogens making them more prone to osmotic damage. Keratinocytes in the skin also express pattern recognition receptors (PRRs) to recognize pathogens and produce cytokines and antimicrobial peptides. The sebaceous glands present in the dermis layer contain lactic acid and fatty acids and maintain the skin

pH acidic that also restricts the growth of many pathogens. The tight junctions present in the epithelial surface of the skin, lung, guts, and urogenital tracts also provide physical barrier against the pathogens. The mucous layer present at the interior of the epithelial surfaces also provides protective covering against invading pathogens. Mucin and other glycoproteins secreted in the mucous layer prevent the adherence of pathogen to the epithelium and are subsequently cleared by the cilia. The antimicrobial peptides defensins present in the mucosal layer also kill the pathogens.

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Influenza virus has a unique ability to bind tightly with the mucous membrane of the respiratory tract due to the presence of a surface molecule, which enables them to escape the ciliary action of the epithelial cells.

5.2.2 Physiological Barriers

The physiological barriers of pathogens include body temperature, pH, and several other soluble factors. An increase of core body temperature to the tune of 1–4 °C was proved to be detrimental to many pathogens. Some animals have inherent capabilities to resist infections due to their high body temperature (e.g., chicken is naturally resistant to anthrax). The pyrogenic response helps to induce certain cytokines (IL-6) that help in lymphocyte trafficking. "Gastric bactericidal barrier" comprising gastric HCl has the ability to inactivate microorganisms entered during ingestion. Lysozyme of saliva and tears has the ability to cleave the cell wall of bacteria. Virus-infected cells produce interferons, a group of signaling proteins that improve the antiviral defense of neighboring cells.

5.2.3 Immune Effector Cells

These are phagocytic cells (granulocytes, monocytes/macrophages, natural killer cells, and dendritic cells), endothelial cells, epithelial cells, lymphoid cells, and platelets. The phagocytes engulf pathogens and kill them by oxygen-dependent and oxygen-independent mechanism.

Granulocytes: Neutrophils, eosinophils, basophils, and mast cells are collectively known as granulocytes due to their granular cytoplasm. These cells are involved in pathogen recognition, engulfment, and phagocytosis. They possess a variety of microbicidal enzymes like lysozyme, collagenase, and elastase.

Mononuclear Phagocyte System (MPS): MPS consists of circulating monocytes and tissue macrophages. Monocytes after maturation migrate to the tissue and differentiate into tissue macrophages, which have more intracellular organelles and increased phagocytic capabilities with higher hydrolytic enzymes compared to monocytes. There are several tissue macrophages named according to their tissue locations such as histiocytes in skin, alveolar macrophages in lungs, Kupffer cells in liver, mesangial cells in kidney, microglial cells in CNS, and osteoclasts in bone. Tissue macrophages serve a variety of functions like phagocytes and antigen-presenting cells. They also have tissue-remodeling capacity through the secretion of matrix metalloproteinases and matrix proteins like collagen and elastin. Cytotoxic factors secreted by macrophages help in tumor immunity. There are three classes of macrophages.

Type 1 activated macrophages are concerned with Th 1 immune response and destroy pathogens by nitric oxide (NO) and oxygen-dependent phagocytosis.

The alternatively activated macrophages are unable to produce NO and hence lack phagocytic properties. They produce extracellular matrix proteins and are mainly involved in tissue remodeling.

Type 2 activated macrophages are stimulated in response to IgG and secret IL-10, IL-4, TNF-α, and IL-6.

Dendritic cells (DCs): These are the antigen-presenting cells that reside in the skin and mucosal surfaces. They take the antigen by means of endocytosis, phagocytosis, pinocytosis, and macropinocytosis; carry the antigen from peripheral lymph nodes; and present it to primary lymph nodes. The antigen processing and presentation by dendritic cells are achieved through major histocompatibility complex II. Other important functions of dendritic cells include regulation of cell-mediated immune response and induction of immune tolerance at peripheral lymph nodes. Immature DCs (imDCs) and precursor dendritic cells (pre-DCs) are the two subsets of dendritic cells. imDCs are seen in bone marrow as their precursors are hematopoietic stem cells. A portion of the imDCs then migrate to the epidermis of the skin and become Langerhans cells, while other portions migrate to the dermis and other tissues and differentiate into interstitial imDCs. The mature dendritic cells are potent T-cell activators and inform T cells about the information of pathogens; thus, dendritic cells act as a bridge between innate and adaptive immune response.

Innate lymphoid cells (ILCs): These cells are involved in inflammation. They do not have antigen specificity due to lack of T-cell receptor or any other cell surface markers. Their primary role is to produce cytokines. They are subdivided into three groups. Group I cells comprise ILC1 and natural killer (NK) cells and produce type 1 cytokines. Group 2 ILCs are abundant in liver, spleen, mesenteric lymph nodes, and Peyer's patches. They produce type 2 cytokines and are associated with

anthelminthic response. Group III ILCs are lymphoid tissue inducer (LTi). They are mostly present in mucosal tissue and maintain a cross talk between intestinal microbiota and gut immune system. The disruption of homeostasis between gut microbiota and gut immune system leads to severe inflammatory bowel diseases like colitis and Crohn's disease.

Natural killer (NK) cells: NK cells are responsible for cell-mediated immune response due to their cytotoxic activity. They possess a unique property called "negative recognition." The surface receptors of NK cells are inhibitory receptors. These receptors suppress the cytotoxic activity of NK cells in the presence of MHC antigens, and when the infected or malignant cells have decreased expression of MHC antigen, they are recognized by NK cells and undergo cell lysis by perforins secreted from NK cells.

Epithelial and endothelial cells: They express PRRs that recognize pathogen-associated molecular patterns (PAMPs) of pathogens. In addition, they also secrete cytokines like IL-1, IL-6, and IL-8 and antimicrobial peptides.

Platelets: Platelets are the components of blood coagulation mechanism but they also express PRR on their surface and secrete cytokines and chemokines to recruit leukocytes at the inflammatory sites. Platelets can interact with endothelium and leukocytes by P-selectin, an adhesion molecule, and initiate pro-inflammatory events.

5.2.4 Pattern Recognition Receptors

The PRRs are able to sense the pathogen-associated molecular pattern (PAMP), conserved molecular pattern of a pathogen. They are subdivided into four classes.

Toll-like receptors (TLRs) are expressed on all immune effector cells. They are able to recognize external pathogen-associated molecular patterns (PAMPs) and internal damage-associated molecular patterns (DAMPs). Till date, around ten TLRs have been identified. Some of them are expressed on cell surface (TLR-1, 2, 4, 5, and 6), and some are intracellular and localized in endoplasmic reticulum (ER), endosomes, and lysosomes (TLR-3, 7, 8, 9, and 10). The intracellular TLRs are also called nucleic acid sensors due to their ability to sense dsRNA and ssRNA of the pathogens.

C-type lectin receptors (CLRs) are mainly recognized bacterial sugar moieties but are able to identify molecules associated with dead cells. They are of two types, membrane CLRs like Dectin-1 and -2 and soluble CLRs like collectins. The ligands of CLRs are β-glucans, mannose, oligosaccharides, and other microbial carbohydrates.

The nucleotide-binding oligomerization domain (NOD) receptors (NLRs) are intracellular PRRs that recognize peptidoglycans and DAMPs and induce synthesis and secretion of cytokines.

Retinoic acid inducible gen-I (RIG)-like receptors (RLRs) are also intracellular PRRs mainly responsible for antiviral immune response. They have the ability to sense viral dsRNA.

5.2.5 Inflammatory Serum Proteins/Acute-Phase Proteins (APPs)

There are several proteins that act as the mediators or inhibitors of inflammatory process. They are also called acute-phase proteins (APPs). They are mainly synthesized in the liver and their concentrations are increased (or decreased) at the rate of 25% or more at the time of inflammation. They therefore act as a suitable biomarker of inflammation. There are two classes of APPs, viz. positive APPs and negative APPs. The concentrations of positive APPs are increased during inflammation (within 1–2 days). Based on the degree of increment, positive APPs can be categorized as major (usually present in very low concentration but may increase up to 100–1000-fold within 24–48 h and rapidly decline thereafter), moderate (increase five- to tenfold within 48–72 h and decrease at a slower rate than major APPs), or minor (increase only 50–100% above basal levels at a gradual rate). The concentrations of negative acute-phase proteins decrease by 25% upon inflammation within 24 h. Albumin and transferrin are the two main negative APPs. The species variations in terms of major and minor APPs along with their functions have been depicted in Table 5.3.

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Hp and SAA can be used as markers of early detection of subclinical mastitis in cows.

5.2.6 Antimicrobial Peptides (AMPs)

They are used by many organisms as the first line of defense against pathogens. They are multifunctional peptides with bacteriostatic, bactericidal, and cytolytic properties. They are promptly synthesized after infection and kill a wide range of pathogens. Various AMPs along with their functions have been presented in Table 5.4.

