Manzoor M. Khan

Immunopharmacology

Second Edition

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Dedicated to my family

Preface

Since the publication of the first edition, there have been significant advances in the understanding of the basic function of the immune response and their relevant clinical applications. Some of the key discoveries include the identification of the new subset of helper T cells, new cytokines and their networks, and novel signal transduction mechanisms. For example, the identification of TH17 subset of helper T cells, in addition to TH1 and TH2 cells, not only advanced our understanding of the function of the basic immune response but our awareness of the possible etiology and pathogenesis of diseases such as allergy, asthma, rheumatoid arthritis, and other autoimmune/immune system-based diseases. The newly identified powerful cytokine networks that regulate both innate and acquired immune responses emerged as a result of the finding of the new cell types such as innate lymphoid cells and iNKT. Identification of the new cytokines and their networks has advanced our knowledge of the mechanisms involved in the maintenance of tissue homeostasis, including inflammation and tissue repair during stress and injury.

From the clinical application's perspective, there have been significant advances in oral immunotherapy for allergic disease, the treatment of HIV infection, the development of new monoclonal antibodies and their fragments to treat human diseases, and the immune cell-based therapies for cancer. The development of HIV vaccines has seen dramatic changes over the last few years. There has been a shift from a sole focus on T-cell vaccines to a holistic approach that pertains to the induction of both humoral and cellular elements. This entails induction of antibodies – both binding and neutralizing – to prevent infection. The cellular vaccination produces a safety net of CD8 T-cell responses to suppress the replication of the virus in the infected patients; as both of the effector arms are aided by helper T cells. The concept of immunotherapy was in infancy when the first edition was written, and since then major advances have been made not only with several major clinical trials but also with the approval of Sipuleucel-T by the FDA for the treatment of cancer in 2010. Furthermore, CAR T-cell therapy to treat cancer is in infancy with great expectations. As a result, the gap between early scientific knowledge and the late development of immune-based therapies is gradually narrowing.

Consequently, the significance and magnitude of these advances warranted a revision of this contribution. The revised edition will provide an in depth understanding of the recent advances and discoveries of the function on the immune response and their applications in the development of novel therapies to treat human diseases.

As we entered the twenty-first century, major advances in the arena of recombinant DNA, hybridoma, and transgenic technologies had revolutionized not only the understanding of the etiology and pathogenesis of a number of debilitating and life-threatening diseases but also provided novel modes of treatment. Whether it is the clinical application of recombinant cytokines, their agonists or antagonists, monoclonal antibodies, regulatory T cells, gene therapy, or the concept of T-cell vaccines, these all required the understanding of an evolving discipline that worked on the interface of immunology, pathology, pharmacology, and genetics called immunopharmacology. The initial emphasis of the discipline was the development of the drugs, which suppressed immune response to prevent tissue rejection after organ transplantation. The field, once considered restricted only to protect the host from invading organisms by mounting immune and inflammatory responses, evolved exponentially as we gradually learned about the exciting and sometimes adverse role of the products of the immune response in a very wide range of physiologic and pathologic settings ranging from cardiovascular, pulmonary, and gastrointestinal to neurological functions. A number of these products and therapies, based on their understanding continue not only to become symptomatic and curative therapeutic agents but have extensively contributed to the early diagnosis of a number of dreadful disorders.

This book is written for the graduate students in pharmacology and the professional students in pharmacy and medicine. The introductory chapter is aimed for the students who have not previously taken a course in basic immunology. Chapters 2 and 3 focus on cytokines, their receptors, pharmacology, and clinical applications. The next section is devoted to the pharmacology of immune regulatory agents, monoclonal antibodies, etiology, mechanisms of IgE-mediated responses, and immunotherapy for allergic disease. The following section includes chapters on the mechanisms of allograft rejections with description of the requirements for different types of clinical tissue transplantation and immunologic basis of acquired immunodeficiency disease. In the chapter on AIDS, the emphasis has been on the life cycle of HIV, available therapeutic options and the difficulties associated with the development of a vaccine for AIDS, and why an HIV vaccine does not fit the paradigm for the classical vaccine development. The last part of the book includes chapters on regulatory T cells and their therapeutic potential followed by the last chapter on the challenges and use of gene therapy to treat human disease.

Omaha, NE, USA Manzoor M. Khan
April 1, 2016

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Contents

Chapter 1
Overview of the Immune Response

Abstract This chapter introduces the components of the immune response that includes differences in innate and acquired immune systems and their differences. Specifically, physical, chemical, and cellular barriers, complement system, and Toll-like receptors are discussed in reference to the innate immune system, whereas humoral and cell-mediated responses are described when explaining the acquired/adoptive immune response. The details include the different isotypes of antibodies involved in humoral immune response and various types of cells that participate in humoral versus cell-mediated immune responses. Additionally, the concepts of antigen recognition, antigen presentation, and molecules involved in these processes, including the major histocompatibility complex, are described. Lastly, mechanisms of cellular migration and immune tolerance are discussed. The emphasis is that how the immune system recognizes and responds to the invading pathogens and what type of system is in place and sequentially develops to perform these tasks.

Keywords Immunogen • Antigen • Innate immunity • Physical barriers • Chemical barriers • Cellular barriers • Complement system • Toll-like receptors • Acquired immune response • Bone marrow • Thymus • Lymphoid organs • Antibodies • IgG • IgA • IgM • IgD • IgE • Immunoglobulins • Lymphoid cells • B cells • T cells • Helper T cells • Cytolytic T cells • TH1 • TH2 • TH9 • TH0 • TH17 • NKT cells • ϒδ T cells • Memory T cells • Regulatory T cells • Natural killer cells • $CD4^+$ • $CD8^+$ • $CD3^+$ antigen-presenting cells • Macrophages • Dendritic cells • Neutrophils • Basophils • Eosinophils • Mast cells • Adhesion molecules • Integrins • Selectins • Cadherins • TCR • γδ TCR • TCR-1 • TCR-2 • Antigen recognition • Antigen processing • Antigen presentation • CD28 • CD40 • B7 • MHC molecules • HLA-A • HLA-B • HLA-C • HLA-D • HLA-DP • HLA-DR • HLA-DQ • MHC class I molecules • MHC class II molecules • Immune tolerance • Central tolerance • Peripheral tolerance

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1.1 Introduction

The immune system is a defense system that protects from infectious organisms and cancer. It is made up of a variety of cells, proteins, tissues, and organs. The cells participating in immune response are designed to recognize and eliminate invading agents. If not eliminated, these invading microorganisms may cause disease. The recognition is a very specific process that enables the body to recognize nonself molecules so the second phase of the process, an immune response, may initiate. Under normal circumstances, an immune response is not generated against self, which are the body's own proteins and tissues. The immune system for each individual is unique, and it employs small and efficient tools to recognize invading organisms that lack a central control. However, they are widely distributed in the body. The recognition of the nonself is not perfect and absolute detection of every pathogen is not required. Immune system is able to recognize molecules, which it has never seen before and can produce an effective response against them. It has been suggested that this system is scalable, resilient to subversion, robust, and very flexible and degrades.

After the recognition of the nonself, the effector phase is generated, which is characterized by the generation of a response against the invading microorganism in which a variety of cells and molecules participate, resulting in the neutralization and/or elimination of the pathogen. Some memory is retained of that pathogen and a second exposure to the same organism results in the development of a memory response. This response has a quick onset and is fiercer, resulting in a more efficient elimination of the pathogen.

1.2 Immunogens and Antigens

Immunogens are any agent capable of inducing an immune response. Their characteristics include foreignness, high molecular weight, and chemical complexity. Antigens are any agent capable of binding specifically to the components of immune response. They include carbohydrates, lipids, nucleic acids, and proteins. Most immunogenic molecules, which induce an immune response, require both T and B lymphocytes. Because T lymphocytes mature in the thymus, these immunogens are called thymus-dependent antigens. Certain types of molecules can induce the production of antibodies without the apparent participation of T lymphocytes. These molecules are referred to as the thymus-independent antigens. The portion of an antigen that binds specifically with the binding site of an antibody or a receptor on lymphocytes is termed an epitope.

1.3 Components

1.3.1 Innate Immunity

This is a general protection that is also termed as natural immunity, which is present at birth in all individuals. A species is armed with innate immunity that provides an individual the basic resistance to disease. This is also called nonspecific immunity

and is the initial defense against infections. It is characterized as broad-spectrum responses, with limited repertoire of recognition molecules and a lack of memory component. Since it is the first line of defense, it is present at birth, it is nonspecific, and it does not allow an increase in resistance after repeated infections. It destroys vast amounts of microorganisms in a short time, with which an individual comes in contact with every day, and protects from causing the disease. There are three types of barriers for the innate immunity, physical barriers, cellular barriers, and chemical barriers.

1.3.1.1 Physical Barriers

The physical barriers include the skin, mucous membranes, epidermis, and dermis. The skin maintains a low pH because of lactic and fatty acids. The mucous membranes in the respiratory system, urogenital system, and gastrointestinal system create a substantial surface barrier. Epidermis and dermis constitute additional physical barriers. The dermis also produces sebum, which maintains an acidic pH due to its lactic and fatty acid content.

