SECTION I

Introduction to Immunology

CHAPTER 1

Overview of the Immune System

Martin F. Flajnik, PhD • Steven M. Holland, MD • Nevil J. Singh, PhD

As we edit this book during a global pandemic, we are acutely aware of the remarkable prowess of the immune system. It is critical to keep us safe, in the face of constant threats from the myriad potentially pathogenic microorganisms that inhabit the world. A half-billion years of arms race between such microbes and the mechanisms used by the immune system to neutralize the threats they pose, has resulted in the evolution of a complex and multilayered system of defense (Chapters 3-5). It begins with layers that offer a nonspecific barrier to pathogens and becomes increasingly specific, crowned by the adaptive immune system that can discriminate narrowly between even minor variants of a particular microbe (Fig. 1.1). The tragic examples of acquired immunodeficiency syndrome (AIDS) or inherited immunodeficiencies illustrate the consequences of impaired adaptive immunity. Patients with AIDS (Chapter 45) and children with severe combined immunodeficiency (SCID) fall victim to infections that are of little or no consequence to those with functioning immune systems. The immune system also monitors threats that arise from within our bodies. It recognizes and rejects spontaneous tumors and is involved in healing wounds. Elegant recognition mechanisms allow the immune system to make very specific receptors against almost any target, a process exploited commercially to develop antibodies for many applications. Yet these extensive capabilities also force the immune system to constantly walk a tightrope, to avoid damaging healthy cells in the body—via autoimmunity—while still attacking diverse threats to its well-being. Rigorous control mechanisms have emerged to keep autoimmunity at bay, which can break down and result in autoimmune diseases (including insulin-dependent diabetes mellitus, multiple sclerosis, rheumatoid arthritis, etc). In addition, excessive inflammatory pathology from damaging healthy cells can occur while attacking an infection even in the absence of autoimmunity; thus, other layers of regulation have emerged to prevent or dampen such autoinflammation.

Fundamental Immunology was conceived in 1984 by Dr. William E. Paul to provide an authoritative presentation of the basic elements of the immune system; of the means by which the mechanisms of immunity act in a wide range of clinical conditions, including recovery from infectious diseases, rejection of tumors, transplantation of tissue and organs, autoimmunity and other immunopathologic conditions, and allergy; and how the mechanisms of immunity can be marshaled by vaccination or biologic therapies to confront tumors and pathogens. This edition perpetuates the long tradition championed by Dr. Paul, to recruit experts in each field to provide an in-depth contemporary summary of our understanding of these topics in the following 49 chapters.

The purpose of this opening chapter is to provide readers with a general introduction to the immune system. It should be of particular importance for those with a limited background in immunology, providing them with the preparation required for subsequent chapters of the book and the capacity to then follow the basic and translational immunological literature. Rather than providing extensive references in this chapter, each of the subjects will designate the chapters that deal in detail with the topic under discussion. Those chapters will not only provide an extended treatment of the topic but will also furnish the reader with a comprehensive reference list.

THE IMMUNE RESPONSE IN A NUTSHELL

Microbes gain access to our bodies through tissue surfaces that are exposed to the environment, such as the skin, lung epithelium, and inner linings of the intestines. The first layers of defense against pathogen entry are the **barriers** presented by these surfaces themselves (Fig. 1.1). These structural barriers represent the most effective protections we possess, and microbes which colonize the outside of the barrier surface can often enter a mutualistic relationship, becoming commensal flora. As long as a more distal layer of immunity is