Table 5.3 Species variations in terms of major and minor APPs along with their functions

Acute-phase proteins	Functions
Positive APPs	
Haptoglobin (Hp) (Major APP in cow and minor APP in horse, pig, cat, dog, and mice)	Carries free hemoglobin after extravascular hemolysis Inhibits chemotaxis and phagocytosis
Serum amyloid A (SAA) (Major APP in cow, horse, cat, dog, and mice)	Recruits inflammatory cells at the site of inflammation via chemotaxis Stimulates the secretion of pro-inflammatory cytokines Inhibits lymphocyte proliferation Helps in lipid transport
Ceruloplasmin (Cp) (Minor APP in dog and pig)	Helps in copper transport Stimulates wound healing by collagen formation and maturation Acts as an antioxidant Inhibits endothelial attachment of neutrophils
C-reactive protein (CRP) (Minor APP in dog and pig)	Promotes bacterial attachment with complement Induces phagocytosis Stimulates cytokine release Inhibits chemotaxis
Alpha-1-acid glycoprotein (AGP) (Minor APP in cat, dog, cow, and mouse)	Anti-inflammatory agent Decreases neutrophil functions
Negative APPs	
Albumin	Regulates colloidal osmotic pressure Reduces albumin production during inflammation and increases the amino acid availability for production of positive APPs
Transferrin	Iron transport protein Decreases free iron for bacterial survival
Adiponectin	Regulates energy status of an animal Anti-inflammatory agent

Table 5.4 Antimicrobial peptides and their functions

Antimicrobial peptides	Source	Functions
Lactoferrin	Mucous membrane, biological fluids like tears, colostrum, milk, and semen	Lactoferrin binds with the lipopolysaccharide of bacterial cell wall and chelates iron (Fe^{3+}) to permeabilize membrane and cell breakdown
Lysozyme	Body secretions like tears, saliva, and milk Also produced by neutrophils and macrophages	Hydrolysis of 1,4-β-glycosidic linkages between *N*-acetylmuramic acid and *N*-acetyl-D-glucosamine of cell wall peptidoglycan
Defensins	Neutrophils, monocytes, macrophages, keratinocytes, paneth cells including mucosa of respiratory, digestive, urinary, reproductive systems	Promotes phagocytosis, chemotactic activity, cytokine production, degranulating mast cells
Histidine-rich glycoprotein (HRG)	Liver, monocytes, macrophages, and megakaryocytes	Antiangiogenic and antitumor properties, chemotaxis, cytokine production
Major basic protein (MBP)	Granules of eosinophils	Antibacterial, antihelminthic, and cytotoxic properties, induces hypersensitivity reactions
RNase 7	Skin	Broad-spectrum antimicrobial activity

5.2.7 The Complement System

The complement system is one of the major components of the innate immune response composed of several interlinked proteins that serves a wide array of functions like pathogen recognition, regulation inflammatory processes, killing of the pathogen, and removal of damaged cells. Another major function of complement system is the regulation of adaptive immune responses. Thus, complement system acts as a bridge between innate and adaptive immune responses.

5.2.7.1 Components of Complement System

The complement system consists of several proteins synthesized primarily from liver, macrophages, and neutrophilic granules. In 1963, when the complement system was first discovered, it consisted of only nine proteins labeled by the letter "C" followed by the numbers and their activated forms were designated by added symbol "a" (C1a is an activated form of C1). Till then, a variety of proteins have been identified under complement system. As per the last nomenclature recommended by the International

Complement Society (ICS), Complement Nomenclature Committee, and European Complement Network (ECN) boards, there were 50 different proteins and protein complexes (for derailed review, refer Kemper et al. 2014). The complement proteins comprise 5–10% of total plasma proteins. The sizes of complement proteins vary from 24 (D) to 460 kDa (C1q).

5.2.7.2 Mechanism of Action of Complement System

The complement system remains inactive in an uninfected animal. They can be activated either by PAMP or through antigen-bound antibodies. The activation leads to a series of reaction cascades, which ultimately produce a key protein named C3b.

5.2.7.2.1 Activation

There are three different mechanisms by which complement can be activated.

Alternative pathway: The main regulatory protein of alternate pathway is C3, which is synthesized in liver and macrophages. It has the highest abundance in serum among the complement components. The alternate pathway operates through three major steps, initiation, amplification, and regulation. In the initiation process, C3 proteins undergo autoactivation by a process called "tickover." The "tickover" of C3 facilitates the conformational changes in C3 and generates C3(H_2O), which in turn binds with another factor B. The C3(H_2O)·B complex undergoes cleavage by another serine protease named factor D. Factor D cleaves factor B into Ba and Bb. Bb itself acts as another serine protease that cleaves C3 into its active form C3b. Once C3b is generated, it again associates with factor B to generate more C3 and the proteolytic cycle continues. Another serum protein named properdin stabilizes these protein:protein interactions during the amplification process.

Lectin pathway: This lectin pathway activates after the recognition of oligosaccharide molecules on the surface of pathogen. There are five types of pattern recognition proteins (PRPs) that specifically bind with oligosaccharide moiety of pathogens, namely mannan-binding lectin (MBL), collectin-11 (CL-11), ficolin-1, ficolin-2, and ficolin-3. The first two PRPs bind with glucose, mannose, and *N*-acetyl-glucosamine, whereas ficolins recognize acetyl groups of bacterial membrane glycoproteins like *N*-acetyl-glycine, *N*-acetyl-cysteine, and acetyl-choline. The PRPs are complexed with MBL-associated serine proteases (MASPs), namely MASP-1, MASP-2, and MASP-3. The binding of PRP with the carbohydrate moiety results in the activation of MASPs. Activated MASP-2 splits C4 into C4a and C4b. Complement factor C2 then binds with C4b to form a complex called C4b2. The bound C2 then undergoes cleavage by MASP-2 and yields C4b2b. The protease C4b2b then splits C3 into C3a and C3b.

Classical pathway: Unlike alternative and lection pathways that involve innate immune response, the classical pathway of complement activation acts in conjunction with adaptive immune response as it is induced by antigen and antibody complexes along with other proteins like CRP, amyloid proteins, and apoptotic bodies. The major complement proteins of classical pathway are C1, C2, C4, C1 inhibitor (C1-Inh), and C4-binding protein (C4bp). The classical pathway activates when C1 binds with Fc portion of an Ag-Ab complex. C1 proteins have three subunits C1q, C1r, and C1s. C1q is like a strand, and two molecules each of C1r and C1s are located between the C1q strand. To become active, at least two C1q strands have to bind with antibody molecules. The interaction leads to conformational change in C1q, which activates C1r. C1r acts as C1s and exposes its active site to convert C1s subunits as an active enzyme. C1s then cleaves C4 into C4a and C4b. C4b acts as a receptor for C2, and C4b-bound C2 acts as a substrate for C1s and cleaves into C2a and C2b. C2a being smaller diffuses into the plasma, and larger C2b remains attached with C4b. This C4b-C2b complex cleaves C3 into C3a and C3b.

5.2.7.2.2 Amplification

The C3b thus produced by three pathways then interacts with complement factor C5 to become C3b5, which is cleaved by C3bBb into C5a and C3b5b. Factors C6 and C7 then join with C3b5b to form C5b67. Formed C5b67 then interacts with C8 to form C5b678 and further C5b6789 after combining with C9. C5b6789 is the terminal complement complex (TCC) or membrane attack complex (MAC), which forms a hole in the microbial cell membrane and induces osmotic lysis of the microbes.

5.2.7.2.3 Regulation

The regulation of alternate pathway is facilitated by factor H and factor I. Factor H blocks the binding of factor B to factor C3b, and factor I inactivates C3b to iC3b. The sialic acid blocks the alternate pathway by inducing the binding of factor H with C3b, and microorganisms lacking sialic acid are killed, but the host cells that possess a sialoglycoprotein named glycophorin A are protected. The classical pathway is regulated through C1 inactivator (C1-INH), a glycoprotein that blocks C1r and C1s. CD55 or decay accelerating factor present in all blood corpuscles and endothelial cells binds with C3 and C5 convertases and induces their decay, thus protecting the normal cells from complement attack. The

Table 5.5 Functions of different complement components

Function	Complement components
Lysis	The lytic complex (C5b6789) ruptures bacterial cell membrane
Opsonization and phagocytosis	C3b and C4b have opsonizing potential and C3b-coated microorganisms bind with CRI of phagocytes and undergo phagocytosis
Chemotaxis	Complement-derived chemotactic factors are C3a: Attracts eosinophils C5a: Chemotactic for macrophages, neutrophils, and eosinophils C567: Attracts neutrophils and eosinophils Bb: Attracts neutrophils
Activation of mast cells	C3a, C4a, and C5a activate mast cells to release histamine, and heparin causes vasodilation and increased tissue permeability
Removal of apoptotic cells	Apoptotic cells lack CD46 and CD59 complement inhibitors and bind with C1q to activate classical pathway and subsequently undergo phagocytosis
Inflammation	Complement-derived C3a and C5a stimulate the production of pro-inflammatory cytokines like TNF-α, IL-1β, and IL-6
Blood coagulation	C5a inhibits fibrinolysis and induces blood coagulation by augmenting the expression of tissue factors and plasminogen activator inhibitor
Regulation of immune functions	The adaptive immune response is increased by C3d

C4-binding protein (C4BP) in the plasma inhibits C3 convertase (C4b2a). CD35 (CR1) expressed in RBCs, phagocytic cells, T and B cells, kidney podocytes, and peripheral nerves helps to clear immune complexes and presents complement activation. There are some regulatory proteins namely protectin (CD59), clusterin, and vitronectin that interfere with TCC formation.