1.3.1.2 Chemical Barriers

A number of endogenous chemicals provide effective barriers in innate immunity. They include hydrolytic enzymes of saliva, low pH of the stomach and vagina, and proteolytic enzymes in the small intestine. Additional examples include cryptidins, α-defensins, α-defensins and interferons, and surfactant proteins A and D. Cryptidins and α-defensins are produced in the small intestine, and α-defensins are produced by the skin and respiratory tract.

1.3.1.3 Cellular Barriers

The cellular barriers include macrophages, dendritic cells, eosinophils, phagocytes, and natural killer cells. Some of these cells internalize macromolecules that they encounter in the circulation or in tissues. This internalization takes place either by pinocytosis, receptor-mediated endocytosis, or phagocytosis. The pinocytosis involves nonspecific membrane invagination. In contrast, receptor-mediated endocytosis involves specific macromolecules, which are internalized after they bind to respective cell surface receptors. Endocytosis is not cell specific and carried out probably by all cells.

As opposed to endocytosis, phagocytosis is more cell specific and results in the ingestion of particulate as well as whole microorganisms. The cells involved in phagocytosis include monocytes and macrophages, neutrophils, and dendritic cells. Furthermore, fibroblasts and epithelial cells can also be induced to assume phagocytic activity.

1.3.1.4 Complement System

The complement system is a part of the innate immune response that facilitates the ability of antibodies and phagocytes to clear invading organisms. Its ability remains the same and does not improve overtime. Nonetheless, it can play a supportive role in the acquired immune response. The complement system is composed of a number of small proteins present in blood. It is synthesized by the liver and circulates as pro-proteins, which are its inactive precursors. After induction, proteases in the complement system cleave specific proteins. This causes the secretion of cytokines and begins a series reactions culminating in enhanced response, as well as induction of the membrane attack complex, which is cytotoxic in nature. The complement system is composed of more than 35 proteins and protein fragments. They include serum, serosal, and cell membrane receptor proteins.

As shown in Fig. 1.1, the complement system is activated by three distinct biochemical pathways. These pathways include the classical complement pathway, the alternative complement pathway, and the lectin pathway. The hepatocytes, macrophages, monocytes, and epithelial cells of the gastrointestinal and urogenital tract synthesize the components of the complement system. The homologous variant protease C3-convertase is produced by all three pathways of activation. Antigen-bound antibody molecules drive the activity of the classical complement pathway. Six units of IgG with the exception of IgG4 or one unit of IgM can activate the complement pathway. The pathway is initiated after the Fc region of the antibody binds to C1 component. It can also be triggered by additional mechanisms such as the binding of the C-reactive protein to polysaccharides of microbial agents. C1 is the initial enzyme, which is made up of two subunits, with calcium-dependent interaction.

Antigens alone and C3 hydrolysis are the stimuli of the alternative complement pathway and the lectin pathway. The alternative pathway is in a continuous activated state, though at low levels. It does not depend on the binding of antibodies to the pathogen. Lectin pathway is homologous to the classical pathway and is activated by attachment of the mannose-binding lectin to mannose residues on the surface of the invading pathogen. This activates mannose-binding lectin-associated serine proteases. Component C3 is cleaved and activated by C3-convertase in all three pathways. This results in the formation of C3a and C3b, which catalyze a series of additional pathways. The internalization and opsonization of an invading organism by phagocytes is facilitated by binding of C3b to the surface of the pathogen. The recruitment of inflammatory cells is mediated by C5a that is an important chemotactic protein. Both C3a and C5a can degranulate mast cells and are anaphylactic in nature. They also cause contraction of the smooth muscles and increase vascular permeability. The formation of membrane attack complex involves the activation of membrane attack pathway orchestrated by C5b and includes C5b, C6, C7, C8, and C9. The membrane attack complex is cytolytic in nature and is the final product of the complement pathways. The osmotic lysis of the target cell is the result of the formation of a transmembrane channel by the membrane attack complex. Complement-coated invading organisms or their fragments are removed by the phagocytic cells.

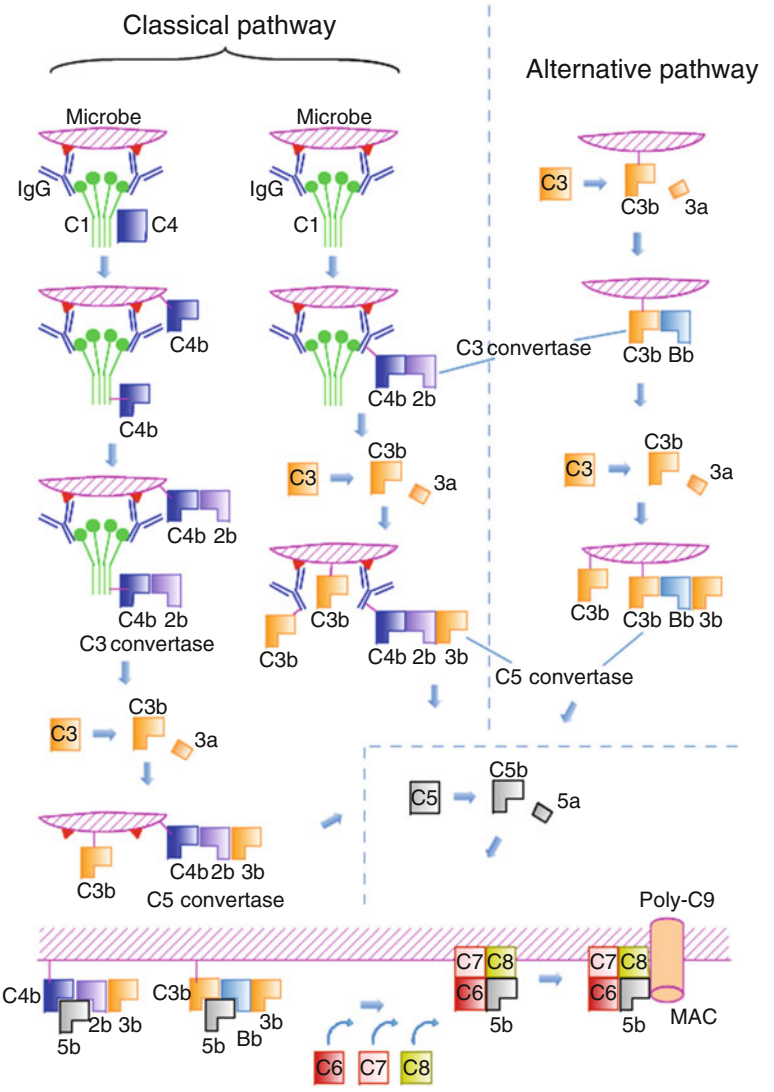

Fig. 1.1 The classical and alternative complement pathways with the late steps of complement activation (Source: Tossh_eng, under the terms of GNU free documentation license)

The functions of the complement system include opsonization (preparation to eat, increasing the ability of the phagocytes to remove the antigen), chemotaxis (attracting mono- and polymorphonuclear phagocytes), agglutination (clumping of the antigens), and cell lysis. Complement system could be deleterious to the host and thus needs to be strictly regulated. This is achieved by complement control proteins, which are present in blood plasma and host cells.

The role of complement system is suggested in a variety of immune-mediated diseases such as asthma, rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, autoimmune heart disease, inflammatory bowel disease, and Barraquer–Simons syndrome. Furthermore, there is evidence for the involvement of complement system in neurodegenerative conditions and other diseases of the central nervous system, including Alzheimer's disease. In HIV/AIDS the complement system is activated to cause additional damage to the self. Mutations and polymorphisms in complement system are responsible for a number of other disease states.

1.3.1.5 Toll-Like Receptors

Toll-like receptors (TLRs) are a family of polypeptides, which are crucial in innate immunity. They are generally expressed on antigen-presenting cells, including macrophages and dendritic cells. TLRs recognize microbes and activate immune response. Their subtypes include TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR11, TLR12, and TLR13. They are a type of pattern recognition receptors and recognize molecules, which pathogens share (pathogen-associated molecular patterns). In combination with a cytokine receptor (IL-1R), they form a receptor superfamily that is called "interleukin-1 receptor/Toll-like receptor superfamily." A common domain is shared by all members of this family, which is referred to as Toll-IL-1 receptor (TIR) domain. There are three subgroups of TIR domains. The receptors for cytokines that are produced by antigen-presenting cells (macrophages, monocytes, dendritic cells) form the first subgroup. Classical TLRs that bind to microbes form the second group. TIR domains consisting of cytosolic adopter proteins make up the third subgroup and are also involved in signaling of the proteins of the other two subgroups (Fig. 1.2).

The ligands for TLR include bacterial lipopolysaccharides (LPS), lipoproteins, flagellin, the unmethylated CpG islands of bacterial and viral DNA, double-stranded RNA of viruses, as well as other molecules. It is considered that TLRs act as dimers. For full ligand sensitivity, they may also rely on other co-receptors. TLR signaling is divided into two pathways, the MyD88-dependent and TRIF-dependent pathway. The MyD88-dependent response occurs on dimerization of TLR receptor and, with the exception of TLR3, is used by all other TLRs. It activates NF-κB and mitogen-activated protein kinase (MAPK). The adopter protein MyD88 is recruited after ligand binding and conformational changes in the receptor. IRAK1, IRAK2, and IRAK4 are then recruited by MyD88. TRAF6 is then phosphorylated and activated by IRAK kinases. The binding to IKK-β results from the polyubiquitination of TAK1 and IRAK kinases. After binding, TAK1 phosphorylates IKK-β, resulting in the phosphorylation and degradation of IκB, which allows migration of NF-κB into the cell nucleus. This cascade results in the transcription and translation of pro-inflammatory cytokines.