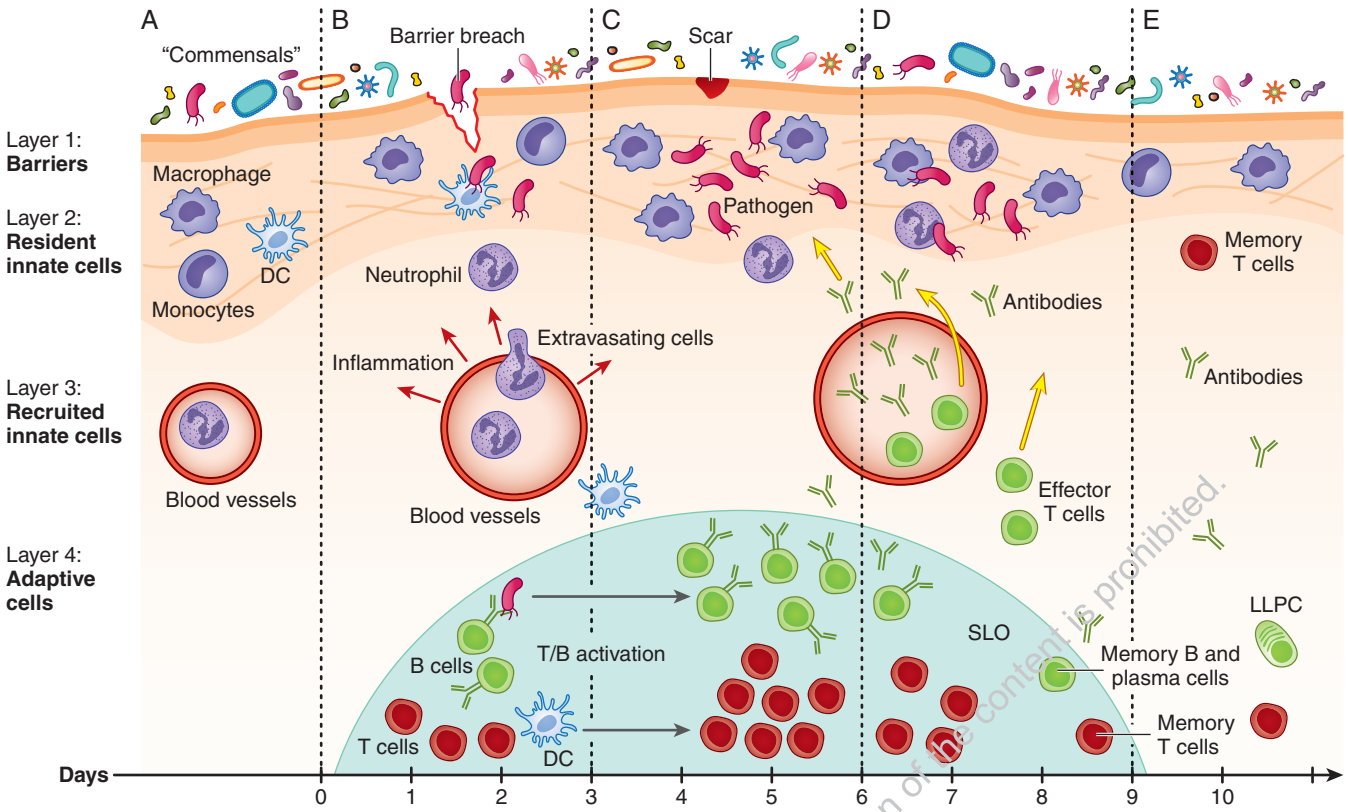


FIG. 1.1. Stages of an immune response. A: Prior to the infection, commensals exist in homeostasis with the host outside the barrier, such as skin. Macrophages, dendritic cells (DCs), and monocytes are resident innate cells within the tissue. **B:** When the barrier is breached by a microbe, resident cells are activated by pattern recognition receptors, sending signals such as cytokines and chemokines to recruit innate cells like neutrophils from the blood via extravasation (also called transendothelial migration). DCs begin to move through the lymph to the draining lymph node (SLO [secondary lymphoid organ]). **C:** Invading innate cells destroy the microbes, while the adaptive response is initiated with the activation of T cells (red) and B cells (green) in the SLO, followed by clonal proliferation and the first production of antibodies. Scars may be formed via homeostatic mechanisms at the breach site. **D:** Innate cells become armed with antibodies for microbe destruction and the breach is healed. Effector T cells and natural killer cells may also come to the site. **E:** Memory B and T cells are generated that may stay in the tissue for some time and disseminate to SLO all over the body. Plasma cells as long-lived plasma cells (LLPC) can persist for months or years.

successful in repelling pathogens, no more central layer in the immune system is activated. When a microbe breaches the barrier, **preformed effector molecules** such as the Alternative Complement (C′) Pathway or constitutively produced lectins might target the pathogen for destruction. The next layer of defense that is activated is **cells of the innate immune system**. Unlike barriers and preformed effectors, which are always ready to resist invasion (ie, "always on" or constitutively protective), this next layer consists of cells that are typically quiescent and positioned just below the barrier tissues. Once they detect pathogens, tumors, or the tissue-damage caused by either, these innate immune cells are activated and mount an effector response that includes phagocytosis, secretion of noxious compounds, and production of proteins that promote downstream immunity. Although these cells broadly sense invasion or damage (using pattern recognition receptors [PRRs]), they are relatively nonspecific in their activation. These PRRs are found in all organisms (Chapters 3-5, 12), not just vertebrates, and this early response is therefore the evolutionarily more ancient form of defense. During this initial response, the site of infection also starts generating **inflammation**. In addition to making the infection site inhospitable to some microbes, inflammation also promotes blood flow to the area, disrupts tight junctions of blood vessel endothelia, and **recruits another layer of circulating innate immune cells** to the venue. This early innate response (barriers, preformed effectors, locally activated cells, and recruited effector cells) is the major defense mechanism for the first 2 to 3 days after an infection. If the pathogen (or tumor or irritant) continues to pose a threat, **cells of the adaptive immune system** are then enlisted for defense. The reason why these cells (T cells and B cells) are delayed in the response is because they are initially activated away from the site of infection, in specialized secondary lymphoid organs (SLOs) (Chapter 8), and the frequency of cells specific for the pathogen/tumor is very low (so-called precursor frequency). After almost a week of activation, cellular proliferation, and maturation, these cells can contribute cellular effectors (T cells) or antibodies (made by B cells), which leave the SLO and circulate back to the tissue site where the pathogen resides (Fig. 1.1). A considerable upgrade that comes with the adaptive immune response is that, unlike the PRR used by the innate system to detect pathogen or damage, their receptors (T cell receptors [TCRs] and antibodies or immunoglobulins [Ig]) are extremely specific for their target. If these sequential (and cooperating) layers are eventually effective and the pathogen

is cleared, some of the T cells and B cells that were activated in this process persist for a long time in the body as **memory cells**. Immunological memory allows the immune system to mount a much more rapid response when the same pathogen is again encountered (ie, it does not require the extra week of activation and maturation to mount the response). Obviously, this sequence of events requires an extensive choreography to ensure that all the "right cells" develop in the body, arrive at appropriate tissue sites at the required time, use specific receptors to be activated, deliver critical sequential effector responses, and incorporate sufficient control mechanisms to suppress the response at the appropriate time. We will now summarize these cell types, processes, and pathways, and discuss the overarching principles evolved in the immune system to operate or deploy all of these armaments appropriately.

KEY PRINCIPLES UNDERLYING AN IMMUNE RESPONSE

While we have historically viewed the immune system as a protective force charged with attacking and repelling pathogens and tumors, it is increasingly clear that it plays a more complex role in keeping the body healthy. In some cases, the latter mandate even requires the system to "ignore" some microbes (for example, commensals or even chronic infections [Chapter 38]) especially if the costs of continued inflammatory responses are high. It participates in healing wounds, facilitating organ development, reinforcing neurological learning, etc. Considering these broad functions, the central principles governing the operation of the mammalian immune system are summarized below. Developing an understanding of these general concepts can be helpful for students, as they try to digest the significance of each detailed mechanistic process in the chapters that follow.

1. *Barriers—The immune system relies on a strong system of passive barriers to avoid mounting an offense.* Resisting infection or the damage due to infection by offering structural barriers around the body is an evolutionarily conserved defense strategy. Some sensitive organs (eg, the brain) have additional layers of selective barriers. Most of the environmental threats the body faces are kept out by these barricades. In turn, the immune system is primarily organized around these barrier functions, to catch threats that manage to get past.
2. *Tolerance—Multiple mechanisms and cross-checks operate to avoid attacking healthy parts of the body.* If pathogens breach immunological barriers, subsequent protection requires immune cells to be activated to perform their defensive roles. Critically, such activation requires sensing the threat using cellular receptors. Innate cells use receptors to conserved molecules (patterns) found in many pathogens or unhealthy cells. The adaptive immune system uses many more specific receptors that surgically identify minute features on each threat. In both instances, it is important that immune cells are not activated in response to normal body cells or targets in healthy tissues. Such discrimination is not trivial since pathogens can also mutate the targets of immune attack to evade it. The innate PRRs are typically quite conserved over evolution (Fig. 1.2) and have presumably been optimized over eons to identify microbes or damage but spare healthy cells. T and B cell receptors (AKA antigen receptors) are