5.2.7.2.4 Functions of Complement System

Upon activation, complement system generates a wide array of products that ultimately leads to lysis of pathogens but the products of complement cascade have a wide range of functions detailed in Table 5.5.

5.2.8 Cytokines

Cytokines are the low-molecular-weight (smaller than 30 kDa) proteins or glycoproteins synthesized from leukocytes or other cells of the body and act as soluble mediators to regulate immunity. The cytokines exert its action after binding with its receptors in the target cells and subsequently activate the intracellular signaling cascade that ultimately alters the gene expression of target cells and causes differentiation, proliferation, and activation of the target cells. The cytokines act in autocrine, paracrine, and endocrine fashion.

5.2.8.1 Properties of Cytokines

The cytokines have properties like the following:

Pleiotropy: When a cytokine has different effects on different types of target cells, the cytokine is said to be pleiotropic. IL-4 produced from activated T_H cells causes proliferation, differentiation, and activation of B cells but only proliferates thymocytes and macrophages.

Redundancy: When two or more cytokines exert similar effect on single target cells, the effect is said to be redundant. IL-2, IL-4, and IL-5 produced from T_H cells cause proliferation of B cells.

Synergy: It is the cooperative effect of cytokines. Here, the cytokines in combinations have more pronounced effect compared to their individual effect. IL-4 and IL-5 produced from T_H cells induce B cells to produce IgE, but neither IL-4 nor IL-5 has individual effect to induce B cells for IgE production.

Antagonism: When the effect of a cytokine is inhibited by another cytokine, then the effect is called antagonism. The effect of IL-4 on B cells is inhibited by IFN-γ.

Cascade reaction: When one cytokine induces a target cell to produce one or more cytokines that in turn stimulates another target cell to produce other cytokines, it is called cascade reaction. IFN-γ produced from activated T_H cells stimulates macrophages to secrete IL-12 that in turn stimulates activated TH cells for IFN-γ, TNF, and IL-2 secretion.

5.2.8.2 Classification of Cytokines

There are six different cytokine families, namely interleukins, chemokines, interferons, tumor necrosis factors (TNF), colony-stimulating factors (CSF), and transforming growth factor-β. There are several subgroups under different families.

Interleukins: They are so named with a thought that it was synthesized by leukocytes but later, it was found that interleukins can be produced from a variety of cells. They play a pivotal role in hematopoiesis, activation,

and differentiation of immune cells. They also have pro-inflammatory properties and help in leukocyte migration and adhesions. Till date, 40 interleukins have been identified and named alphabetically from IL-1 to IL-40.

Chemokines: These are chemotactic cytokines and attract the leukocytes to the site of infection. Structurally, chemokines are subdivided into four families based on the N-terminal cysteine residue.

CC chemokines: They have two adjacent cysteine residues at N-terminal region. Twenty-eight CC chemokines have been identified (named from CCL-1 to CCL-28) so far, and majority of them are chemotactic for monocytes.

CXC chemokines: They are characterized by two cysteine residues separated by an amino acid at the N-terminus. There are 17 CXC cytokines, namely CXCL-1 to CXC-17. They attract neutrophils at the site of infection.

C chemokines: They have two cysteine residues, one at N-terminal region and another at downstream. There are two C chemokines (XCL-1 and XCL-2).

CX3C chemokines: CX3C chemokines are having two cysteine residues at N-terminus separated by three amino acids. Besides their role in chemotaxis, they are also involved in cell adhesion. Till date, a single CX3C chemokine has been identified (CX3CL1).

Interferons (IFN): They emerged as antiviral proteins, but later, their roles in immunomodulation and cancer immunology have been identified. IFNs are classified into three types, type I (IFN-α and IFN-β), type II (IFN-γ), and type III (IFN-λ1, 2, and 3).

Tumor necrosis factor (TNF): TNF is produced from activated natural killer (NK) cells, macrophages, and T lymphocytes with diverse physiological functions in cell proliferation, differentiation, and carcinogenesis. It is also a pro-inflammatory cytokine. The TNF is classified into TNF-α and TNF-β. TNF-α is produced from monocytes, macrophages, and T cells and has functions as inflammatory mediators and cell adhesions. TNF-β is produced mainly from activated lymphocytes and has functions similar to TNF-α.

Colony-stimulating factor (CSF): These are responsible for differentiation of leukocytes in the bone marrow. There are four families of CSF, namely granulocyte–colony-stimulating factor (G-CSF), macrophage–colony-stimulating factor (M-CSF), granulocyte–macrophage-colony-stimulating factor (GM-CSF), and multiple-colony-stimulating factor (also called IL 3). M-CSF is responsible for differentiation of macrophage precursors. G-CSF is responsible for differentiation of granulocyte precursors. GM-CSF is produced from lymphoid and nonlymphoid cells and helps in maturation and differentiation of both granulocytes and monocytes. The role of multiple-colony-stimulating factors is the differentiation of hematopoietic stem cells into myeloid progenitor cells. There are two other CSF like erythropoietin and thrombopoietin.

Transforming growth factor-β (TGF-β): It can be produced from a variety of cells including T cells and monocytes. The main function of TGF-β is the inhibition of cellular growth and production of extracellular matrix. TGF-β also acts as a negative regulator of T-cell and macrophage activation.

5.2.8.3 Pro- and Anti-inflammatory Cytokines

The cytokines secreted in response to an infection mainly by the macrophages and that upregulate the inflammatory response are called pro-inflammatory cytokines. They are pyrogenic in nature and stimulate acute-phase reactions. IL-1 and TNF-α are predominant pro-inflammatory cytokines. The anti-inflammatory cytokines are responsible for the downregulation of inflammatory process. IL-6 is a potent anti-inflammatory cytokine that inhibits the effects of IL-1 and TNF-α. IL-4 and IL-10 are also anti-inflammatory cytokines. The overproduction of pro-inflammatory cytokines compared to anti-inflammatory cytokines leads to autoimmune diseases.

5.2.8.4 Mechanism of Action of Cytokines

The cytokines exert its effects after binding with target cell receptors. There are six classes of cytokine receptors, namely type I cytokine receptors, type II cytokine receptors, chemokine receptors, tumor necrosis factor receptor (TNFR) superfamily, TGF-beta receptors, and immunoglobulin (Ig) superfamily. The receptors are associated with tyrosine kinases called Janus kinases (JAKs) and transcription factors called signal transducer and activator of transcription (STAT). The binding of cytokine with its receptor leads to the activation of JAK by phosphorylation. Phosphorylated JAK combines with STAT, and the complex then moves to nucleus, binds with DNA regulatory site, and activates transcription. The transcription of DNA causes protein synthesis, and the response of cytokine is generated.

5.2.8.5 Functions of Cytokines

The functions of different cytokines, their sources, and target cells have been detailed in Table 5.6.

5.2.8.6 Pathogen Recognition and Inflammatory Signaling in Innate Immune System

Innate immune system, being the first line of defense, recognizes the pathogen during the initial stage of infection and subsequently eliminates the pathogens from the host body. To achieve this, the innate immune system utilizes pattern recognition receptors (PRRs) that recognize pathogen-associated molecular patterns (PAMPs), distinct structure present on the pathogen (Table 5.7).

Table 5.6 Cytokines, their sources, target cells, and functions

Name	Source	Target cells	Functions
Interleukin-1	Macrophages, monocytes, B lymphocytes, endothelium	Macrophages, neutrophils, NK cells, endothelium, and hypothalamus	• Chemotaxis of macrophages and neutrophils • Stimulation of B cells to produce antibody • Stimulation of NK cells to destroy pathogens • Stimulation of endothelium to secrete vasoactive peptides to increase vascular permeability • Stimulation of nervous system to induce including fever, anorexia, and fatigue • Stimulation of hepatic cells for production of acute-phase proteins
Interleukin-2	T_H cells	T cells, NK cells	• Stimulation of T-cell proliferation and NK cell activity
Interleukin-3	T_H cells, mast cells, and NK cells	Bone marrow, mast cells	• Stimulation of leukocyte and erythrocyte production • Stimulation of mast cell to release histamine
Interleukin-4	T_H cells, mast cells, and NK cells	B cells and macrophages	• Stimulates the differentiation of B cells into plasma cells • Stimulation of MHC expression
Interleukin-5	T_H cells, mast cells	B cells and eosinophils	• Stimulates the differentiation of B cells into plasma cells • Stimulates the proliferation and differentiation of eosinophils
Interleukin-6	Macrophages, monocytes, T_H cells, and bone marrow cells	B cells, monocytes, macrophages, hypothalamus, and hepatic cells	• Stimulates the differentiation of B cells into plasma cells • Stimulation of nervous system to induce including fever, anorexia, and fatigue • Stimulation of hepatic cells for production of acute-phase proteins
Interleukin-7	Macrophages, thymus, and bone marrow	Bone marrow stem cells and neutrophils	• Stimulates hemopoietic stem cells into progenitor B and T cells • Chemotaxis of neutrophils
Interleukin-8	Macrophages	Neutrophils	• Chemotaxis of neutrophils
Interleukin-9	T_H cells	Select T cells	• Stimulation of select T cells
Interleukin-10	T_H cells	Macrophages, APC	• Inhibits IL-1 synthesis • Downregulation of the expression of MHC
Interleukin-11	Bone marrow	PHSC, hepatocytes	• Growth and differentiation of PHSC • Produces APP
Interleukin-12	Macrophages, B cells	Cytotoxic T cells, NK cells	• Regulates T-cell response • Stimulates NK cell proliferation
Interleukin-13	T_H cells	Macrophages	• Inhibits pro-inflammatory cytokine production • Stimulates proliferation of NK cells and T cells
Interleukin-15	T cells	T cells, B cells, NK cells, and intestinal epithelial cells	• Stimulates proliferation of T cells, B cells, and gut epithelium • Stimulates cytokine production
Interleukin-16	T cells	T_H cells, eosinophils	• Induces MHC expression • Chemotaxis of eosinophils
Interleukin-17	T_H cells	Bone marrow, macrophages, splenocytes, and synovial cells	• Stimulates the release of TNF-α • Stimulates cytokine production • Stimulates the proliferation of granulocytes
Interleukin-18	Monocytes, macrophages	NK cells, monocytes, macrophages, and T_H cells	• Stimulates the release of cytokines like TNF-α, IL-1, IL-8, and IFN-γ
Tumor necrosis factor-alpha (TNF-α)	Macrophages	Tumor cells, monocytes, and macrophages	• Destroys the tumor cells • Stimulates the release of IL-1, IL-2, IL-6
Tumor necrosis factor-beta (TNF-β)	T cells	Tumor cells, neutrophils, and macrophages	• Destroys the tumor cells • Stimulates phagocytosis • Controls fatigue, pyrexia, and anorexia