TRIF-dependent pathway is used by TLR3 and TLR4. dsRNA activates TLR3 pathway and LPS activates TLR4 pathway. The adaptor TRIF is recruited after the activation of TLR3 by dsRNA. TBK1 and RIPK1 are activated by TRIF. IRF3 is

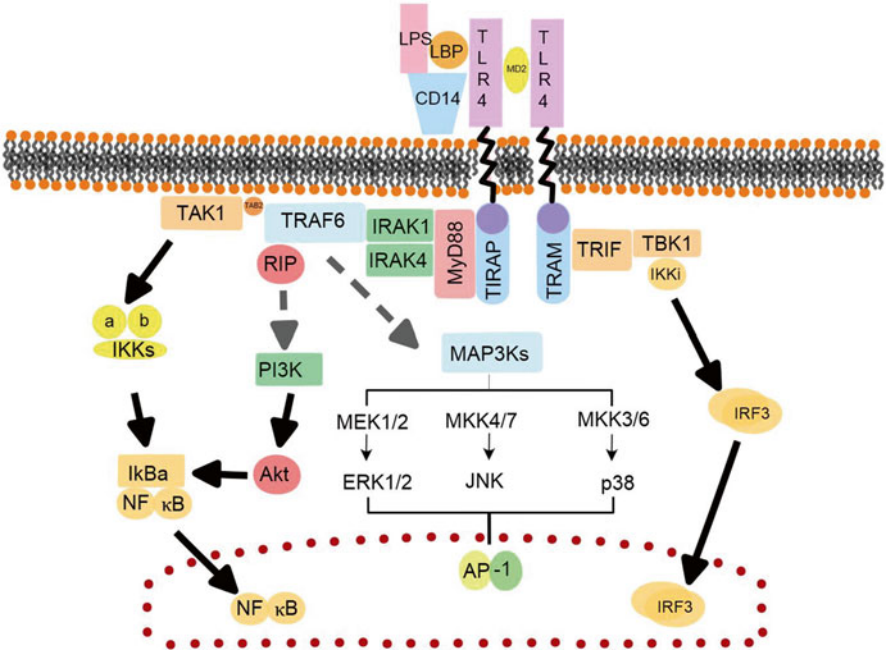

Fig. 1.2 Toll-like receptor pathways (Source: Niels Olson – Licensed under CC BY-SA 3.0 via Wikimedia Commons)

phosphorylated by the TRIF/TBK1 signaling complex. This results in the migration of IRF3 into the nucleus, where it causes the transcription and translation, eventually resulting in the production of type I interferon. TLR signaling results in the augmentation or inhibition of genes, which produce the inflammatory response. Signaling by TLR activates or regulates a very large number of genes. All four adaptors are used by TLR4 that is unique for this subset of receptors. TIR domain-containing adaptors TIRAP and MyD88 are recruited by TLR4, MD2, and LPS. This causes the activation of NF-κB and MAPK. A signaling complex is then formed between TRAM, TRIF adaptors, and TLR4-MD2-LPS complex, after the TLR4-MD2-LPS complex goes through endocytosis. IRF3 is activated as a result of this TRIF-dependent pathway. This results in the production of type I interferon. However, the production of pro-inflammatory cytokines requires both early and late phases of NF-κB activation. The signal transduction pathways for TLR are shown in Fig. 1.2.

There are multiple outcomes after TLRs are activated by microbes. In response to viral infection, the infected cell may undergo apoptosis. There may also be a release of antiviral cytokines, such as interferons. While TLRs are involved in the release of cytokines, they play no significant role in the phagocytosis of the microbes. In case of bacterial antigens, pro-inflammatory cytokines are produced. The invading pathogens may also be uptaken by the antigen-presenting cells and after undergoing processing presented to naïve helper T cells.

In addition to their role in innate immunity, TLRs also play a key role in acquired immune responses. Their functions are broad and they affect tissue homeostasis. TLR signaling has been implicated in many inflammatory diseases, including asthma, allergic rhinitis, autoimmune diseases, and other immune-mediated inflammatory diseases. The pathological manifestation may result from either overactive TLR signaling or insufficient signaling. A number of TLR ligands, both agonists and antagonists, are being studied in animal models, preclinical and clinical settings. Since TLRs work together, their function is complex. Our information for their therapeutic implications is still rudimentary and requires significant additional understanding.

1.3.2 Adaptive/Acquired Immune Response

This immune response occurs when the body encounters an antigen and/or pathogen. With this response, the body protects itself from future encounters with the same antigen/pathogen so they will not cause disease. The response is more complex than the innate immune response. It requires the recognition and processing of the immune response. After the antigen is recognized, the adaptive immune response employs humoral and cellular responses specifically designed to eliminate the antigen. This response also includes a memory component, which allows improved resistance against that specific antigen during subsequent infections. T lymphocytes, B lymphocytes, and macrophages participate in the acquired immune response. The lymphocytes (T and B cells) are central to all acquired immune responses, because of their specificity in recognizing the pathogens. This recognition can take place either inside the tissue or in blood or tissue fluids. B cells recognize antigens by synthesizing and releasing antibodies, which specifically recognize antigens. T lymphocytes do not secrete antibodies but have a wide range of regulatory and effector functions.

1.3.2.1 Antibodies

The antibodies are proteins that are produced during the immune response. They identify and eliminate foreign objects such as bacteria and viruses. They are synthesized in response to specific antigens and only bind to the antigen against which they are produced. Antibodies are glycoproteins and are also called immunoglobulins. They are synthesized and secreted by B lymphocytes, which produce antibodies after their activation resulting from exposure to an antigen. The antibodies can circulate freely or in the bound form attached to the cells, which possess Fc receptors.

The antibody molecule (monomer) is a Y-shaped structure (Fig. 1.3). It consists of two identical light and heavy chains, which are connected by disulfide bonds. In the native state, the chains are coiled into domains, each of which consists of 110

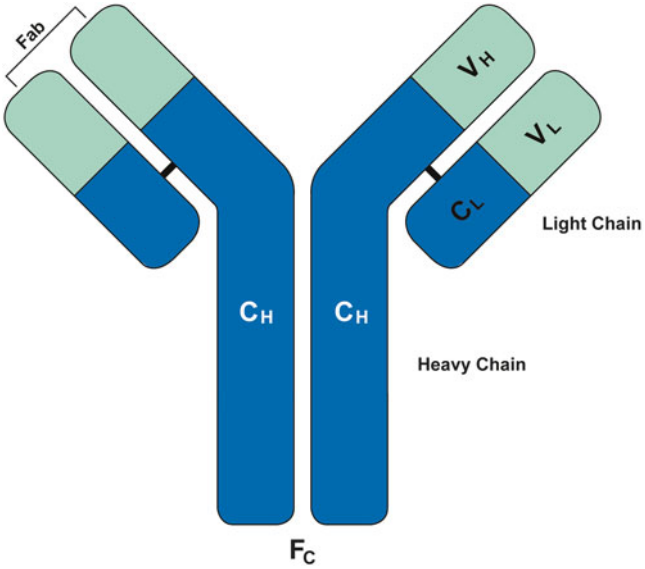

Fig. 1.3 The immunoglobulin molecule. Each immunoglobulin molecule is composed of two identical heavy (CH + VH) and two identical light chains (CL + VL). The antigen binds to the Fab region which varies according to the specificity of the antibody. The rest of the domains (*blue*) are constant. The classes of the antibody molecules differ based on the Fc region of the heavy chain

amino acids. Both light and heavy chains are made up of constant and variable regions. The two identical light (L) chains and two identical heavy (H) chains are held together by disulfide bonds. Both light and heavy chains have constant and variable regions. Two major classes of L chains are kappa and lambda and the ratio of κ and λ chains varies from species to species. Papain splits the immunoglobulin molecules into three fragments of about equal size. Two fragments are antigen-binding fragment (Fab) and the third fragment is fragment crystallizable (Fc). The variable regions of both heavy and light chains form the antigen-binding site (Fab). The Fab region varies according to the specificity of the antibody. The antibodies are very diverse molecules and their differences reside predominantly in the variable region. This variability enables each antibody to recognize a particular antigen.

There are five different types of heavy chains, which correspond with five different classes of antibodies. The constant region of the heavy chain is identical in all antibodies of the same class. The classes of the immunoglobulin molecules differ based on the Fc region of the heavy chain, which are responsible for the different functions performed by each class. Thus the constant region confers on each class of antibody its effector function.

As depicted in Table 1.1, there are five different classes of H chains. Each chain differs in antigenic reactivity, carbohydrate content, and biological function. The nature of the H chain confers the molecule its unique biologic properties. A distinctive set of glycoforms characterizes each immunoglobulin. The glycoforms render

Table 1.1 Classes (isotypes) of H chains

Immunoglobulin class (isotype)	Heavy chain
IgM	μ
IgG	γ
IgA	α
IgD	δ
IgE	ϵ

broad differences in the frequency, form, and locality of oligosaccharides, which are responsible for the diversity of immunoglobulins. Since these glycoform populations can be identified on a regular basis, any alteration in their characteristics suggests a disease state and could be a potential therapeutic tool. The oligosaccharides possess critical recognition epitopes. This provides the immunoglobulins with additional functional repertoire. The effector function of immunoglobulins is thus regulated by these sugar molecules.