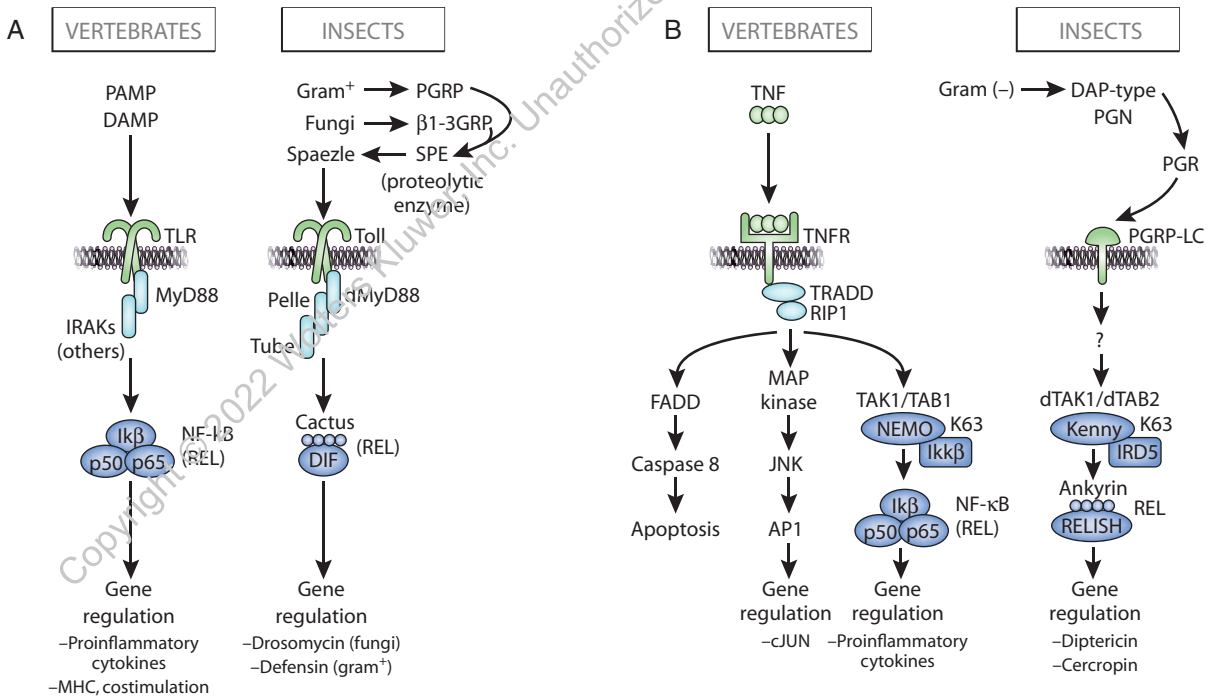


FIG. 1.2. Comparison of innate immune response induction, intracellular pathways, and immune outcome in insects (eg, *Drosophila*) and vertebrates (eg, human). Note that the initiation of the innate response and the outcome(s) in insects and vertebrates are quite different for both the toll/toll-like receptor pathway (*left*, **A**) and the immune deficiency/tumor necrosis factor pathways (*right*, **B**), but the intracellular signaling pathways are well conserved evolutionarily in all animals.

educated and regulated by a variety of processes to avoid strong reactivity against healthy self-tissues. While the term "immunological tolerance" (or self-tolerance) usually applies to the latter, the general principle of tolerating healthy tissues operates in recognition steps for most cells in the immune system.

3. *Layering—The immune system sequentially engages and coordinates the functioning of increasingly threat-specific tiers of defense.* As discussed above, if the pathogen evades one layer of defense, then the immune system has another one called to action (Fig. 1.1). Understanding this tiered approach to immune functions involves dissecting how each layer is sequentially activated. In general, cells in the earlier layers activate the subsequent ones. This process, however, also involves a "bridging" of the layers, such that all the early-activated cells continue to cooperate to neutralize the ongoing threat. For instance, adaptive immune cells (T cells) require critical cells in the preceding layer (that is, activated innate cells such as dendritic cells [DCs] [Chapter 34]) to be activated. But once fully activated, effector T cells look to the earlier layers by further recruiting and arming innate cells to respond more robustly. This cross-connectivity and coordination between different cell types requires sophisticated cell-to-cell communication mechanisms. Typically, this involves messenger proteins called cytokines and chemokines (Chapters 9-12) secreted and sensed by cells in different layers.
4. *Customization—Each immune response is tailored to both the nature of the threat and the specific tissue location affected.* There are a range of insults that can threaten our body including toxins, viruses, bacteria, parasites, tumors, etc, and the immune system deploys different weapons to fight each of these effectively. Phagocytosis and degradation may be appropriate for a small toxic particle, but this does not work for defense against a large worm. An intracellular virus requires different mechanisms for elimination than an extracellular fungus. Secondly, the precise tissue in which the immune system must battle a pathogen is important to consider: immune responses to similar pathogens or tumors in different organs (eg, skin vs gut) vary considerably. Accordingly, there is an extensive "decision-making" process (usually during effector-differentiation), in which the immune system customizes the various molecules and cells to be deployed, based on a variety of considerations including the particular pathogen and the tissue microenvironment. Information about these factors is also passed on between cells from one layer to the next as the response develops.
5. *Homeostasis—Timely termination of the response and return to a resting state is a key prerogative.* As important as rapidly initiating a strong immune response is to control infections; continued responses after pathogen clearance can be deleterious. Indeed, some of the immunopathology associated with infections represents dysfunction of this arm, for example, the lung failure associated with exacerbated responses to influenza or SARS-2. Accordingly, a variety of molecules and specialized cells have evolved to control this phase. The principles of tolerance and response-termination can often be difficult to separate out. One hallmark is that the former operates to prevent both the initiation and maintenance of a response; but termination mechanisms come to the fore after the effector phase has fulfilled its mandate by eliminating or controlling the threat. The impetus to preserve homeostasis can be thought of as a teleological objective of the immune system. Its participation in wound healing, avoiding metabolic imbalances, etc are likely to be driven by this goal. Homeostasis does not mean that the immune system (and the body) always returns to its "preinfection" or naïve state; instead after each infection or threat management, the system reaches a new resting state by processes which are still being defined.
6. *Memory—The immune system retains a memory of its previous responses*: The first time a pathogen is repulsed (called the "primary response") the adaptive system takes weeks to do so. This might give the pathogen longer to establish, grow, and damage the body. In contrast, the next time the same pathogen attacks, the "secondary" immune response is much more efficient (Chapter 30). Vaccinations are the clinical strategy to mimic this "first exposure," albeit usually using nonpathogenic formulations, so that when the infection does come about, the system uses this memory to mount a secondary response (Chapter 40). While memory is largely described in the context of the adaptive immune system, a process of "training" is also thought to condition cells of the innate immune system. As discussed in Principle #5, the formation of additional memory (or innate training) after each threat, is part of the new homeostatic state of the immune system. Multiple cellular and molecular mechanisms operate to maintain this state.
7. *Clonality—The overall immune response is a collaboration between many independently operating single cells.* Perhaps a defining characteristic of the immune response is that all of the above principles are executed by individual cells, belonging to different lineages (for example, macrophages, neutrophils, T cells, B cells) (Fig. 1.3). While organ systems like muscle, liver, and lung are made up of distinct lineages of cells as well, these are relatively hard-wired to operate as a unit. The "free-living" nature of the cells in the immune system also allows them to be much more phenotypically plastic, albeit within the limits of the genetic program imbued within its lineage. For instance, a macrophage in the brain may make different responses than one in the heart, but neither can ever make antibodies, a function specific of B cells. Yet, the same macrophage can "behave" differently in response to cues that may be available in certain tissue microenvironments, or to threats in that niche at a particular time or during its history of interactions with other immune cells (like T cells). This principle is also important to a key tenet of the adaptive immune system: each T or B cell expresses a unique antigen-specific receptor and its progeny inherit that receptor. This leads to concepts based on clonality, such as clonal expansion, clonal deletion, clonal contraction discussed below.