(continued)

Table 5.6 (continued)

Name	Source	Target cells	Functions
Interferon-alpha (IFN-α)	WBC	Uninfected cells and hypothalamus	• Inhibits viral replication • Stimulates sickness behavior
Interferon-beta (IFN-β)	Fibroblasts	Uninfected cells	• Inhibits viral replication
Interferon-gamma (IFN-γ)	T cells and NK cells	Uninfected cells, macrophages, and B cells	• Inhibits viral replication • Increases the expression of MHC • Activates macrophages

Table 5.7 Pathogen-associated molecular pattern and pathogen recognition receptor (PRR)

Pathogens	PAMPs	PRRs
Viruses	Surface glycoproteins	TLR2 and TLR4
	Viral DNA	TLR9
	Viral ssRNA	TLR7, TLR8, and RIG-I
	Viral dsRNA	RLRs, TLR3, and NLRs
Gram (+) bacteria	Peptidoglycans	TLR2 and NLRs
	Bacterial DNA	TLR9 and NLRs
	Lipoproteins	TLR2
	Lipoteichoic acid	TLR2
Gram (−) bacteria	Bacterial DNA	TLR9 and NLRs
	Porin	TLR2
	Peptidoglycans	TLR2 and NLRs
	Lipopolysaccharide	TLR4
	Flagellin	TLR5
Fungi	Zymosan	TLR2
	β-glycans	TLR2
	Mannan	TLR2 and TLR4
Protozoa	DNA	TLR9
	GPI anchors	TLR2 and TLR4

Besides PAMPs, the PRRs of innate immune system also recognize damage-associated molecular patterns (DAMPs). DAMPs are endogenous molecules released in response to stress or tissue injury and are potent stimulators for noninfectious inflammation. Different DAMPs and their receptors are presented in Table 5.8.

Through the binding of ligands (PAMPs or DAMPs), PRRs are stimulated, facilitate downstream signal transduction, and lead to transcriptional activation of genes for pro-inflammatory cytokines, chemokines, cell adhesion molecule, and IFNs. This pro-inflammatory signaling pathway also activates the adaptive immune response.

5.2.9 Inflammation

It is a complex tissue reaction against tissue damage or pathogenic microorganisms that results in clearance of the invading pathogens through the activation of the components of the innate immune system. Rubor (redness), calor (heat), dolor (pain), and tumor (swelling) are four cardinal signs of inflammation stated by the Roman physician Celsus. These signs can be well explained by the major events of inflammation such as the following:

Vasodilation: It is mediated by nitric oxide (NO) and vasodilatory prostaglandins. Pro-inflammatory cytokines such as IL-1 and TNF-α produced from activated leukocytes stimulate inducible nitric oxide synthase (iNOS) and cyclo-oxygenase (COX-1 and -2). iNOS produces NO from L-arginine. NO in turn causes smooth muscle relaxation. Prostaglandins (PGI2, PGD2, PGE2, and PGF2α) and prostacyclins are the vasodilatory prostaglandins synthesized from arachidonic acid by the action of COX-1 and -2. Both these NO and prostaglandins cause vasodilation, and engorgement of the capillary network leads to redness (rubor) and increased tissue temperature (calor).

Increased capillary permeability: The alteration in the capillary permeability is mediated by the release of certain inflammatory mediators such as histamine, bradykinin, leukotrienes, and platelet-activating factor (PAF). Together, the increased vascular permeability and capillary hydrostatic pressure lead to leakage of protein-rich fluid (exudate) in the interstitium of the inflammatory site. Accumulation of exudates causes edema or swelling that allows the delivery of antibodies and other acute-phase proteins in inflamed site.

Leukocyte migration: See functions of neutrophils.

5.2.10 Phagocytosis

Phagocytosis is the ability to ingest or engulf other cells and particles by some specialized cells called phagocytes. The process was discovered by Élie Metchnikoff (1845–1916) during his studies on some marine organisms. He was the pioneer to develop the concept cellular immunity and was awarded the Nobel Prize in 1908 together with Paul Ehrlich for notable contribution in the field of immunology. In unicellular organisms, phagocytosis is a process of cell nutrition, but for multicellular organisms, it is a means to kill the

Table 5.8 Damage-associated molecular pattern (DAMP) and pathogen recognition receptor (PRR)

Origin	DAMPs	Receptors
Extracellular matrix	Fibronectin	TLR4
	Fibrinogen	TLR4
	Decorin	TLR2, TLR4
	Heparan sulfate	TLR4
Cytosol	Heat-shock proteins	TLR2, TLR4
	S100 proteins	TLR2, TLR4
Nuclear	Histones	TLR2, TLR4
	DNA	TLR9
	RNA	TLR3, TLR7, TLR8, RIG-I
Mitochondria	mtDNA	TLR9
	mROS (reactive oxygen species)	NLRs
Granule	Defensins	TLR4
Plasma membrane	Syndecans	TLR4
	Glypicans	TLR4

pathogen and cellular debris and thus plays an important role in innate immunity as well as tissue homeostasis. Based on these two functions, phagocytes can be classified as preferential phagocytes (neutrophils, macrophages, monocytes, dendritic cells, and osteoclasts) that act to eliminate pathogens. The nonprofessional phagocytes (epithelial cells, fibroblasts, and endothelial cells) are mainly involved in the elimination of apoptotic bodies.

A phagocyte can recognize a pathogen either directly by PRRs present on them or indirectly through some molecules that form a bridge between the phagocyte and the particle to be ingested. These are called opsonins such as antibodies (IgG) and complement components, and the process is called opsonization. The direct PRRs of phagocytes are Dectin-1, mannose receptors, CD14, and scavenger receptor A (SR-A) that recognize polysaccharides, mannans, lipopolysaccharide, and lipoteichoic acid, respectively. Fcγ receptors (FcγR) are the opsonic receptors that bind with the Fc portion of IgG molecules.

After the interaction between phagocytes and ingesting particles, a series of signaling events initiate that leads to the remodeling of membrane and cytoskeleton of the phagocytes to form pseudopods that cover the particle and a depression called phagocytic cup is formed at their point of contact. The target particle is then surrounded by the membrane, and it closes at the distal end to form phagosome. The phagosome thus formed interacts with endosomes and lysosomes and finally fuses to form phagolysosome.

The cytotoxic effects of phagocytes are achieved through oxygen-dependent and oxygen-independent mechanisms. The phagocytes produce a number of reactive oxygen and nitrogen intermediates with potent microbial activity. The production of reactive oxygen and nitrogen intermediates occurs through a metabolic process called respiratory burst, which activates peroxidase enzymes. The reactive oxygen intermediates are superoxide anion ($O_2^{\bullet -}$), hydroxyl radicals ($OH^{\bullet}$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), and monochloramine (NH_2Cl). The reactive nitrogen intermediates are nitric oxide (NO), nitrogen dioxide (NO_2), and nitrous acid (HNO_2). In oxygen-independent mechanism, the killing of pathogens is achieved through defensins, tumor necrosis factor, and hydrolytic enzymes.

5.3 Acquired/Adaptive Immunity

As the name implies, this type of immunity is not by birth rather acquired after previous antigenic exposures. Acquired immune responses are capable of selective elimination of pathogens. Acquired immune responses have some cardinal features like *specificity*; they have the capability to distinguish different classes of microorganisms. *Diversity* is another important feature of acquired immunity by which it can recognize a wide array of antigens and microorganisms. The acquired immune system remembers the initial exposure of an antigen and comes with higher immune resistance during its second exposure by *memory*. It also has a unique ability to *discriminate the self and nonself antigens* and to react accordingly.