The antibody molecules have two distinct functions. The first is to bind the pathogen such as virus or the bacteria against which immunoglobulin was produced, and the second is to recruit other cells and molecules, such as phagocytes or neutrophils, to destroy the pathogen to which the antibody is bound.

1.3.2.2 Classes of Immunoglobulins

There are five different classes of immunoglobulins: IgG, IgM, IgA, IgD, and IgE. The structures are shown in Fig. 1.4.

IgG: Immunoglobulin G is present in lymph fluid, blood, cerebrospinal fluid, and peritoneal fluid. It is composed of 2 γ chains of 50 kD and 2L chains (κ or λ) of 25 kD with a total molecular weight of 150 kD. The functions of IgG include agglutination and formation of precipitate, passage through the placenta and thus conferring immunity to the fetus, opsonization, antibody-dependent cell-mediated cytotoxicity (ADCC), activation of complement, neutralization of toxins, immobilization of bacteria, and neutralization of virus.

IgM: The gene segment that encodes the μ constant region of the heavy chain occupies the front position among other constant region gene segments, and consequently, IgM is the first immunoglobulin produced by mature B cells. Its molecular weight is 900 kD and is a five-chain structure. All chains consist of 2L and 2H chains and have five antigen-binding sites. It is synthesized in appreciable amounts by children and adults after immunization or exposure to thymus-independent antigens. Elevated levels usually indicate a recent infection. IgM does not pass across the placenta but is synthesized by the placenta. Its elevated levels in the fetus are indicative of congenital infection. It is the best agglutinating and complement-activating antibody and possesses high avidity. Sometimes it is referred to as a natural antibody, since it is often bound to specific antigens, even when there was no prior immunization.

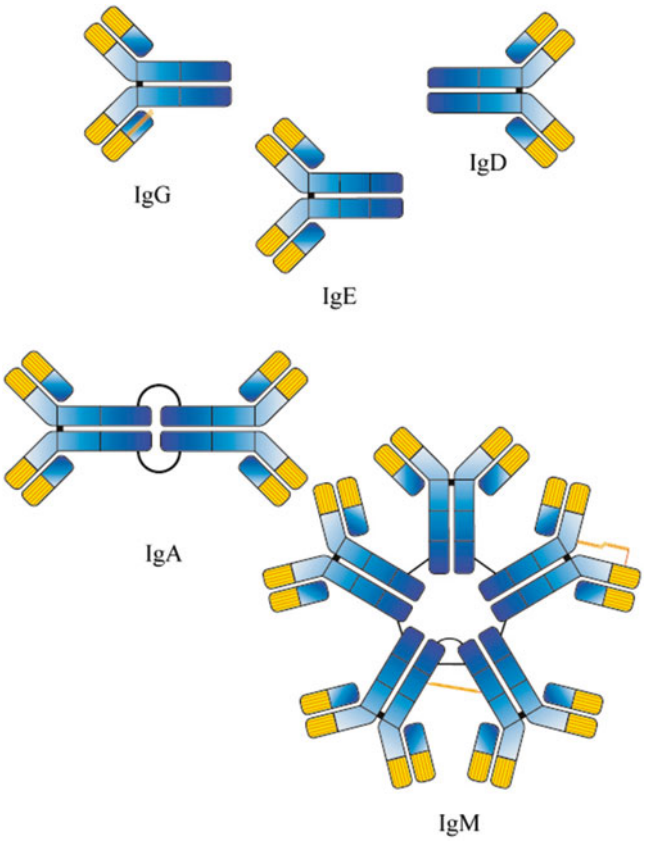

Fig. 1.4 Classes of immunoglobulins. This figure depicts five classes of immunoglobulins IgG, IgE, IgD, IgA, and IgM. IgA can be present as a monomer or dimer molecule, whereas IgM exists as a pentamer

IgA: Immunoglobulin A is a major immunoglobulin in external secretions (saliva, mucus, sweat, gastric fluid, and tears). It is a major immunoglobulin of colostrum and milk; it has a molecular weight of 165 kD and is present in both monomeric and dimeric form. It is present in two isotypes, IgA1 (90 %) and IgA2 (10 %). The bone marrow B cells produce IgA1, which is present in serum. The B cells located in the mucosa synthesize IgA2, which is present in secretions. Chemically the heavy and light chains of IgA2 are bound by non-covalent bonds and not connected by disulfide bridges. It plays an important role in mucosal infections, bactericidal activity, and antiviral activity. Plasma cells produce polymeric IgA, and mucosal epithelial cells express polymeric Ig receptor, resulting in high levels of IgA in mucosal areas. This is followed by its transportation across mucosal epithelial cells, where it is separated from its receptors resulting in its release into secretions. Its effects are achieved after interaction with specific receptors including FcR1, Fcμ/micro R, and CD71. However, certain pathogens block the protective properties of IgA.

IgD: Immunoglobulin D causes the differentiation of B cells to a more mature form and is expressed on the surface of B lymphocytes. It is present in a monomeric form with a molecular weight of 180 kD.

IgE: Immunoglobulin E is associated with type 1 hypersensitivity and allergic disease. Its molecular weight is 200 kD. It also plays a role in host defense against parasitic infections. IgE binds to specific Fc receptors on the cell surface of mast cells, basophils, eosinophils, macrophages, monocytes, and platelets. Two main types of Fc receptors for IgE include FcεRI and FcεRII. The former is a high-affinity receptor, whereas the later, also termed as CD23, is a low-affinity receptor. FcεRI receptors are present on mast cells and basophils, whereas FcεRII are present on B cells, although their expression can also be induced on other cell types including monocytes, macrophages, eosinophils, and platelets by TH2 cytokine, interleukin (IL)-4. IgE serves as a stimulus for the upregulation of both receptors. Binding of IgE to its receptors on mast cells results in the release of a variety of endogenous mediators including several cytokines, and the symptoms can vary from a mild allergic response to potentially life-threatening anaphylactic shock. Normal physiologic levels of IgE are low, but under atopic conditions, its levels rise as a result of an isotype switch from IgG to IgE. This is in response to an antigen and under the influence of TH2 cell-derived cytokines.

1.4 Cell Cooperation in the Antibody Response

After exposure to an antigen, its recognition by the immune system is followed either by production of an immune response or the development of tolerance, depending on the circumstances. The immune response could be humoral, cell mediated, or both. On second and subsequent encounters with the same antigen, the type of response is determined by the outcome of the first response. However, the quantity and quality of both responses are very different.

1.4.1 Primary and Secondary Antibody Responses

After administration of an antigen for the first time, there is an initial lag phase where antibodies are not produced. This is followed by a period in which the antibody titer rises logarithmically to a maximum and subsequently declines. The decline is due to either the breakdown or clearance of the antibodies.

The primary and secondary responses differ in four ways:

1. *Time course*. The secondary response has a shorter lag phase and an extended plateau and decline.
2. *Antibody levels*. The antibody levels are ten times higher in the secondary response as compared to the primary response.

3. *Antibody class*. The major proportion of the primary response is made up of IgM, whereas the secondary response consists almost entirely of IgG.
4. *Antibody affinity*. The affinity of the antibodies is much greater in the secondary response as opposed to the primary response, which is termed as "affinity maturation."

1.5 Cells Involved in the Immune Response

The immune system is composed of a variety of different cell types and organs that are involved in specifically recognizing nonself antigens to eliminate them. Phagocytes are an important defense, which participates in both innate and acquired immune responses. The lymphoid cells render the high degree of specificity involved in the recognition of nonself antigens and are part of the acquired immune response. All cells participating in the immune response arise from pluripotent stem cells and are divided into the lymphoid lineage – consisting of lymphocytes – and the myeloid cells, consisting of phagocytes (monocytes and neutrophils) and other cells.

There are three different kinds of lymphocytes that have specific functions: T cells, B cells, and natural killer cells. T cells develop in the thymus, while B cells develop in the adult bone marrow. The thymus and the bone marrow are the primary lymphoid organs where lymphocytes acquire specific cell surface receptors, which give them the ability to recognize antigens. The natural killer cells are cytotoxic lymphocytes that develop in the bone marrow. The phagocytes are made up of either monocytes (macrophages) or polymorphonuclear granulocytes, which include neutrophils, eosinophils, and basophils.

1.5.1 Lymphoid Cells

All lymphoid cells originate during hematopoiesis from a common lymphoid progenitor in the bone marrow. Their formation is known as lymphopoiesis. B cells mature in the bone marrow, while T cells mature in the thymus. The bone marrow and the thymus are called primary lymphoid organs. This is followed by migration via circulation into the secondary lymphoid tissue (spleen, lymph nodes, tonsils, and unencapsulated lymphoid tissue). The average human adult has about 10^{12} lymphoid cells, and lymphoid tissue as a whole represents about 2 % of the total body weight. Lymphoid cells represent about 20 % of the total white blood cells present in the adult circulation. After culmination of the immune response, many mature lymphoid cells live a very long life as memory cells.

Morphology Lymphocytes possess a large nucleus with little to no basophilic cytoplasm. Differences are seen in the nuclear (N) to cytoplasmic ratio, the degree of cytoplasmic staining with histological dyes, and the presence or absence of azurophilic granules.

Markers Most of the lymphocytes express specific cell surface makers on their cell surface. Some are present for a short duration, while others are responsible for their characterization. Such molecules can be used to distinguish various cell subsets. The selected antigenic markers are depicted in Table 1.2.