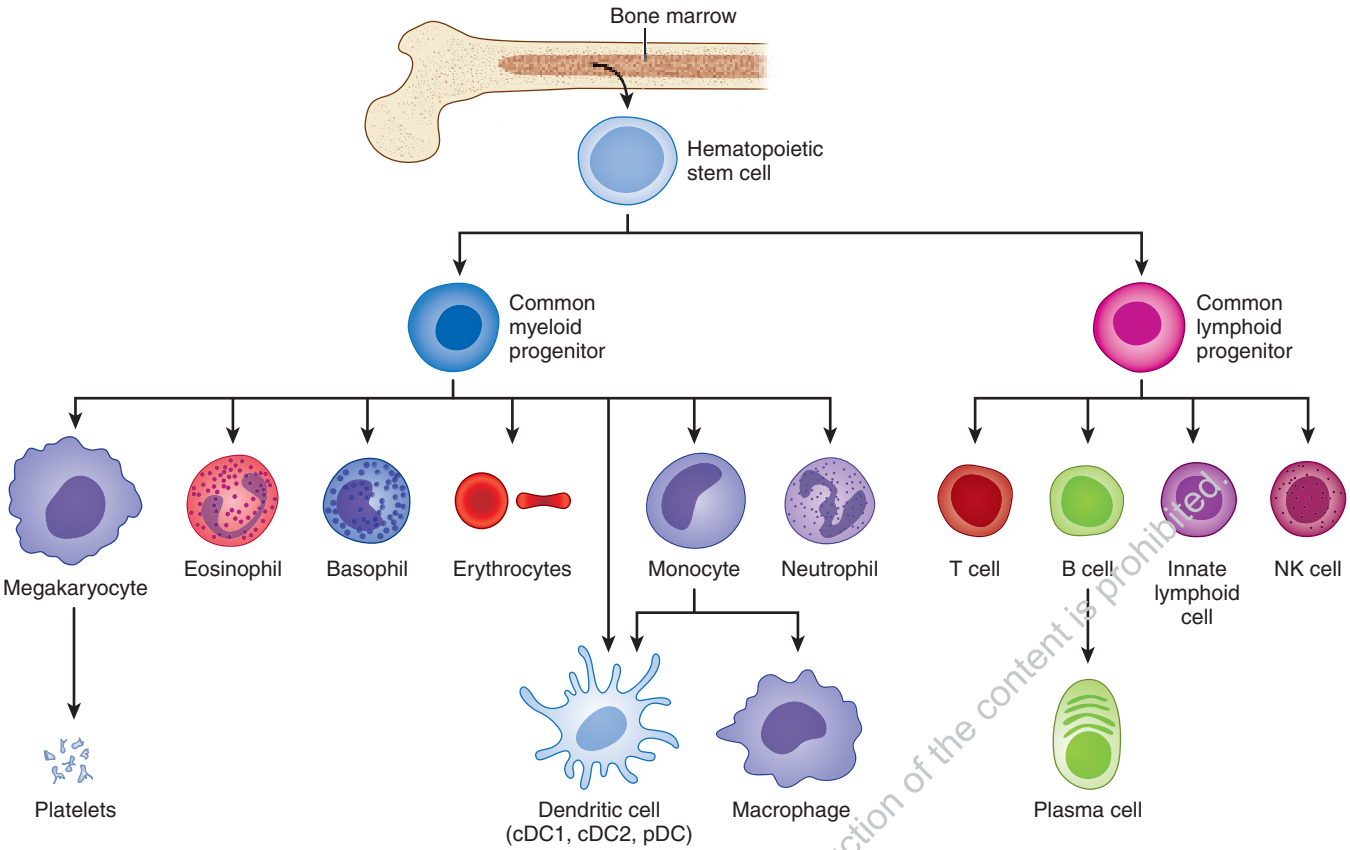


FIG. 1.3. Blood cell types. Hematopoietic stem cells (HSC, light blue) reside in the adult bone marrow and give rise to all of the blood cell types. The common myeloid progenitor (CMP, dark blue) gives rise to the cells of the myeloid lineage displayed as they appear with a histological staining procedure (except dendritic cells, DC, also light blue). DC can be derived from monocytes or from a dedicated precursor; they are divided into three types: cDC1, cDC2, and plasmacytoid DC (pDC). The common lymphoid progenitor (CLP, fuchsia) gives rise to cells of the lymphoid lineage, including lymphocytes with rearranging antigen receptors (T cell, red; B cells, green) and those that do not (natural killer [NK] cells, maroon; innate lymphoid cells [ILCs], purple). When B cells are stimulated by antigen, they become secretory plasma cells (also green). Note that the cells in the lymphoid lineage are artificially colored.