5.3.1 Components of Adaptive Immunity

Adaptive immune responses are brought about by different classes of lymphocytes, namely B and T lymphocytes. The immune responses mediated through B lymphocytes are called *antibody mediated or humoral immune response* as B cells are capable of producing antibodies (or immunoglobulins) upon antigenic exposure, which bind with antigens and make them vulnerable for destructions. *The cell-mediated immune responses* are mediated through T

lymphocytes that produce signals to activate phagocytic cells to destroy them.

T cells: They are so named due to their maturation in the thymus after derived from hematopoietic stem cells in bone marrow. They express antigen-binding receptors at their surface called T-cell receptor (TCR). The TCRs are able to recognize the correct antigen fragments after processed and presented through antigen-presenting cells (APC). Dendritic cells, macrophages, fibroblasts, and epithelial cells can act as APCs. These APCs express a surface protein called major histocompatibility complex (MHC) that recognizes self and nonself antigens.

Major histocompatibility complex (MHC): These are the cell surface proteins encoded by a group of genes present in chromosome 6 in humans. The main function of MHC is to discriminate between self and nonself. MHCs are of two types; MHC class I (also known as human leukocyte antigen [HLA]) is expressed in all nucleated cells and is responsible for processing and presentation of endogenous (intracellular) peptides. In contrast, MHC class II expresses only some immune effector cells like macrophages, dendritic cells, and B cells and is responsible for processing and presentation of exogenous (extracellular) peptides. The MHC-antigen complex activates TCR and stimulates the differentiation of T cells into different subsets.

B cells: B cells are matured in the bone marrow (or liver during fetal life), but in birds, the maturation of B lymphocytes takes place in the bursa of Fabricius, a lymphoid organ found near the cloaca. B lymphocytes require antigenic exposure before final maturation after which they become immunocompetent. They have unique antigen-binding receptors and are able to interact directly with the antigens without the involvement of APCs. After interaction with foreign antigens through the receptors, B cells undergo proliferation and differentiations into plasma cells that are capable of producing antibodies. Plasma cells are short lived. Therefore, a portion of B cells are differentiated into long-lived memory B cells, which are able to respond quickly on re-exposure.

5.3.2 Lymphoid Organs

The organs at which lymphocytes are produced and matured are called lymphoid organs. There are two classes of lymphoid organs.

Primary/central lymphoid organs: In primary lymphoid organs, the lymphocytes are produced and undergo maturation. The primary lymphoid organs are thymus, bone marrow, and bursa of Fabricius.

Secondary/peripheral lymphoid organs: In peripheral lymphoid organs, the lymphocytes interact with antigens. They include lymph nodes, spleen, tonsils, and gut and mucosal associated lymphoid tissues.

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Recently, some abnormal lymph node-like structures were identified at the sites of chronic inflammation, cancers, and transplanted organs with graft rejection. These are called tertiary lymphoid organs (TLOs) or ectopic lymphoid structures (ELSs). They secrete a cytokine called lymphotoxin β (LTb), which induces the differentiation of the stromal cells into lymphoid organs. In some cases, the development of TLO at the site of tumor showed better prognosis.

5.3.2.1 Primary Lymphoid Organs

Thymus: It is a bilobed structure situated above the heart. The lobes are encapsulated and are divided into lobules by connective tissue strands called trabeculae. The lobules are divided into outer cortex and inner medulla. There are stromal cells between the cortex and medulla composed of epithelium, dendritic cells, and macrophages. The thymic epithelial cells are also called nurse cells that surround the thymocytes in the cortex.

Thymus is mainly responsible for maturation of T cells. The progenitors of T cells derived from PHSC enter into the thymus as thymocytes and become immunoreactive and antigen-competent T cells. During the course of the development of thymocytes, they express antigen-binding receptors, and the T cells capable of recognizing foreign antigens and MHC molecule will be selected and released. The selection involves two steps. In the first step, there is positive selection of T cells that recognize self MHC. The T cells that are unable to recognize self MHC molecule will undergo apoptosis. In the second step (negative selection), thymocytes with affinity receptor for self-antigen and self MHC are eliminated. So, ultimately the T cells that recognize both foreign antigen and MHC molecule are selected. This is called immune tolerance, and the thymus is called the organ of tolerance.

Bone marrow: It is a spongy tissue situated inside the bones. It is the primary hematopoietic organ. Initially, almost all the bones contain red bone marrow that creates blood cells. But, during the course of ageing, the marrow of long bones becomes fatty tissue and the hematopoiesis decreases. But the marrow of flat bones like ribs, sternum, and pelvis is active and hematopoiesis continues.

Bone marrow is the principal site for B-cell maturation. A portion of B cells enter into the secondary lymphoid organs to differentiate plasma cells upon antigenic stimulation. Other activated B cells become memory B cells or

long-lived plasma cells reside in spleen and bone marrow. These are the persistent source of antibodies.

Bursa of Fabricius: It is the primary lymphoid organ of birds situated dorsal to the rectum and anterior to sacrum. It has a communication with cloaca through a short duct. The organ is composed of 12–20 longitudinal folds packed with numerous follicles separated through connective tissue layer. Each follicle contains B lymphocytes, dendritic cells, epithelium, and macrophages.

The bursa of Fabricius is the main site for antigen-committed B-cell maturation. Pre-bursal stem cells enter in the bursa around the seventh day of embryonic life and become bursal stem cells that undergo rapid differentiation in bursal microenvironment and become antigen-specific B cells and self-renewing post-bursal stem cells.

5.3.2.2 Secondary Lymphoid Organs

Lymph nodes: There are several lymph nodes strategically located in different anatomical locations to receive immunological signals from the body and provide an ideal microenvironment for immune cell communication. Each lymph node is divided into outer cortex that contains B cells. The inner medullary region contains both T and B cells. The paracortex between cortex and medulla contains T cells and dendritic cells. Both T cells and B cells enter the lymph nodes through endothelial venules and leave the node through efferent lymphatic vessels. T cells interact with dendritic cells during their movement, and their continual interactions facilitate to recognize foreign antigens entrapped in dendritic cells. Antigen-primed T cells then divide and induce immune reactions to eliminate it. Some of the dividing T cells also travel to B-cell-rich cortex and promote B-cell division and maturation to produce antibodies.

Spleen: It is one of the main secondary lymphoid organs in the left abdominal cavity beneath the diaphragm. It is the largest lymphatic organ of our body. Spleen is responsible for trapping of blood-borne antigens. Blood enters the spleen through splenic artery and leaves through splenic vein. The spleen is surrounded by a tissue capsule, which extends inward in the form of trabeculae to divide the spleen into two compartments, the red and white pulp. These red and white pulps are separated by marginal zone. The white pulp consists of mainly T lymphocytes surrounding splenic arteries and forms periarteriolar lymphoid sheath (PALS). The red pulp consists of sinusoids filled with blood and populated by macrophages. The marginal zone is populated by lymphocytes and macrophages. The red pulp consists of venous sinuses filled with blood and cords of lymphatic cells, such as lymphocytes, erythrocytes, and macrophages. The defective and old erythrocytes are destructed in the red pulp of spleen by the macrophages. When the antigens in the blood reach the spleen, they are trapped by dendritic cells situated in the marginal zone and carried to T-cell-rich PALS where the antigens are presented to TH cells with class II MHC molecules. Activated TH cells induce B-cell activation. Activated B cells and TH cells then migrate to primary follicles situated in the marginal zone. The primary follicles are differentiated into secondary follicles upon antigenic exposure. The secondary follicles are like lymph nodes containing germinal centers populated by B cells and plasma cells surrounded by lymphocytes.

Mucosal associated lymphoid tissue (MALT): They are situated in the mucous membranes of the gastrointestinal, respiratory, and urogenital systems. They are populated with plasma cells. Due to the large surface area of the mucosal lining of the different systems of our body, the populations of plasma cells in the MALTs are far more than bone marrow, spleen, and lymph nodes together. They resemble the structure of lymph nodes composed of lymphoid follicles, interfollicular region, subepithelial dome region, and follicle-associated epithelium. M cells are specialized cells responsible for transport microorganisms and soluble molecules from the intestinal lumen to the subepithelial region. There are several MALTs named on the basis of their anatomical positions such as gut-associated lymphoid tissue (GALT), bronchus-associated lymphoid tissue (BALT), nasopharynx-associated lymphoid tissue (NALT), lacrimal duct-associated lymphoid tissue (LDALT), conjunctiva-associated lymphoid tissue (CALT), larynx-associated lymphoid tissue (LALT), and salivary duct-associated lymphoid tissue (DALT) (Table 5.9). Functionally, MALTs are divided into effector sites and inductive sites. The inductive sites act as secondary lymphoid tissue where maturation of B cells occurs in response to antigen-primed T cells. GALT, BALT, DALT, and CALT are inductive sites. Effector sites are present in all mucosal tissues and contain T cells (CD4+), plasma cells specific for IgA secretions (few IgG- and IgM-secreting plasma cells are also present), and fewer numbers of B cells, dendritic cells, and macrophages.

5.3.3 Antibody

The interaction of B cells with an antigen leads to proliferation and differentiation of B cells to develop plasma cells. These plasma cells secrete antibodies specific to that particular antigen that travels in the blood to neutralize the antigens.