1.5.1.1 B Cells

These lymphocytes (Fig. 1.5) are unique due to their ability to secrete immunoglobulins. The word "B" refers to "bursa of Fabricius," an organ where B cells mature in birds. In humans, B cells are produced in the bone marrow. The development of B cells occurs at various stages, which include progenitor B cells, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, immature B cells, and mature B cells. Each stage is characterized by rearrangement of certain genes and expression of receptors. In particular, immature B cells start to express IgM receptors and mature B cells express IgD receptors as well.

The cell surface of each receptor possesses unique receptors called B-cell receptors (BCR), which have a membrane-bound immunoglobulin and will bind to a particular antigen. Following encounters with an antigen and recognition of a second signal from TH cells, B cells differentiate into plasma cells. Memory B cells are also formed from activated B cells, which are antigen specific, and quickly respond when they encounter the same antigen a second time, producing secondary immune response. All antigens do not require a second signal from TH cells to activate B cells, which is termed T-independent activation.

A single B cell has approximately 1.5×10^5 antibody molecules on its cell surface, which are all specific for a particular antigen. Other molecules expressed on mature B cells include B220, MHC class II molecules, CD21, CD32, CD35, CD40, CD80, and CD86. B220 is a form of $CD45^-$ $CD45R^-$ and is used to identify B cells despite not being exclusive for B cells. CD40 interacts with its ligand on TH cells, and this interaction is crucial for the development of B cells to differentiate into either antibody-secreting cells or memory cells.

After recognizing antigens through membrane-bound antibodies, there is B-cell proliferation and differentiation for about 4–5 days. This results in the production of plasma and memory cells. One of the five classes of antibodies is produced and secreted by plasma cells, which do not possess membrane-bound antibodies. Plasma cells survive for about 1–2 weeks.

B cells can be activated by both T-cell-dependent and T-cell-independent manner. For T-cell-dependent activation, a certain subset of effector T cells produced in response to an antigen can induce B cells, through a mechanism identified as immunologic synapse. Most antigens are T-cell dependent suggesting that maximal antibody synthesis depends on T-cell help. For T-cell-dependent antigen, the initial signal is emitted by cross-linking of the antigen to the B-cell receptor. The costimulation resulting from T cells constitutes the second signal. T-cell-dependent antigens are peptides, which are present on top of the MHC class II molecules of B cells and are presented to either TH2 cells or follicular helper T cells. The B cell presents the

Table 1.2 Selected antigenic markers on leukocytes

Antigen	Molecular weight (kD)	Distribution	Function
CD1	43–49	Dendritic cells, B cells	T-cell response
CD2	45–58	T cells, NK cells	T-cell activation
CD3	20–28	T cells, NKT cells	TCR expression and signal transduction
CD4	55	T cells, NKT cells	MHC class II-restricted immune recognition
CD5	58	T cells, B cells	Modulation of TCR and BCR signaling
CD8	32–34 each monomer α and β	T cells, TC class I-restricted T cells	Co-receptor
CD11a	180	Leukocytes	α chain of LFA-1-(adhesion molecule)
CD11b	160	Monocytes, granulocytes	α chain of complement receptor CR3 (MAC-1)
CD11c	150	Monocytes, granulocytes	α chain of p150, 95 (complement receptor/adhesion molecule)
CD16	50–80	NK cells, macrophages, neutrophils	Fc receptor subunit (low affinity). Phagocytosis, AD-antigen and ADC-cytotoxicity
CD21	130 (soluble) 145 (membrane bound)	B cells, follicular dendritic cells	Receptors for various antigens. Involved in signal transduction
CD22	140	Mature B cells	Adhesion and signaling
CD23	45	B cells, follicular dendritic cells, monocytes	IgE synthesis regulation, induction of inflammatory cytokines
CD25	55	Mitogen-induced T cells, monocytes/macrophages. Anti IgM-induced B cells	α subunit of IL-2 receptor
CD28	90	Most peripheral T cells. $CD3^+$ thymocytes	Co-stimulator for T-cell activation
CD29	130	Leukocytes	β subunit of VLA-1 integrin
CD32	40	B cells, monocytes, granulocytes	IgG molecule (Fc region) antigen-binding receptor
CD40	48 monomer	B-lineage cells, follicular dendritic cells. Endothelial cells, macrophages	B-cell growth, differentiation, isotype switching. Induction of cytokine release and adhesion molecules
CD45	180–220	Hematopoietic cells	T- and B-cell activation
CD45R	220, 205	B cells, T-cell subsets, granulocytes, monocytes	Restricted leukocyte common antigen
CD80	60	Activated B and T cells, macrophages	Co-stimulator of T-cell activation
CD86	80	B cells, monocytes, dendritic cells	Co-stimulator for T-cell activation

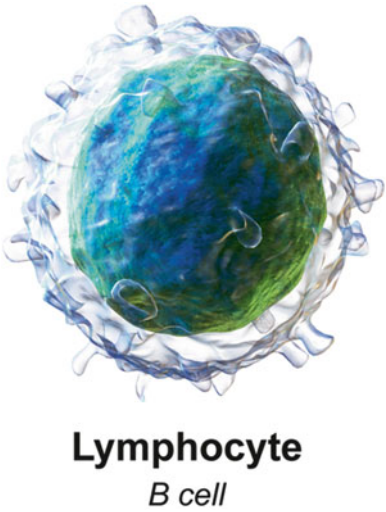

Fig. 1.5 Structure of a B lymphocyte (Source: Blausen.com staff. "Blausen gallery 2014." *Wikiversity Journal of Medicine*. doi:10.15347/wjm/2014.010. ISSN 20018762. – Own work. Licensed under CC BY 3.0 via Wikimedia Commons)

same antigen to the primed TH cells. As a consequence, T cells secrete cytokines that cause the proliferation and differentiation of B cells into plasma cells. Some cytokines cause class switch recombination (isotype switching).

The T-cell-independent B-cell activation involves type 1 T-cell-independent activation and type 2 T-cell-independent activation. T-cell-independent activation of B cells is rapid, but does not involve class switch recombination and germinal center formation. Germinal centers are located within secondary lymphoid organs. At this site, mature B lymphocytes divide, differentiate, and undergo mutation. For type 1, T-cell-independent activation takes place after an antigen is bound to B cells, and secondary activation is achieved through Toll-like receptors. The B cell produced is IgM restricted and is specific for the TLR-binding antigen. The expression of antigens on the surface of invading pathogen is in an organized and repetitive state, which forms the basis of type 2 T-cell activation. These antigens induce specific B cells through cross-linking of antigen receptors in various ways.

1.5.1.2 T Cells

T lymphocytes (Fig. 1.6) develop in the thymus and are consequently called T cells. However, their precursors are found in the bone marrow. The presence of the T-cell receptor distinguishes T cells from other lymphoid cells. There are presently two defined types of TCR; TCR-2 is a heterodimer of two disulfide-linked polypeptides with a molecular weight of 90,000 kD (α and β), and TCR-1 is structurally similar but consists of γ and δ polypeptide. Each chain has a constant and a variable region. These chains are characterized by an intrachain disulfide bridge and some sequence homology with the immunoglobulin domains. Both receptors are associated with a complex of polypeptides making up the CD3 complex. Thus, a T cell is defined

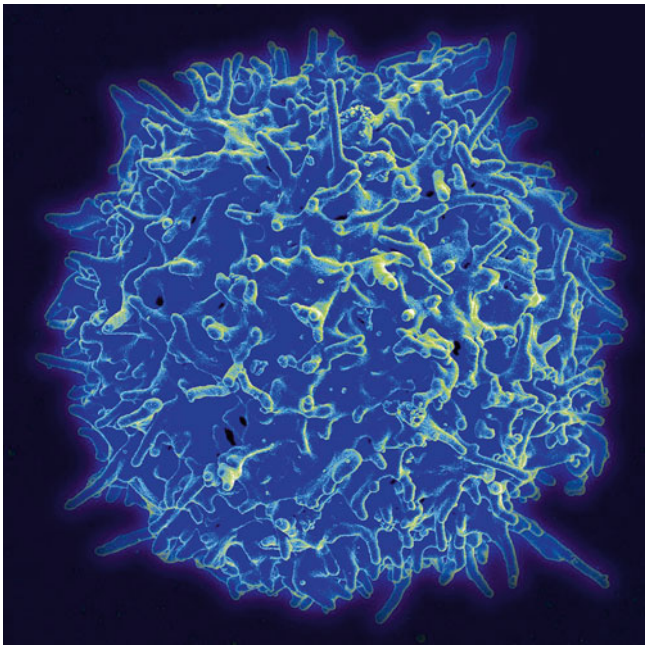

Fig. 1.6 Structure of a T lymphocyte (Source: NIAID, public domain)

either by TCR-1 or TCR-2, which is associated with CD3. Approximately 95 % of blood T cells express TCR-2 and up to 5 % have TCR-1. The TCR-2 cells are composed of two glycoprotein chains α and β and are subdivided into two distinct populations, the TH cells that are $CD4^+$ and Tc cells, which are $CD8^+$. $CD4^+$ T cells recognize antigens in association with major histocompatibility complex (HC) class II molecules, whereas $CD8^+$ T cells recognize antigens in association with MHC class I molecules.