KEY STAGES OF THE IMMUNE RESPONSE

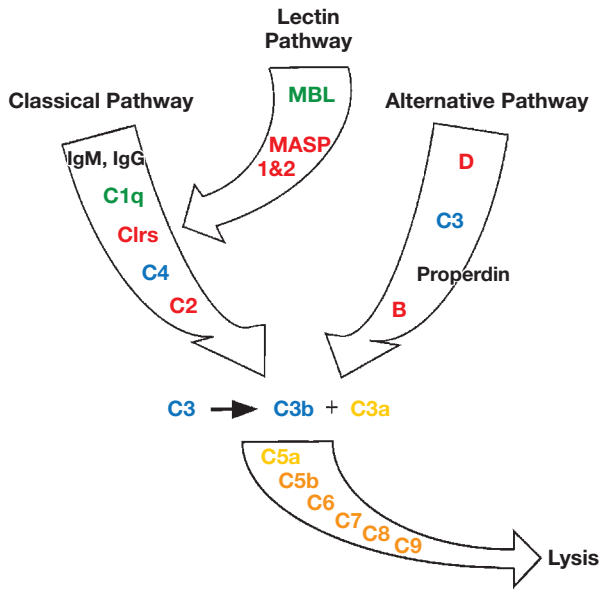
In this section, we discuss how the tiered approach to defense operates during a typical immune response (Fig. 1.1). For simplicity, we focus mostly on responses to a microbial pathogen, but a similar flow of events is triggered by toxins, tumors, and other threats as well.

Barrier Defenses

The initial obstacles to pathogen entry are provided by specializations in the linings of these entry sites that provide physical, chemical, and mechanical barriers (Fig. 1.1). Physical obstacles include dead cells on the epidermis of the skin and tight junctions between epithelial cells. In addition, specialized chemical secretions such as mucus (a carbohydrate-rich secretion that lines the gut, lung, and reproductive tracts) and antimicrobial peptides secreted by the barrier cells provide a chemical barrier. Finally, mechanical movements by cilia and tissues themselves help move larger pathogens out of the body. The features of the mucosal barrier and cells involved in mucosal immunity are discussed in Chapter 38. Barriers are a major mechanism of defense in plants (Chapter 5) and invertebrates (Chapter 3), which possess innate mechanisms of defense but no (or not much) adaptive immunity. In addition to blocking pathogen entry, the barriers also form a physical limit for a relatively continuous system that joins all the cellular structures in the body. In addition to blood, an additional system of fluid recirculation known as the lymphatic system (Chapter 8) allows the tissue fluid that bathes all cells to circulate. This lymphatic fluid drains via lymphatic vessels into specialized SLO called lymph nodes. This also carries to the subbarrier tissue the second layer of protection known as the complement system.

The Complement System (Chapter 13)

Complement (C′) is a complex system of at least 30 proteins, including binding proteins, proteolytic enzymes, regulatory and inflammatory molecules, and cell surface receptors capable of destroying pathogens (Fig. 1.4). C′ can be activated by immune complexes of IgM or IgG bound to pathogens in the Classical C′ Pathway, or by lectins such as mannose-binding lectin (MBL) or ficolin (or others) in the Lectin C′ Pathway. A third pathway, the Alternative C′ Pathway, is so-named



- C3b: Opsonization
- C3a, C5a: Inflammation
- C5b-9 (MAC): Lysis
- Regulators: factor H, DAF, MCP, CD59, CR1, factor I
  - Protect Self
  - Prevent uncontrolled inflammation
  - Preserve Complement

FIG. 1.4. The complement (C′) pathways. The pathways leading to the activation of C3 via its cleavage are the Classical, Lectin, and Alternative pathways, noted in the arrows at the top of the figure. The Lysis pathway is shown below. Binding proteins (IgM, IgG, C1q, MBL) are shown in green; serine proteases (C1r, C1s, MASP1, MASP2, Factor D, Factor B, C2) are shown in red; thioester-containing proteins (C3, C3b, C4) are shown in blue; anaphylatoxins (C3a, C5a) are shown in yellow; membrane-attack complex (MAC) shown in orange. The C′ cascades and their regulation is described in the text and to the right of the figure.

because it was the last pathway to be discovered (and actually not believed in for several years!), yet it is the most ancient pathway dating back (at least) to corals (Chapter 4). Activation of the C′ pathway can result in enhanced uptake of pathogens via phagocytosis, cell lysis, and/or induction of inflammation. The central player of all three C′ pathways is C3, a multidomain, highly expressed (>1 mg/mL in serum) serum protein that is the *only* extracellular defense molecule capable of forming a covalent attachment to cells via a thioester bond.

The ancient Alternative Pathway requires no other binding protein for its activation besides C3. The thioester bond is normally sequestered in the C3 molecule and when exposed it is hydrolyzed by water molecules and then degraded by C′ serum inhibitory proteins (the binder Factor H and the serine protease Factor I). However, if there is a biological surface such as that displayed by a bacterium devoid of regulatory proteins to block C′ activation, the thioester can make a covalent attachment to hydroxyl or amide groups on the pathogen. The bound C3 is protected from degradation by Factor H/I in the serum and by properdin, which is attracted to C3 on surfaces devoid of sialic acid, like those found on bacteria. The membrane-bound C3 molecule now attracts the serine protease Factor B, which itself then becomes a substrate for the serine protease factor D. When Factor B is cleaved into Bb (and another small fragment), its enzymatic activity is activated. The heterodimer C3b/Bb is now the active protease, AKA C3 convertase, of the Alternative Pathway, which can amplify the response by cleaving many C3 molecules into C3a and C3b to expose the thioester bond and densely coat the pathogen surface with covalently bound C3b. C3b receptors on neutrophils and macrophages bind to the molecules displayed on the bacterium and **phagocytosis is enhanced, a process called opsonization**. The smaller, soluble fragment of C3 released by cleavage, **C3a, induces an inflammatory response as described above**. If two C3b molecules are associated with the Bb, the protease specificity is modified and the serum protein C5, a homologue of C3 that lost its thioester (Chapter 4), is cleaved into C5a and C5b; thus, this $C3b_2Bb$ complex is now called the "C5 convertase." The larger fragment C5b attracts the C′ components C6-C9 forming a pore called the membrane-attack complex (MAC), with multiple C9 molecules actually making up the pore. **MAC causes direct osmotic lysis of cells** and C5a, like C3a, induces an inflammatory response. C3a and C5a are historically called anaphylatoxins, since at very high levels (especially C5a) they can produce symptoms of anaphylaxis.