Table 5.9 Different MALTs and their locations

Name		Site
GALT	Peyer's patches	Mucosa and submucosa of the gastrointestinal tract with more abundance at jejunum
	Isolated lymphoid follicles	Antimesenteric border of the small intestine
	Cryptopatches	Intercryptal lamina propria of the small intestine
	Lymphoglandular complexes	Colon
NALT		Caudoventral portion of the left and right nasal passages
BALT		Bifurcation of bronchial tree between a bronchus and an artery (absent in dogs, cats, and Syrian hamsters)
Tonsils		Oro- and nasopharynx (absent in rodents)

Table 5.10 Serum levels of different immunoglobulins in different species

Species	IgG	IgM	IgA	IgE
Cattle	1700–2700	250–400	10–50	–
Sheep	1700–2000	150–250	10–50	–
Horse	1000–1500	100–200	60–350	4–106
Pig	1700–2900	100–500	50–500	–
Dog	1000–2000	70–270	20–150	2.3–4.2
Cat	400–2000	30–150	30–150	–
Chicken	300–700	120–250	30–60	–

Source: Tizard (2013)

Therefore, antibodies are antigen-binding proteins produced by plasma cells. Antibodies are found in body fluids with maximum abundance in blood serum.

5.3.3.1 Structure of Antibody

Antibodies are glycoprotein in nature. They are mostly obtained from γ-globulin fractions of plasma protein and hence called immunoglobulins. They are heterodimer consisting of two identical heavy (H) and two identical light (L) molecular weight of 50,000 and 25,000, respectively. Each L chain is linked with H chain by a disulfide bond to form a heterodimer (H-L). There are also noncovalent interactions such as hydrogen bond and hydrophobic bonds between H and L chains. The other H and L chains are joined in a similar fashion to form another H-L heterodimer. These two identical H-L heterodimers are joined by a disulfide bond and non-covalent interactions to form a "Y"-shaped heterotetramer $(H\text{-}L)^2$ by a hinge region. The tip of the "Y," the amino-terminal ends of both H and L chain containing 110–130 amino acids, varies greatly among different antibodies. This variable portion of the antibody is called V regions (V_L for L chain, V_H for H chain). The portion of V region which shows maximum variability among different antibodies is called complementarity-determining regions (CDRs) for both H and L chains. Like variable region, there is a constant (C) region at the tail of the "Y" for both L (C_L) and H (C_H) chains. Functionally, the immunoglobulin has two different regions, fragment antigen-binding (Fab fragment) and fragment-crystallizable region (Fc region) identified after digestion with the enzyme papain. The Fab region is a low-molecular-weight fraction (45,000 Da) having antigen-binding property. Another comparatively higher molecular weight fraction (50,000 Da) has no antigen-binding activity and is called Fc fragment ("fragment, crystallizable").

5.3.3.2 Immunoglobulin Classes

There are five classes of immunoglobulins identified in mammals. They are immunoglobulin G (IgG), IgM, IgA, IgE, and IgD. Different classes of immunoglobulins are identified by their amino acid sequences in the constant region of the heavy chains. Normal serum levels of different immunoglobulins in different species have been presented in Table 5.10.

Immunoglobulin G (IgG): IgG has the smallest immunoglobulin molecule with highest abundance in the blood among other immunoglobulin classes. They are having molecular weight of about 180 kDa containing two identical heavy chains (γ) and two different light chains (either κ or λ). IgG is produced from plasma cells of lymph nodes, spleen, and bone marrow. They can pass through capillary barriers and are thus found largely at the inflammatory sites when vascular permeability is increased. Based on their heavy-chain sequences, there are four IgG subclasses, namely IgG1, IgG2, IgG3, and IgG4. IgG1 has the highest concentration in the plasma, and the rest are numbered in accordance to their serum proportions, and IgG4 has the lowest concentration. Each IgG subclass also has different functional properties. IgG1, IgG3, and IgG4 are able to cross placental barriers easily and thus protect the fetus. IgG1 and IgG3 are primarily involved in opsonization due to their high affinity for Fc receptors,

whereas IgG2 has the lowest affinity. The IgG3 is the most potent immunoglobulin for complement activation followed by IgG1. IgG4 is unable to activate complement system.

Immunoglobulin M (IgM): The IgM is secreted in the form of a pentamer consisting of five monomers each of 180 kDa; hence, the total molecular weight is about 900 kDa. They are primarily produced by plasma cells residing at secondary lymphoid organs. Their proportion in the serum is about 5–10% of the total immunoglobulins. Each IgM monomer consists of two light chains (κ or λ) and two identical heavy chains (μ). Five IgM monomers join together in circular fashion with the help of a small peptide chain of 15 kDa named J-chain. IgM has ten antigen-binding sites due to their pentameric structure. IgM is the first immunoglobulin produced in response to an infection, and it is also the first immunoglobulin synthesized in neonates. IgM is a potent complement activator compared to IgG and also helps in opsonization and viral neutralization, but due to their large size, they are found in very less concentration in the body fluids and rarely reach the site of inflammation.

Immunoglobulin A (IgA): They constitute around 10–15% of the total serum immunoglobulin and are mainly secreted from plasma cells located at the surfaces of the body such as skin, mammary gland, intestinal wall, and respiratory and urinary system. Thus, IgA is mostly found in the external secretions like milk, tears, saliva, and mucus. IgA is secreted as a dimer consisting of two single monomers having a molecular weight of 180 kDa, so secreted IgA has 360 kDa joined by J-chain. Each IgA monomer has two light chains and two heavy chains (α). IgA protects the mucous membrane of digestive, respiratory, and urinary systems against *Salmonella*, *Vibrio cholerae*, and *Neisseria gonorrhoeae* and polio, influenza, and reoviruses. As IgG is mostly secreted in milk, it protects the newborn from infection during pre-weaning periods.

Immunoglobulin E (IgE): It was so named as it is induced by the E antigen of ragweed pollen. It has a molecular weight of about 190 kDa composed of two heavy chains (ε chain) and two light chains. Like IgA, it is also synthesized by the plasma cells under the skin, but in serum, it has extremely low concentration. IgE is mainly involved in allergic manifestation. It binds with Fc receptors of basophils and mast cells along with antigen and activates them. The activation of basophils and mast cells causes degranulation with the release of pharmacologically active substances that mediate allergic response. IgE is also involved in anti-parasitic defense. IgE has the shortest half-life (2–3 days).

Immunoglobulin D (IgD): It has a molecular weight of about 180 kDa and is composed of two δ heavy chains and two light chains. It is predominantly a membrane-bound immunoglobulin expressed in mature B cells with a very little serum concentration (30 μg/mL). It constitutes about 0.2% of the total immunoglobin in serum. IgD induces basophils to release pro-inflammatory and antimicrobial mediators.

5.3.3.3 Immunoglobulin Variants

Isotypes: The isotypes of immunoglobulins are determined by the constant regions of the heavy chain, and the constant region determinants are called isotypic determinants. The variations in the constant region are due to variations in the genes that encode the heavy chains. Bovine IgG has three isotypes such as IgG1, IgG2, and IgG3 encoded by IGHG1, IGHG2, and IGHG3 genes, respectively. The different isotypes of immunoglobulins have different physical and functional properties. IgG2 is a potent agglutinin, whereas IgG1 does not have such a function. Subclasses of different immunoglobulins in different species have been presented in Table 5.11.

Allotypes: The allotypic immunoglobulin variants are generated due to allelic variations. In the previous section, we discussed bovine IgG isotypes like IgG1, IgG2, and IgG3 encoded by IGHG1, IGHG2, and IGHG3 genes, but there may be multiple alleles for IGHG gene which encode subtle amino acid differences and they are called allotypic determinants. IgA2 subclass has two allotypes designated as A2m(1) and A2m(2).

Idiotypes: The idiotypic immunoglobulin variants are resulted due to variations in the amino acid sequences in the variable regions of light and heavy chains.

Table 5.11 Subclasses of different immunoglobulins in different species

Species	IgG	IgA	IgM	IgE	IgD
Cattle	G1, G2, G3	A	M	E	D
Horse	G1, G2, G3, G4, G5, G6, G7	A	M	E	D
Sheep	G1, G2, G3	A1, A2	M	E	D
Pig	G1, G2a, G2b, G3, G4	A	M	E	D
Dog	G1, G2, G3, G4	A	M	E1, E2	D
Cat	G1, G2, G3, G4 (?)	A	M	E1, E2 (?)	D
Mice	G1, G2a, G2b, G3	A1, A2	M	E	D

Source: Tizard (2013)

Immunoglobulin Superfamily These are a group of proteins having immunoglobulin-like domains containing 70–110 amino acids similar to variable and constant regions of immunoglobulin molecule. They share the common ancestral primordial gene that encodes immunoglobulin domains. The proteins under the immunoglobulin superfamily are Ig-α/Ig-β heterodimer of B-cell receptor, T-cell receptor, T-cell accessory proteins (CD2, CD4, CD8, and CD28), MHC class I and II molecules, cell adhesion molecule, and platelet-derived growth factors.