Helper T Cells

Helper T cells ($CD4^+$ T cells) play the most crucial role in acquired immunity. The development of helper T cells takes place in the thymus, after positive and negative selection. In the thymus, cells carrying both $CD4^+$ and $CD8^+$ cells are eliminated, and only cells recognizing either MHC class I or MHC class II molecules are retained. The cells recognizing the antigen in the context of MHC class II molecules become the helper T cells. $CD4^+$ T cells are activated through class II-restricted antigens. They regulate a number of immune responses including T-cell proliferation, isotype switching of the antibodies, generation and effector function of cytolytic T cells, induction of NK cell activity, and inducing bactericidal activity of phagocytes. Most of their effects are mediated via secretion of cell-specific

cytokines. They are called $CD4^+$ T cells, as they express CD4 receptors. However, there are some other cells that also express CD4 receptors, but they are considered as exception to the rule. While there are two major subsets of helper T cells, TH1 and TH2, additional subsets including TH17, TH9, Treg, TH22, and THαβ have also been identified.

Induction of $CD4^+$ T cells results in their expansion and differentiation into effector as well as regulatory subsets. $CD4^+$ effector T cells, TH1 and TH17, and Treg cells function in metabolically different ways. In murine models, inflammatory effector T cells maintain enhanced levels of glycolytic genes and depend on high glycolytic rates, whereas Treg cells are oxidative for their division, differentiation, and survival. They also depend on the mitochondrial electrons. The bifurcation between T-cell glycolytic and oxidative metabolism depends on pyruvate dehydrogenase. Pyruvate dehydrogenase kinases suppress the activity of pyruvate dehydrogenase. Pyruvate dehydrogenase kinase 1 is present in TH17 cells, and in low amounts in Treg cells, but not in TH1 cells. The antagonism or deletion of pyruvate dehydrogenase kinase 1 specifically inhibits TH17 cells and augments Treg cells. It is apparent that $CD4^+$ subsets employ specific metabolic programs, and the inhibition of these pathways should regulate diverse T-cell subsets, which may have clinical implications.

The major two subsets of helper T cells are differentiated on the basis of cytokines that they secrete, as a result of their proliferation. TH1 cells are involved in cytotoxicity and regulate the killing of the invading pathogens, specifically intracellular bacteria and protozoa. They are characterized by the secretion of IFN-ϒ and are induced by IL-12, but they also secrete additional cytokines including TNF-α and TNF-β. The effector cells for TH1 immune response include cytolytic T cells, macrophages, IFN-ϒ, CD4 T cells, and IgG B cells. STAT4 and T-bet are the predominant transcription factors for TH1 cells. TH2 cells support B-cell activation, isotype switching, IgE production, recruitment, and induction of various cell types and are antiparasitic by natural design. This function is performed by TH2 cells through secreting a variety of cytokines, including IL-4, IL-5, IL-10, and IL-13. IL-2, IL-3, IL-25, IL-31, and GM-CSF are secreted by both subsets. The proliferation of TH2 cells is induced by IL-4. STAT6 and GATAs are the major transcription factors for TH2 cells. However, there is also IL-4-/STAT6-independent signaling, which may result in TH2 differentiation in a complementary way. Eosinophils, basophils, mast cells, B cells, and IL-4/IL-5 CD4 T cells are the principal effector cells. TH2 cells are predominantly involved in the etiology and pathogenesis of allergic disease and asthma.

TH17 is a subset of helper T cells that produces IL-17. These cells are responsible for establishing immunity to the microbial agents at epithelial/mucosal barriers (Fig. 1.7). TH17 cells develop separately from TH1 and TH2 cells. They are induced either by IL-6 and TGF-β or IL-23 and IL-1β. Their effector cells include neutrophils, IL-17 $CD4^+$ T cells, and IgM/IgA B cells. STAT3 and retinoic acid receptor-related orphan receptors G are their main transcription factors. The effector cytokines include IL-17, IL-21, and IL-22. Deficiency/lack of TH17 cells leaves a host susceptible to opportunistic infections. IL-22 acts on epithelial cells to produce certain proteins that are bactericidal. In response to distinct cytokines, TH17 cells

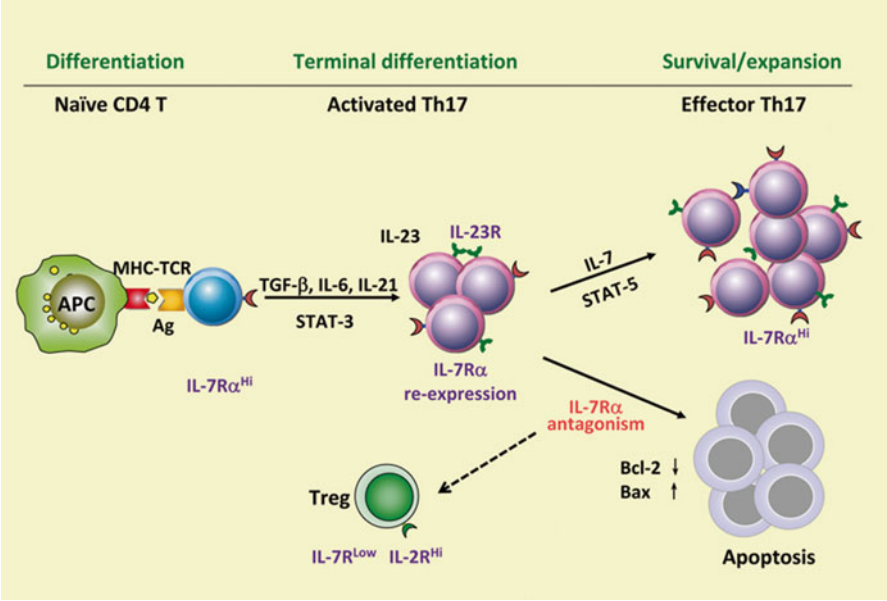

Fig. 1.7 This figure depicts the differentiation, survival, and proliferation of TH17 cells. The role played by IL-17 in the survival and proliferation of TH17 cells is also shown. Other cytokines including IL-6, IL-21, and IL-23 are also involved in the differentiation of TH17 cells. During T-cell activation and differentiation, IL-7 receptors are expressed on TH17 cells (Reproduced with permission, Source: Leung S, Liu X, et al. (2010). The cytokine milieu in the interplay of pathogenic TH1/TH17 cells and regulatory T cells in autoimmune disease. *Cellular and Molecular Immunology*, 7: 182-189. Nature Publishing Group)

will develop either into protective or pro-inflammatory cells. The pro-inflammatory TH17 cells are produced by IL-23 and IL-1 β, and the protective TH17 cells are generated by IL-6 and TGF-β, which are the T regulatory 17 cells.

TH17 cells are involved in the production of two members of IL-17 family. These members are IL-17A and IL-17F, which play a role in the recruitment, activation, and migration of neutrophils (Fig. 1.7).

The steroid receptor-type nuclear receptor RORϒt is required for IL-17 production, a cytokine released by TH17 cells. In addition to RORϒt, another member of the retinoid nuclear receptor family, RORα, which is also selectively expressed in TH17 cells, plays a similar but not identical role in the differentiation of TH17 cells. These observations suggest that two lineage-specific transcription factors are involved in the differentiation of TH17 cells. STAT3 plays a role in the induction of RORϒt. IL-6, IL-21, and IL-23 play a crucial role in this process via specifically activating STAT3.

The development of TH17 cells and Treg cells is interconnected reciprocally. This was demonstrated by experiments where TGF-β in the presence of either IL-6 or IL-21 resulted in the development of TH17 cells and in inhibition of Treg development. The presence of both TGF-β and IL-6 or IL-12 results in a strong

production of IL-17 cells from the naïve T cells. However, this response is not produced if the aforementioned combination is not used.

IL-23 also plays an important role in inducing the synthesis of IL-17 by activated T cells. A full sustained development of TH17 cells requires IL-23. For a productive and sustained TH17 response, the presence of IL-23 is required, despite the observation that IL-23 is not a differentiation factor for this T-cell subset. In contrast both IFN-ϒ and IL-4 are inhibitors of TH17 differentiation. TH17 cells are involved in inflammation and tissue injury in autoimmune diseases that include rheumatoid arthritis, Crohn's disease, juvenile diabetes, multiple sclerosis, autoimmune uveitis, and psoriasis.

TH9 cells were originally considered to be included in TH2 cells and they secrete IL-9. Since both IL-4 and IL-9 are rarely secreted by the same cell, IL-9-producing cells are classified as a separate subset of helper T cells. IL-9 is secreted by TH9 cells in response to IL-4, TGF-β, and IL-1 and inhibited by IFN-ϒ. The development of TH9 cells is also induced by other stimuli, which include IL-25, tumor necrosis factor receptor superfamily member 4 (TNFRSF4 or Ox40), cyclooxygenase (COX)-2, 1,25-dihydroxyvitamin D3, calcitonin gene-related peptide (CGRP), thymic stromal lymphopoietin (TSLP), Jagged 2, and programmed cell death ligand (PD-L)2. The development of TH9 cells is mediated by a series of complex pathways. IL-2 receptors, IL-4 receptors, and TGF-β-dependent signal transduction pathways are required for their development. The CD28-derived signals are important in the induction of IL-9 production by TH9 cells. Their development is transcriptionally regulated by IRF4 and PU.1. IL-9 plays a role in the development of allergic and autoimmune diseases. Furthermore, they may also play a role in cancer, since in animal models they augment immune response against melanoma (Fig. 1.8).