The Lectin and Classical C′ Pathways follow the same pattern as described above, except these pathways are initiated by molecules that bind to the pathogen, carbohydrate-binding proteins (lectins) acting as PRR for the Lectin Pathway and IgM or IgG antibodies for the Classical Pathway. Enzymes associated with the lectins (MBL-associated serine protease 1, 2 or MASP 1, 2) cleave another C3 homologue, C4, into C4a and C4b exposing the thioester bond of C4 as well as a site for binding to the serine protease C2, a homologue of Factor B. C2 is then also cleaved by MASP and the generated C4bC2a heterodimer is the C3 convertase, homologous to C3bBb, which cleaves C3 to initiate the same downstream cascade events detailed above. A molecule similar in structure to MBL, C1q, binds to antibodies on the pathogen surface and initiates the Classical C′ Pathway. Enzymes homologous to MASPs, C1r and C1s, cleave C4 and C2 to form the C3 convertase C4bC2a (Fig. 1.4).

As mentioned, the process of activation of the C′ cascade is highly regulated. Because activated C3 can bind to any OH or NH_2 group, self-molecules can also be targeted. Besides Factor H and Factor I, other regulatory proteins either in the serum or at the cell surface (eg, C1 esterase inhibitor, decay accelerator factor, membrane cofactor protein, CD59) prevent uncontrolled C′ activation. In addition, the regulatory proteins control the C′ cascade once it is initiated to prevent overactivation leading to excessive inflammation, as mentioned above. Abnormalities in these regulatory proteins

are associated with clinical disorders such as hereditary angioedema, paroxysmal nocturnal hemoglobinuria, and macular degeneration, to name just a few.

C′ activation also has profound effects on adaptive immunity. Antigens decorated with C3 fragments can be transported into SLO via several different pathways, for loading onto FDC and subsequent antigen-specific B cell activation (see below). In addition, C′-decorated antigen can bind to both BCR and C′ receptors on antigen-specific B cells, which lowers the threshold for B cell activation, that is, the adaptive system is alerted that the antigen has been declared "foreign" by the innate system. Finally, recent work has shown that C′ is important for regulating synapse formation in the nervous system, and in certain phases of T cell activation. Considering the complexity of the C′ system, it should come as no surprise that it can be recruited for other functions.

The C′ system is an elegant demonstration of the evolution of a biochemical process that seems to be "irreducibly complex"; yet we now understand the precise events in the vertebrate lineage that resulted in this seemingly intact unit. As discussed in Chapter 4, gene duplication and neofunctionalization of the loci encoding the binding proteins, serine proteases, pore-formers, and regulatory proteins sequentially welded together rigid biochemical modules. For example, the founding member C3 can be traced back to corals, while the vertebrate paralogues C4 and C5 have assumed new functions in the Classical Pathway and cell lysis, respectively.

Innate Immunity by Tissue-Resident Cells (Chapters 12, 15, 18, 34-38)

The cells that can detect the pathogen soon after barrier breach are those that are already present at or beneath the barrier tissues (Figs. 1.1 and 1.3). During development of the organism, a few lineages of immune cells (see below) are programmed to migrate to and then remain at these sites prior to pathogen attack. These cells include innate cells including subsets of macrophages and innate lymphoid cells (ILCs, Chapter 18) but also certain semiadaptive (Chapter 35) cells like γδ T cells. While innate cells are capable of rapid responses, they must be activated by sensing the intruders. This means that such cells require dedicated receptors to sense and trigger them. In the innate system, these sensors are families of PRR defined above (Fig. 1.2) (Chapter 12). Based on the tolerance principle (see Principle #2 in the above section) of recognizing threats while avoiding attacking healthy tissue, **PRRs sense two categories of molecules**. The first are some that tend to be highly conserved in groups of pathogenic microbes and hence referred to as **pathogen-associated molecular patterns (PAMPs)**. The second are patterns that become evident only when a cell is unhealthy (stressed, damaged, infected, or otherwise in "danger") and called **danger-associated molecular patterns (DAMPs)**. PRRs include several families of molecules, of which the most intensively studied are the toll-like receptors (TLRs) and the nucleotide oligomerization domain–like receptors (NLR). Each TLR recognizes a distinct set of substances: TLR4 recognizes lipopolysaccharides (LPS), TLR3 double-stranded ribonucleic acid (RNA), and TLR9 unmethylated CpG-containing DNA. The key is that substances like LPS are found in most gram-negative bacteria but not vertebrate hosts. In the case of RNA and DNA, the sensors are placed at locations where they would not be detected if a cell is healthy. Additionally, the recognized substances are generally indispensable to the infectious agent. So, the microbial sensors provide a highly efficient means to recognize potential pathogens. Vertebrates have inherited, *en masse*, the basic families of PRR found in the invertebrates (Chapters 3, 4). Plants also use PRR with similar domain structures as those found in vertebrates, but with extensive modifications to the point that even when the molecules appear to be derived from a common ancestor, the PRR were more likely to have been derived by convergence (eg, NLR, Chapter 5). Plant PRR can detect PAMPs but also metabolic changes within the cell that has been infected, which can also occur in vertebrates. The innate system per se can provide an effective means to control or eliminate pathogens. Indeed, invertebrates and plants rely almost entirely on the innate immune system to cope with microbial infection.

The interaction of a TLR with its ligand induces a series of intracellular signaling events, of which activation of the transcription factor NF-κB is pivotal (Fig. 1.2). Many genes that are part of the innate cell's response to infection or damage are controlled by promoters with NF-κB-response elements. Macrophage activation for instance involves the production of the cytokine tumor necrosis factor alpha (TNFα), which can kill infected cells but also activate local epithelia and endothelia to become inhospitable to microbes. TNFα also promotes the vascular changes critical for the next step of inflammation and enhances the macrophage's phagocytic activity and the induction of antimicrobial systems. In addition, the activated macrophage and irritated epithelia secrete chemokines which initiate a process of cellular recruitment from the circulation into the infected site. As mentioned above, induction of an inflammatory response after activation of the innate immune system recruits other cell types, including neutrophils, to the site.