5.3.4 Recognition of Antigens by T and B Lymphocytes

B lymphocytes can recognize the epitopes that present on the surface of the pathogens or soluble factors released by the pathogens without the involvement of MHCs. The immunoglobulins present in the membrane of B lymphocytes specifically bind with the antigens. These membrane-bound immunoglobulins together with disulfide-linked heterodimers called Ig-α/Ig-β are called B-cell receptor (BCR). Both Ig-α and Ig-β chains have long cytoplasmic tails consisting of 61 and 48 amino acids. The Ig-α/Ig-β heterodimer is responsible for intracellular signaling after antigen binding.

In contrast to B cells, virus-infected host cells and cancer cells are recognized by T cells in conjugation with MHC. The antigen-binding sites on the T cells are called T-cell receptors (TCRs). TCRs are analogous to membrane-bound immunoglobins in B cells and also have both V and C regions, but TCR is unable to recognize antigen directly; rather, it recognizes short peptide fragments of antigens, which bind to MHC molecules through a process called antigen processing and presentation.

5.3.5 Antigen Processing and Presentation

The recognition of antigens by the T cells requires antigen processing and presentation by the APCs. In this process, foreign antigens are degraded into smaller peptides to interact with MHC molecules. There are two different mechanisms for exogenous and foreign antigens by which APCs process and present the antigens to T cells.

The *exogenous antigens* are engulfed by the APCs through endocytosis or phagocytosis, degraded by the endocytic processing pathways, and attached with class II MHC molecules. The antigen and MHC II complex then move to the surface of APCs and are recognized by T cells displaying CD4.

The *endogenous antigens* (viral proteins or cancer antigens) are produced with the cells. They are processed with the endoplasmic reticulum and subsequently attach with class I MHC molecules within the endoplasmic reticulum. The complexes are then transported to the cell membrane and recognized by T cells displaying CD8.

5.3.6 Monoclonal and Polyclonal Antibodies

Monoclonal antibodies bind to a single epitope, and polyclonal antibodies are the heterogeneous immunoglobulin mixture against a single antigen and can bind with different epitopes of a single antigen. Monoclonal antibodies are produced from the same clone of B cells, whereas polyclonal antibodies are produced from different B-cell clones. Monoclonal antibodies have high specificity and reproducibility, but the production of monoclonal antibodies is time consuming and expensive. The polyclonal antibodies have high affinity, and cost of production is also less compared to monoclonal antibodies. Monoclonal antibodies are used in therapeutics, and polyclonal antibodies are used in research applications.

5.4 Hypersensitivity and Immunological Disorders

Hypersensitivity is the pathological consequence that leads to the fatal host responses mediated by the immune system. It occurs when a pre-sensitized immune system of the host overreacts in response to an antigen. Hyperactive immune system thus produces effector molecules that induce inflammatory responses, which is undesirable to the hosts.

P. G. H. Gell and R. R. A. Coombs classified hypersensitivity reactions into four types based on the reactions involved and mediators. They are type I or IgE-mediated hypersensitivity, type II or antibody-mediated hypersensitivity, type III or immune complex-mediated hypersensitivity, and type IV or delayed-type hypersensitivity. Among these four types, first three are categorized under humoral immune responses and the last one is under cell-mediated immune response. Types I, II, and III are also called immediate hypersensitivity due to their earlier onset.

5.4.1 Type I or IgE-Mediated Hypersensitivity

Type I hypersensitivity reactions occur in response to some antigens called allergens that bind with IgE molecules. The allergen-IgE complexes thus produced attach with the mast cells or basophils to cause degranulation and release some inflammatory mediators. It can take 15–30 min from the time of exposure to the antigen. The allergens are of different types. They may be pollens of birch tree, rag seed, or

rapeseed oil; drugs such as penicillin or salicylate; foods like nuts, eggs, or seafood; and insect products like bee venom and animal hair. Type I hypersensitivity reaction is mediated by IgE, which binds with the primary cellular components of type I hypersensitivity reactions of mast cell or basophil. The other cellular components such as platelets, neutrophils, and eosinophils help to amplify the reaction.

5.4.1.1 Mechanism of Type I Hypersensitivity Reaction

Production of IgE antibody: The initial response of type I hypersensitivity is similar humoral response, except the nature of antibody produced. In exposure to normal antigens, IgG or IgM is produced, but in response to allergens, IgE is produced.

Sensitization: The IgE antibody thus produced binds with FcRI receptors present in mast cells or basophils and sensitizes them. It is also called pre-sensitization of immune system against the particular allergen.

Shocking dose of antigen: The second exposure of the allergens is called shocking dose. During the second exposure, the allergens bind with Fab region of IgE molecules attached on the surface of mast cells or basophils during the pre-sensitization stage.

Activation and degranulation of mast cell: Binding of allergens to IgE antibody causes the activation of mast cells or basophils through intracellular signaling cascade by phosphorylation, adenylation, and methylation. The activated mast cells undergo degranulation and release certain pharmacologically active substances of various functions detailed in Table 5.12.

5.4.1.2 Anaphylactic Reactions

Pharmacologically active substances released from mast cells cause vasodilation, smooth muscle contraction, mucus production, and sneezing that leads to allergic response either localized or systemic.

5.4.1.3 Examples of Type I Hypersensitivity Reactions

The manifestation of type I hypersensitivity reactions could be either localized or systemic. The generalized type I hypersensitivity leads to systemic anaphylaxis (anaphylactic shock) with a short period of time after the exposure of the allergens. Lung is the primary target organ for most of the domestic animals, but in dogs, the liver is mostly affected. The clinical signs include excitement, pruritus, salivation, vomiting, dyspnea, convulsions, or even death. The localized type I hypersensitivity includes allergic rhinitis, asthma, allergic enteritis, and atopic dermatitis.

5.4.2 Type II or Antibody-Mediated Hypersensitivity

In this reaction, the antibodies (IgG or IgM) induced against cellular antigens and this antibody-mediated immune reaction lead to cellular damage by either of the three mechanisms.

1. The antibodies bind with the target cell that expresses the antigen. The Fc portion of the antibody binds with Fc receptor of the phagocytic cells and leads to the opsonization. The opsonin then activates phagocytic cell, and the target cell is phagocytosed. In some cases, the Ag-Ab complex activates complement system and terminal complement complex (C5b6789) and lysis of target cells. This is called antibody-dependent cellular cytotoxicity (ADCC). The classical examples are autoimmune hemolytic anemia and erythroblastosis fetalis.
2. The activation of complement complex leads to the formation of C3a and C5a that are extremely chemotactic and attracts neutrophils and eosinophils towards target cells. The reactive oxygen species generated in neutrophils then destruct the cell (e.g., Goodpasture syndrome).
3. Autoantibodies cause cellular dysfunctions without inflammation or cell lysis. In Graves' disease, the

Table 5.12 Pharmacologically active substances of type I hypersensitivity reactions

Mediators	Functions
Histamine	Bronchoconstriction, mucus secretion, vasodilatation, and increased vascular permeability
Tryptase	Proteolysis
Kininogens	Vasodilatation, vascular permeability, edema
Eosinophil chemotactic factor of anaphylaxis (ECF-A)	Attracts eosinophil and neutrophils
Leukotriene B4	Attracts basophils
Leukotriene C4, D4	Similar to histamine but 100 times more potent
Prostaglandins D2	Edema and pain
Platelet-activating factor (PAF)	Platelet aggregation and heparin release

autoantibodies bind with thyrotropin receptors, which leads to overproduction of thyroid hormones.

5.4.3 Type III or Immune Complex-Mediated Hypersensitivity

In this condition, larger amount of Ag-Ab complexes causes tissue-damaging reactions. The reaction usually takes 3–10 h after exposure to the antigen in a pre-sensitized individual. The tissue damage is caused by neutrophils, macrophages, or other phagocytes. At first, the Fc region of the antibody binds with Fc receptors present in the phagocytes, and activation of the receptors leads to the production of inflammatory mediators such as prostaglandins, leukotrienes, nitric oxide, cytokines, and chemokines and promotes inflammation and migration of neutrophils at the site. When the neutrophils try to destroy the immune complexes, they deposit their granular contents in the surrounding structures like basement membrane and collagen fiber and disrupt them. In some cases, the migration of neutrophils occurs in response to chemotactic complement products (C3a and C5a). The severity of the reaction depends upon the amount of Ag-Ab complex and their site of deposition. It may cause localized tissue reactions at the site of deposition. The typical reactions are systemic lupus erythematosus and Arthus reaction in the skin. Sometimes, the immune complexes are deposited in various tissues via blood, and the reactions develop at their site of tissue deposition. The examples are lupus nephritis in kidneys, aspergillosis in lungs, polyarteritis in blood vessels, and rheumatoid arthritis in joints.

5.4.3.1 The Arthus Reaction

It is a localized type III hypersensitivity reaction due to the formation of Ag-Ab complexes after the intradermal injection of an antigen. The immune complexes form local vasculitis. It is seen very frequently after the vaccination against diphtheria and tetanus.

Know More. . . . . .
Some dogs develop "blue eye," corneal edema, and opacity after infected or vaccinated with live canine adenovirus type 1. It usually takes 1–3 weeks after the exposure and resolves by its own after the elimination of the virus.