THαβ helper cells are responsible for providing immunity against viruses. They are induced by IFN-α/IFN-β and/or IL-10. The latter is the key effector cytokine for the THαβ helper subset. The main effector cells include NK cells, cytolytic T cells, IgG B cells, and IL-10 $CD4^+$ T cells. STAT1, STAT3, and IRFs are their principal transcriptional molecules.

TH22 cells are identified as a separate subset of helper T cells since some T cells secrete IL-22 independent of IL-17, specifically $CCR10^+$ T cells. IL-22 belongs to IL-10 family and binds to a heterodimer receptor (IL-10 receptor β chain and the IL-22R); its function is distinct when compared to IL-10. IL-22 is expressed only on nonimmune cells and also induces three MAPK pathways. The production of IL-22 is dependent on IL-23 and transcriptional factors T-bet and AhR in some experimental models. It is involved in epithelial innate responses with dual protective as well as detrimental roles. IL-22 is neither pro- or anti-inflammatory in its effects. The distinct function of TH22 cells is epidermal immunity and remodeling.

Cytolytic T Cells

Cytolytic T cells ($CD8^+$ T cells) kill virus-infected and cancer cells. They are antigen specific and recognize their antigen in the context of MHC class I molecules. The development of cytolytic T cells takes place in the thymus, after positive and

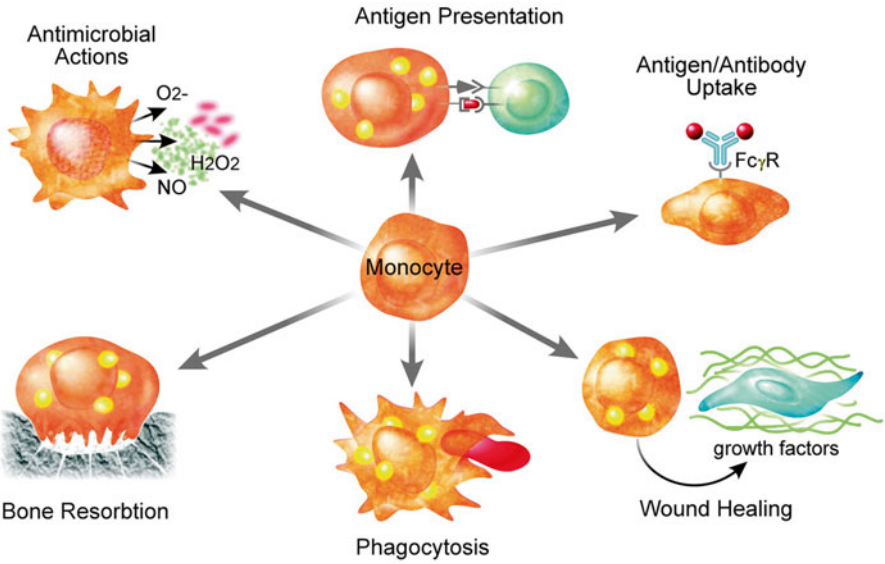

Fig. 1.8 This figure depicts the development and role of TH9 cells. TH9 cells develop from naïve TH cells requiring IL-2 and are induced by TGF-β and IL-4. Their production is augmented by IL-1, IL-5, IL-7, IL-8, and IL-9. TH9 cells via releasing IL-9 play a role in allergic disease, autoimmune disorders, leukemia, and melanoma (Reproduced with permission, Source: Schmitt et al. (2014) Th9 cells, a new player in adaptive immunity. *Trends in Immunology*. 35: 61–68. Elsevier Limited)

negative selection, where the cells carrying both $CD4^+$ and $CD8^+$ cells are eliminated, and only cells recognizing either MHC class I or MHC class II molecules are retained. The cells recognizing antigens in the presence of MHC class I molecules become the cytolytic T cells. They are activated through class I-restricted antigens. After the development into effector cytolytic T lymphocytes, they migrate to the tissues where they are activated in response to coming in contact with the target cells. Upon activation cytolytic T cells will proliferate in response to IL-2. Cytolytic T cells release perforin, granulysin, and granzymes, which are all cytotoxins. These cytolytic effector proteins are present in cytoplasmic exocytic granules. Granzyme, a part of large family of serine proteases, is expressed in a variety of cells involved in innate and acquired immunity. It enters the cytoplasm of the target cells through perforin-mediated entry, and a caspase cascade is activated. Perforin is a pore-forming protein, and these series of events lead to apoptosis by chymase activity of granzyme, of the target cells. In animal models, inactivation of Prf1 gene inhibits cytolytic T-cell effector function and immune response, without compromising the development of T lymphocytes.

The killing of target cells by cytolytic T cells requires TCR, MHC class I molecules, and interaction of adhesion molecules. In particular, interaction of LFA-1 expressed on cytolytic T cells and ICAM molecules expressed on the target cells plays a stabilizing role in this effector function. The TCR-/CD3-associated signal transduction activates tyrosine kinase that causes phosphorylation of PLCϒ, activa-

tion of PKCs, and Ca^{++} influx. As a result there is granule polarization and exocytosis, and after contact cytolytic T cells release their content into the target cell resulting in its killing.

Natural Killer T Cells

Activation of natural killer T cells results in the performance of functions similar to $CD4^+$ and $CD8^+$ T cells. They release cytokines including IL-4, IFN-γ, GM-CSF, as well as others and are also cytotoxic. They are mostly not MHC restricted, instead they recognize antigens that are glycolipids and require CD1d. NKT cells bridge innate and acquired immune responses. They express both αβ T-cell receptor and markers present on natural killer cells such as NK1.1. Natural killer T cells include $NK1.1^+$, $NK1.1^-$, $CD4^+$, $CD8^+$, $CD4^-$, and $CD8^-$ cells. They also express CD16 and CD56 antigens, which are present usually on NK cells. NKT cells are classified into type 1 NKT, type 2 NKT, and NKT-like cells. NKT-like cells are not CD1d restricted and may have MHC or other restrictions. Abnormal NKT cell function may be associated with cancer and autoimmune disease.

Gamma Delta T Cells (ϒδ T Cells)

Gamma delta T cells are a minor subset of T cells that express a distinct T-cell receptor on their surface membrane. Their distribution is high in gut mucosa, included in a population of lymphocytes known as intraepithelial lymphocytes (IELS). This subset has a T-cell receptor, which is made up of one ϒ and one δ chain. In the peripheral blood, the γδ T-cell population is composed of Vγ9/Vδ2 T cells and is unique in the sense that this is specific for a non-peptide microbial metabolite, HMB-PP, which is a precursor of isopentenyl pyrophosphate. The γδ T-cell population produces a rapid response after recognizing HMB-PP. They do not recognize peptide epitopes in the context of MHC molecules after antigen uptake and processing. However, some recognize MHC class Ib molecules. They play a prominent role in the recognition of lipid molecules. Gamma delta T cells are of invariant nature, and perhaps alarm signals such as heat shock proteins cause their induction. In addition, there is a subpopulation of this T-cell subset present in the epidermal compartment of the skin (murine) and termed dendritic epidermal T cells.

They are considered "first-line defense" or a bridge between innate and acquired immune responses. Gamma delta T cells develop in the thymus and in the periphery, in response to signals from other leukocytes. They divide into functionally distinct subsets, after maturation and influence immune cells, as well as healthy tissue, both directly and indirectly. Gamma delta T cells produce host responses to the invading pathogens. Their features classify them in between innate and acquired response, because on one hand they allow a rapid immune response against a foreign antigen and on the other hand rearrange TCR genes to cause junctional diversity. The later function is a part of acquired immunity due to the development of a memory phenotype in response to an antigen. But their restricted TCR may be utilized as a pattern

recognition receptor, and thus they will be classified as part of innate immune response. Based on additional available data, it seems that gamma delta T cells can fit the definitions of both innate and acquired immune responses.

Memory T Cells

Memory T cells belong to a subset of infection and cancer-fighting T cells, which have previously recognized and responded to their cognate antigen. They recognize viruses, bacteria, or cancer cells. Memory T cells are a product of primary immune response, and as a consequence, when they face the same invading pathogen or the tumor cell for the second time, they produce a rapid and potent response. Three distinct subsets of memory T cells have been found, as they can be recognized by the differential expression of L-selectin and CCR7. The first subset is called stem memory T_{scm} cells. They are $CCR7^+$, $CD45RO^-$, $CD62L^+$, $CD45RA^+$, $CD27^+$, $CD28^+$, and IL-7Rα^+. The second subset is called central memory T_{cm} cells. They secrete IL-2, but not IL-4 or IFN-ϒ, and express CCR7 and L-selectin. The third subset is called effector memory T_{EM} cells. These cells produce IL-4 and IFN-ϒ, but do not express CCR7 or L-selectin. T_{CM} and T_{EM} subsets contain antigen-specific memory T cells directed against viruses and other microbial molecules. Both helper and cytolytic T cells contain these subsets of memory T cells. The T_{CM} has some common characteristics, which is also exhibited by memory cell stem cells. There is an increased level of phosphorylation of STAT5 in these cells, which allows their self-renewal. As opposed to T_{EM} cells, the T_{CM} cells are more potent in conferring immunity against viruses, bacteria, and tumor cells.