Inflammation and the Recruitment of Circulating Innate Cells (Chapter 12)

The circulatory system is the major highway by which immune cells and messenger proteins (cytokines, chemokines described below) can be transported from one part of the body to another. Endothelial cells that line the blood vessels typically form a tight barrier, keeping immune cells in the circulation. Within minutes of activation of stromal cells and tissue-resident innate cells at the site of a barrier breach, mediators (cytokines and chemokines) made by these cells engage receptors on vascular endothelial cells. This triggers changes in the shape and rigidity of the blood vessel (via remodeling of the actin cytoskeleton) and alters blood flow in the vessels closest to the site of infection (Fig. 1.1C). Note that C3a/C5a described in the C′ section can also activate mast cells in the tissue to release mediators such as histamine that also causes vascular leakage. Cytokines like IL-1 and TNF produced by activated macrophages then further induce the expression of

cell adhesion molecules in the inner lumen of the blood vessel and further dilate the vessel. The combination of the vascular leakage (which contributes to the localized swelling or edema in inflammation) and release of inflammatory mediators like prostaglandins drastically alters the microenvironment. Circulating neutrophils (Chapter 14), macrophages (Chapter 15), and natural killer (NK) cells (Chapter 18) are now attracted to this vascular niche by chemokines (for example, IL8 [CXCL8] acting on the receptor CXCR1), which further activates cell adhesion molecules on their membranes. The combination of adhesion receptors induces these cells to bind strongly to the endothelia at the site of inflammation and then crawl out into the extravascular space and eventually reach the site of infection, a process called diapedesis or extravasation. The newly recruited cells bring an additional arsenal to the fight. Among these, neutrophils are particularly effective against bacterial pathogens and can even extrude their genomic DNA as sticky NETs (neutrophil extracellular traps) which trap antibacterial peptides and then glom onto and kill microbes. Overall, the action of innate cells at this phase has three defining characteristics. First, although they are triggered broadly by threats (PRR ligands), they are not very specific to the pathogen. This also leads to the second characteristic: that is, they tend to be broad-acting in their effector mechanisms. Cytokines like IL-1 and TNFα as well as mechanisms like NETs can damage some healthy cells in the milieu as they pursue a scorched-earth policy to rid the system of threats. Maintaining such responses for prolonged periods can be deleterious. Third, this response is a one-and-done effort; it does not (and classically cannot) generate specific memory of the pathogen to respond better against the same threat. While there is emerging evidence for epigenetic programming of epithelia and some innate cells that modify their responses to subsequent infections, AKA trained immunity, it is not clear if this constitutes a true memory or rather a maturation process of the immune system.

Innate Myeloid-Derived Cells

Cells of the myeloid lineage that orchestrate innate immunity and alert adaptive immunity include macrophages, DCs, and the granulocytes comprising neutrophils, eosinophils, basophils, and mast cells (Fig. 1.3). One of the goals of cellular immunity is to energize macrophages to eliminate organisms that have established intracellular infections (Chapter 15). Nonactivated macrophages are inefficient in destroying intracellular microbes and are important for homeostasis by removing cellular debris such as apoptotic cells. However, when macrophages are stimulated by interferon (IFN)-γ and other mediators produced by T cells, their antimicrobial capacity is enhanced. These mechanisms include reactive oxygen species (ROS), nitric oxide (NO), proteolytic enzymes, and proinflammatory cytokines. Macrophages can be stimulated by other cytokines, such as IL-4, to adopt a different phenotype, one more programmed to stimulate a healing process to counter inflammation. Macrophages can also serve as APCs and enlist the "help" of activated, cytokine-producing CD4+ T cells (see below). Although macrophages function as APCs for stimulation of activated T cells, they are not particularly effective in the activation of naïve CD4 T cells. In instances in which they are the site of infection or have phagocytosed infectious agents or their proteins, antigens may be transferred to DCs, the major myeloid cell involved in antigen presentation (see below).

Neutrophils play critical roles in a wide range of inflammatory situations, as described below. In their absence, it is exceedingly difficult to clear infections with extracellular bacteria (Chapters 14, 43), and young children with a delay in neutrophil production suffer from recurrent bacterial infections in the blood. Both innate and adaptive responses play important roles in orchestrating the growth, differentiation, and mobilization of these crucial cells. For example, Th17 cells are particularly important because of their role in recruiting neutrophils to sites of immune responses to extracellular microbes.

Mast cells and basophils (Chapters 16, 47) play major roles in the induction of allergic inflammatory responses. They express cell surface receptors for the Fc portions of IgE (FcR) and for IgG subclasses (FcR). These receptors enable them to avidly bind antibody on their surfaces, and when antigens capable of reacting with those antibodies are encountered, the resultant cross-linking of FcεRI results in the immediate release of a series of potent mediators such as histamine, serotonin, and a variety of enzymes that initiate allergic and anaphylactic-type responses. Mast cells can also be activated via their anaphylatoxin receptors C3ar/C5aR. Such stimulation also induces these cells to produce cytokines including IL-3, IL-4, IL-13, IL-5, IL-6, granulocyte-macrophage colony-stimulating factor (GMCSF), and TNFα, which have important consequences in inflammation, and in overstimulation in allergic responses.

Eosinophils (Chapters 17, 42, 47) differentiate under the influence of the cytokine IL-5. They migrate to tissue sites in response to the chemokine eotaxin. Because Th2 cells can produce IL-5 and stimulate the production of eotaxin, eosinophil accumulation is often associated with Th2-mediated inflammation. Eosinophils store proteins in their secondary granules including major basic protein, eosinophil cationic protein, and eosinophil peroxidase. When released, these proteins are responsible for much of the damage that eosinophils mediate both to helminthic parasites—their presumed physiological function—and infected tissues—chronic allergic inflammation in conditions such as asthma.

Innate Cells Initiate Adaptive Immune Responses (Chapters 8, 34)

Following barriers, tissue-resident cells, and recruited innate cells, the fourth layer of immune protection is the adaptive system. Prior to their activation, T and B cells, which make up this system, are not found in peripheral tissue sites but migrate in and out of SLOs. This geographical separation between the initial site and these fourth layer cells necessitates a bridging mechanism. DCs, which also act as sentinel cells patrolling subbarrier tissue sites, perform this role for T cells (Figs. 1.1B and C). DCs use pinocytosis to continually sample their environment for antigens. These antigens are

processed proteolytically and then pieces (peptides) displayed back on the surface of DC on specialized major histocompatibility complex (MHC) molecules (see below). MHC molecules serve as adaptors for peptide display on the cell surface and the TCR can recognize the amalgam formed by the peptide and an MHC protein (pMHC). If a DC were to encounter a PRR ligand (indicating that the antigen it just acquired may be derived from a threat), then the DC also gets activated by this signal as described above for macrophages. The activated DCs upregulate chemokine receptors such as CCR7 on their surface and home to the nearest AKA "draining" lymph nodes following the gradient of CCL19/21, chemokines made by lymph node stroma. The arrival of DC in the draining lymph nodes allows the SLO T cells to sample antigens that were in the original at-risk tissue site. In addition, the activated DCs also make cytokines that dictate T cell differentiation from a naïve precursor cell (Chapter 28). Depending on specific signals delivered to DCs educated by the specific pathogen and the original tissue niche, these DCs will produce cytokines that tailor T cell responses, as prescribed in the fourth principle above. Unlike T cells, B cells are activated by intact antigen, which can enter the SLO by fluid flow through the lymphatics or be carried and/or transported to SLO B cell zones by specialized macrophages.