5.4.4 Type IV or Delayed-Type Hypersensitivity

It is mediated by antigen-specific T cells. The type I reaction differs from other types of hypersensitivity in terms of the time taken to respond. It usually takes 1–3 days after the antigenic exposure and hence is called delayed type. Another important unique feature of type IV hypersensitivity from other types is the involvement of T cells in contrast to antibody as in other three types. Here, in this reaction, the antigens after processing and presentation by ABP stimulate type 1 helper T (TH1) cells to secrete cytokines and chemokines like IFN-γ, TNF-α and -β, and interleukin 2 (IL-2). TNF-α and -β increase the vascular permeability, and IFN-γ causes macrophage activation to secrete their lethal contents and subsequent tissue damage. Type IV hypersensitivity reaction is important for intracellular pathogens where antibodies are unable to reach. The lethal damage to the tissue causes the destruction of pathogens together with the cells. Type IV hypersensitivity reaction is of three types.

The *contact hypersensitivity dermatitis* occurs when an exogenous antigen invades the skin and induces inflammatory reaction in dermis and epidermis. The antigen-presenting dendritic cells and Langerhans cells process the antigen and present to type 1 helper T (TH1) to induce inflammatory reaction.

Tuberculin-type hypersensitivity occurred in response to intradermal injection of purified protein derivative (PPD) named tuberculin (product of tuberculosis bacillus). It is used to detect tuberculosis in animals.

Granulomatous-type hypersensitivity occurs in more serious condition when the recruited macrophages are unable to destroy the antigens. As a result, more number of macrophages are recruited and these macrophages filled with intracellular antigen lead to granuloma.

5.4.5 Immunological Disorders in Animals

5.4.5.1 Thyroiditis

In this condition, the antibodies are generated against thyroglobulin or thyroid peroxidase. The lymphocytes invade the thyroid gland and cause epithelial cell destruction through antibody-dependent cell-mediated cytotoxicity. The clinical manifestations include dry and dull hair coat, loss of hair, scaling, hyperpigmentation, and pyoderma. Thyroiditis is common in dogs that are more susceptible, and susceptible dog breeds are Beagles, Great Danes, and Doberman.

5.4.5.2 Polyneuritis

It is an autoimmune disease against myelin proteins of nerve tissues. It occasionally occurs in horses and dogs. The characteristic symptoms are hyperesthesia and paralysis of tail, rectum, and urinary bladder. Sometimes, facial and trigeminal paralysis also occurs. In dogs, the disease is also called "coonhound paralysis" as it occurs as a result of bite or scratches from raccoons.

5.4.5.3 Uveitis/Blue Eye

It is an autoimmune disorder in response to autoantigen interphotoreceptor retinoid-binding protein and is characterized by the inflammation of uveal tract (iris and ciliary body) of the eye. It can be seen in horses and dogs. In horses, the inflammatory site is infiltrated with Th1 cells and neutrophils along with fibrin and C3 depositions, which may lead to blindness. In puppies, uveitis may develop after vaccination with canine adenovirus (CAV)-2-modified live viral vaccine and the prognosis is good. The characteristic symptoms include conjunctivitis, ulcers in cornea, vascularization, and corneal scarring.

5.4.5.4 Immune-Mediated Hemolytic Anemia

The immune-mediated hemolytic anemia occurs due to the production of autoantibodies against RBC membrane proteins like glycophorins, spectrin, and anion-exchange protein CD233. It occurs in cattle, horses, dogs, cats, and mice. The major clinical manifestations are anemia and others secondary symptoms like fever, jaundice, tachycardia, and splenomegaly.

5.4.5.5 Myasthenia Gravis

It is a skeletal muscle disease that occurs due to the formation of autoantibodies against acetyl choline receptor, which leads to blockage of the receptors and complement-mediated destruction of the receptors, which ultimately causes nerve impulse transmission. The clinical signs are abnormal fatigue, muscle weakness, and exercise intolerance. The species involved are dogs, cats, ferrets, and humans.

5.4.5.6 Systemic Lupus Erythematosus (SLE)

It is an autoimmune disorder due to the development of autoantibodies against nucleic acids, nucleoproteins, and chromatin. These autoantibodies are called antinuclear antibodies. It is a classic example of type III hypersensitivity reaction. The immune complexes are deposited in various tissues like glomeruli to form glomerulonephritis, synovial joints to form arthritis, arteriolar walls leads to fibrosis and skin leads to ulcerative lesions. It is mostly seen in dogs with symptoms like localized ulceration in the skin and mucous membrane, glomerulonephritis, lymph node enlargement, splenomegaly, nervous symptoms like lameness, and lethargy. In equines, SLE is characterized by skin diseases like dermal ulceration, alopecia, and crusting together with anemia.

5.4.5.7 Rheumatoid Arthritis

It is an immune complex-mediated hypersensitivity reaction that occurs in response to the deposition of immune complexes in the joints. It is very common in humans but is occasionally seen in dogs (mostly in toy breeds) characterized by lameness immediately after awaking in the morning in addition to anorexia, depression, and pyrexia.

5.4.5.8 Canine Leukocyte Adhesion Deficiency

It is an autosomal recessive genetic disorder due to mutation in integrins, the cell adhesion molecule. The neutrophils are unable to adhere with vascular endothelium and thus unable to migrate to the site of injury (see Sect. 5.2.9—Leukocyte migration). The affected dogs are prone to recurrent infections. Leukocyte adhesion deficiency is also seen in cows.

Learning Outcomes

- **Antigens**: Antigens are the molecules that bind specifically with the products of immune response (i.e., antibodies or cytotoxic T lymphocytes) induced by immunogens, and this property is called antigenicity. The antigenicity is determined by several factors such as foreignness, chemical structure and molecular size, doses, and route of administration. Antigens can be classified into exogenous and endogenous based on their source. Antigens can also be classified into complete antigen (immunogen), incomplete antigen (hapten), and superantigen based on immune responses.
- **Innate immunity**: Innate immunity is the evolutionary nonspecific defensive reflex against foreign materials owned by birth. It serves as the first line of defense against infection. Components of innate immunity include anatomical barriers (skin and mucosal membrane), physiological barriers (body temperature, pH, and several other soluble factors), immune effector cells (granulocytes, monocytes/macrophages, natural killer cells, dendritic cells, endothelial cells, epithelial cells, lymphoid cells, and platelets), pattern recognition receptors [Toll-like receptors, C-type lectin receptors, nucleotide-binding oligomerization domain (NOD) receptors, and retinoic acid-inducible gen-I (RIG)-like receptor], inflammatory serum proteins/acute-phase proteins (haptoglobin, serum amyloid A, ceruloplasmin), antimicrobial peptides (AMPs), complement system, and cytokines.
- **Adaptive immunity**: Adaptive or acquired immune responses are capable of selective elimination pathogens. It has some cardinal features like specificity, diversity, and memory. It also has a unique ability to discriminate the self and nonself antigens and to react accordingly. Adaptive immune responses are brought about by different classes of lymphocytes, namely B and T lymphocytes. The immune responses mediated through B lymphocytes are called *antibody-mediated or humoral immune response* as B cells are capable

(continued)

of producing antibodies (or immunoglobulins) upon antigenic exposure, which bind with antigens and make them vulnerable for destructions. *The cell-mediated immune responses* are mediated through T lymphocytes that produce signals to activate phagocytic cells to destroy them.

- **Hypersensitivity**: It is the pathological consequence that leads to the fatal host responses mediated by the immune system when a pre-sensitized immune system of the host overreacts in response to an antigen. It can be classified into type I or IgE-mediated hypersensitivity, type II or antibody-mediated hypersensitivity, type III or immune complex-mediated hypersensitivity, and type IV or delayed-type hypersensitivity. Among these four types, first three are categorized under humoral immune responses and the last one is under cell-mediated immune response. Type I, II, and III are also called immediate hypersensitivity due to their earlier onset.

Exercises

Objective Questions

Q1. Arrange the biomolecules in their ascending order of immunogenic response proteins, polysaccharides, lipopolysaccharides, and nucleic acid.
Q2. Acute-phase protein associated with copper transport is _____.
Q3. Which biomolecule protects host cells from complement-associated lysis?
Q4. The primary lymphoid organ of birds is _____.
Q5. Which immunoglobulin is predominant in external secretions?
Q6. Viral proteins are associated with which class of MHC?
Q7. Anaphylactic shock is an example of _____ hypersensitivity.
Q8. Name one autoimmune disease associated with skeletal muscle.

Subjective Questions

Q1. Mention some features of acquired immunity.
Q2. Briefly describe the mechanism of action of cytokines.
Q3. How MHC helps to discriminate self and nonself?
Q4. Explain the inflammatory signs under the light of its cellular events.
Q5. "Thymus is the organ of tolerance" . . . . Justify the statement.

Answer to Objective Questions

A1. Proteins > polysaccharides > lipopolysaccharides > nucleic acid
A2. Ceruloplasmin
A3. Glycophorin A
A4. Bursa of Fabricius
A5. IgA
A6. Class I MHC
A7. Type I
A8. Myasthenia gravis

Keywords for Subjective Questions

A1. Specificity, diversity, memory, discrimination of self and nonself
A2. Receptor binding, intracellular signaling, response
A3. Antigen processing and presentation, exogenous antigen, MHC II, endogenous antigens, MHC I
A4. Vasodilation, redness, tissue permeability, edema
A5. T lymphocyte selection, positive, negative

Further Reading

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