Memory B cells are produced within germinal centers after the primary infection. They are responsible for a rapid and robust secondary immune response, following the reexposure to their specific primary antigen. During primary immune response or first exposure to an invading pathogen exhibiting T-dependent antigen, in the presence of T_{FH} cells within the follicles of secondary lymphoid organs, there is an activation of naïve follicular B cells. This results in the production of antigen-specific foci of B cells. These cells differentiate to become antibody-secreting cells (plasma cells) to fight infection. After the infection is cleared, a fraction of these cells remain as dormitory memory cells. These memory cells have long life, after they go through a mutative and selective germinal center reaction. The activated B cells that do not go through germinal center differentiation are eliminated. With each subsequent exposure to the same antigen, there is a generation of polyclonal secondary response, and increased number of memory B cells remains.

Regulatory T Cells

The Treg cells were previously known as the suppressor T cells and are pivotal in maintaining immune tolerance. Their principal functions include a negative feedback after the generation of an immune response to limit unintended damage and to protect from autoimmunity. These cells are described in detail in chapter 10.

1.5.2 Natural Killer Cells

After B and T cells, NK cells are the third largest class of lymphocytes that were originally identified due to their spontaneous killing ability of tumor cells. They develop in the bone marrow from common lymphoid progenitor and require IL-15, C-KIT, and FLT-3. NK cells share effector function and the ability to produce cytokines with T cells. They were previously referred to as null cells because they do not express either T-cell or B-cell receptors, do not secrete antibodies, and do not possess antigen-recognizing receptors. Their morphology is also different from B and T cells, as they are large granular lymphocytes. These cells make up about 5–10 % of the lymphocytes in human peripheral blood.

NK cells participate in innate immunity and are the first responders against infection and possibly tumors. They are $CD3^-$ and also lack immunoglobulin receptors; however, they carry CD56 antigen, which is used to identify these lymphocytes. NK cells also express CD16, which is a low-affinity receptor for IgG and is not present on mature T cells. They are involved in antibody-mediated cellular cytotoxicity and apoptosis. Following binding of CD16 of NK cells to the Fc portion of IgG, cytoplasmic granules are released, which cause the destruction of the target cell where CD16 facilitates the release of the cytoplasmic granules. Natural cytotoxicity receptors (NCR) are exclusively expressed on NK cells and include NKp30, NKp44, NKp46, as well as NKp80. NKp44 and NKp46 are important in defense against viruses, as they bind to influenza hemagglutinin. Another NK cell surface receptor, 2B4, binds to CD48 and may play a role in the defense against Epstein–Barr virus. A defect in 2B4 function is associated with the X-linked lymphoproliferative syndrome. In addition, NK cells possess receptors for a variety of cytokines including IL-2, IL-12, IL-15, IL-21, IFN-α, and IFN-ϒ.

The killing ability of NK cells is associated with the expression of MHC class I molecules. According to the "missing-self hypothesis," NK cells search for the presence of MHC class I molecules, which are ubiquitously expressed. A decrease in the expression of MHC class I molecules on a cell allows NK cells to kill the target, as it is released from the influence of MHC class I molecules. The ability of NK cells to kill tumors and virally infected cells resides in several inhibitory receptors called immunoglobulin-like receptors and CD94/NKG2 heterodimers. The immunoglobulin-like receptors include KIR, immunoglobulin-like transcripts (ILT), and leukocyte Ig-like receptors (LIR). These receptors bind to HLA and after a cascade of signal transduction, inhibit NK cell stimulation. In addition to these inhibitory receptors, there are also stimulatory receptors that rely on perforin and INF-γ for their function. NK cells produce a number of other cytokines and chemokines, including TNF-α, IL-5, IL-13, GM-CSF, MIP-1 (α and β), as well as RANTES.

These cells are crucial in fighting viral infections as part of early innate response. Severe systemic viral infections, specifically herpes virus, may result from a lack or malfunction of NK cells. Patients infected with HIV have low numbers of NK cells. They also play a role in killing tumors. The patients with Chediak–Higashi syndrome have an increased risk of lymphomas, and this disease is associated with

impaired NK cells, macrophages, as well as neutrophils. A number of cytokines including IL-2, IL-12, IL-15, IL-21, IFN-α, and IFN-β induce NK cells, which results in their proliferation, margination, cytokine production, and cytotoxicity.

1.5.3 Antigen-Presenting Cells

APCs are a heterogeneous population of cells with extraordinary immunostimulatory capacity. Some play an important role in the induction of the function of the activity of helper T cells, and some communicate with other lymphocytes. Cytokines can render the ability to present antigens to a variety of cell types. This results in the expression of MHC class II molecules, which are sometimes lacking on some cells such as endothelial cells. The different types of antigen-presenting cells include macrophages, dendritic cells, B cells, and interdigitating cells. Antigen-presenting cells are mostly derived from the bone marrow and are distributed in lymphoid tissues as well as in the skin. These three types of major antigen-presenting cells are also called professional antigen-presenting cells.

1.5.3.1 Macrophages

Macrophages (Fig. 1.9) participate in innate as well as acquired immune response. As opposed to T and B cells, they are not characterized by any specific cell surface receptors and play an important role in normal tissue repair and aging. They are phagocytes that continuously remove self-proteins, which are degraded and presented to T cells in the context of MHC class II molecules. However, this does not result in the activation of T cells, since in the absence of infection, the expression of MHC class II molecules on macrophages is low and the presence of B7, a costimulatory molecule, is almost negligible. Following infection, there is an upregulation of MHC class II and B7 molecules.

Also termed as mononuclear phagocytic system, monocytes circulate in the blood and macrophages in the tissues. In the bone marrow during hematopoiesis, the progenitor cells for granulocytes–monocytes differentiate into promonocytes. The promonocytes then leave the bone marrow and enter the bloodstream. In the blood, promonocytes mature into monocytes. Monocytes/macrophages are derived from the bone marrow stem cells. After monocytes enter damaged tissue via endothelium by chemotaxis, they differentiate into macrophages. Monocytes undergo multiple changes during differentiation to macrophages; they enlarge several folds, the number of intracellular organelles is increased, they are able to hydrolyze enzymes, and their phagocytic ability is augmented. As shown in Table 1.3, macrophages are classified according to their tissue distribution, where their functions are diverse and tissue specific.

The most important role of macrophages is antigen presentation. However, in addition to antigen presentation, macrophages play several other important roles in

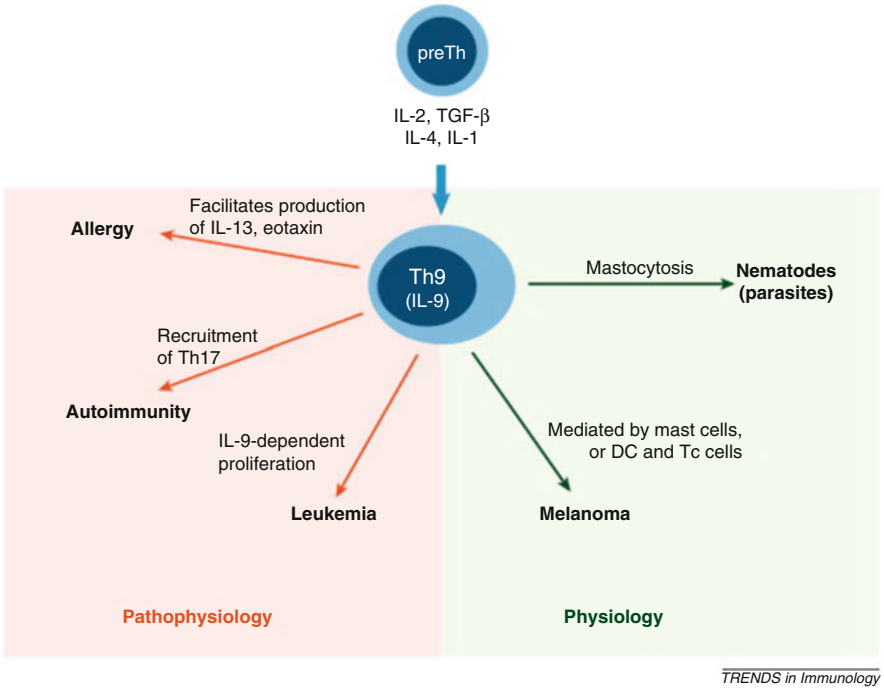

Fig. 1.9 Functions of monocytes/macrophages: macrophages are involved in first line of defense against invading pathogens. This is performed by producing oxidative burst and release of the inflammatory mediators. They are also involved in acquired immune response through their role in antigen presentation and cytokine secretion. They clear immune complexes and cause wound healing by releasing growth factors (Source: Chawla A. *Circulation research*, 2010, 106: 1559–1569, Lippincott Williams & Wilkins, reproduced with permission)

Table 1.3 The types of macrophages and their distribution

Name	Tissue type
Alveolar macrophages	Lungs
Kupffer cells	Liver
Histiocytes	Connective tissue
Microglial cells	Brain
Osteoclasts	Bone
Mesangial cells	Kidney

immune response, which include inflammatory response, antitumor activity, microbicidal activity, lymphocyte activation, and tissue reorganization. Most of their physiopathological effects are mediated via cytokines. In addition to the microbicidal activity, they release oxygen-dependent free radicals, cytotoxins, antimicrobials, and oxygen-independent hydrolases. For tissue reorganization, they secrete collagenases, elastases, and angiogenesis factors (Fig. 1.9).