The Primary Immune Response (Chapters 26, 28)

After the innate phase, cells of the adaptive immune system lymphocytes (T and B cells) are now recruited to the response. T cells, B cells, NK cells, and ILC are derived from a lymphoid precursor cell, and their development is described below. The first time a pathogen triggers adaptive immunity, the T and B cells are in a "naïve" state, that is, they have completed maturation in their developmental organs, but have never encountered their cognate antigen. This first response is therefore called a primary immune response. The term is relevant to adaptive cells since (as we discuss further in the next section) any subsequent reexposure to the pathogen usually elicits a significantly different response than the first one. Innate cells do not typically make that distinction.

Clonality of Lymphocytes

Adaptive immunity is mediated by lymphocytes that recognize antigen with narrow specificity. Each T cell or B cell bears a receptor that is very specific to small regions of an antigen (an epitope or antigenic determinant). The high degree of specificity allows their response to be very surgical as opposed to the brute force methods used by the innate system. It also means that the body must produce millions of different receptors for T and B cells, such that (ideally) every possible antigen can be detected. The fascinating genetic mechanism that makes this possible is unique to the immune system and involves the rearrangement of different gene segments in the developing lymphocyte (see below and Chapters 4, 22, 25, 27). By the time a mature T or B cell arrives in the peripheral SLO, each cell expresses only one of many possible receptors that was generated by VDJ rearrangement. Importantly, although the system has millions of receptors, each T or B cell just expresses only one of them, with ~10,000 copies at the cell surface. When such a mature T or B cell divides, all the daughter cells inherit this unique receptor, and this is the basis of the principle of clonal expansion in the immune system (Fig. 1.1C). When activated T or B cells divide, the number of cells expressing the specific receptor for a particular epitope (from a pathogen or tumor) also increases. Clonal selection also offers a mechanism for tolerance: if one of these receptors happens to be self-reactive, then the cell is induced to undergo apoptosis during lymphocyte development or even as a mature cell, and the self-reactivity is also purged from the system.

Burnet's Clonal Selection Hypothesis

These principles were first laid out as four bedrock tenets of the adaptive immune response by McFarlane Burnet in 1954. While the molecular details and mechanisms have evolved with new knowledge, the basic rules have stayed surprisingly consistent. Updated for current language, the four tenets of this theory are:

1. Each naïve lymphocyte expresses a single receptor with a unique specificity by the time it completes its development.
2. When the mature lymphocyte's receptor engages an antigen (under appropriate conditions), the lymphocyte will be activated, that is, clonal selection.
3. Once activated, the lymphocyte undergoes cell division extensively, such that its daughter cells all express the same receptor (ie, are clones of itself), that is, clonal expansion.
4. Any lymphocyte that can be activated to self-molecules should typically be subjected to tolerance. In Burnet's hypothesis, it was assumed that such cells would undergo cell death during development (a process called negative selection or clonal deletion); but we now know that negative selection is only one of many tolerance processes.

Antigen Recognition by T Cells and B Cells

As mentioned, T cells differ from B cells in their mechanisms of antigen recognition. Ig, the B cell's receptor (BCR), binds to individual antigenic epitopes on the surface of native molecules, be they on cell surfaces or in solution. In that sense the BCR is like any other conventional receptor binding a ligand based on structural complementarity. Antibody and BCRs have evolved in part to bind to and to protect against microorganisms in extracellular fluids.

By contrast, T cells recognize cell-associated molecules and mediate their functions by interacting with *antigen-presenting cells* (APCs) including DCs, macrophages, and B cells. As mentioned above, the TCR does not recognize antigenic determinants on intact, undenatured molecules. It recognizes peptides (p) from the microbial antigen that have been proteolyzed (known as antigen processing) within APCs. This is further described below and in Chapter 19. These peptides are then loaded onto specialized peptide presentation MHC molecules to form pMHC complexes. There are two kinds of T cells bearing TCR named helper (Th) and cytotoxic (CTL), based on their functional and phenotypic differences. CTLs

are identified by surface expression of the protein CD8 and are concerned with killing any cells infected with pathogens or that have turned tumorous. Accordingly, the MHC allelic product that recognizes the proteins that infected/tumor cells express is adept at carrying cytosolic peptides to the surface to display for CD8 T cell recognition. These MHC genes encode for MHC-I (or class I) proteins that are expressed by all nucleated cells in the body. In contrast, Th cells, which express cell-surface CD4, regulate multiple immune cells. So, they are interested in proteins taken up by immune cells from their environment (eg, pathogen particles, extracellular microbes, tumor debris). These proteins are processed by lysosomal proteases and displayed on another set of MHC proteins (MHC-II or class-II) expressed predominantly by specialized APCs (see below).

The Significance of Two-Signal Models

The activation of T and B cells represents a crucial tipping point in the immune response, since that sets in motion a massive amplification of the cellular attack mechanisms. Once activated, these initially rare and quiescent (naïve) antigen-specific T cells can divide once in every 3 to 4 hours, leading to an ~10,000 fold expansion in cell numbers over the next 4 days. Each of these cells can secrete noxious cytokines, kill multiple target cells, or secrete huge amounts of specific antibodies, as the case may be. This is not a force to be unleashed flippantly. Accordingly, the immune system employs multiple checks and balances to make sure that the response is triggered only if necessary and against a threat rather than a healthy self-tissue. Although a bit simplistic, immunologists classify some of these control mechanisms as "two-signal" operations (Chapters 24, 32). This means that the TCR or BCR engaging a specific target antigen only provides the lymphocyte with the first half of the complete signaling required to drive a productive response (Fig. 1.5). Therefore, antigen-derived signals are termed Signal 1. The second signal, which is necessary to complete the process driving full activation,

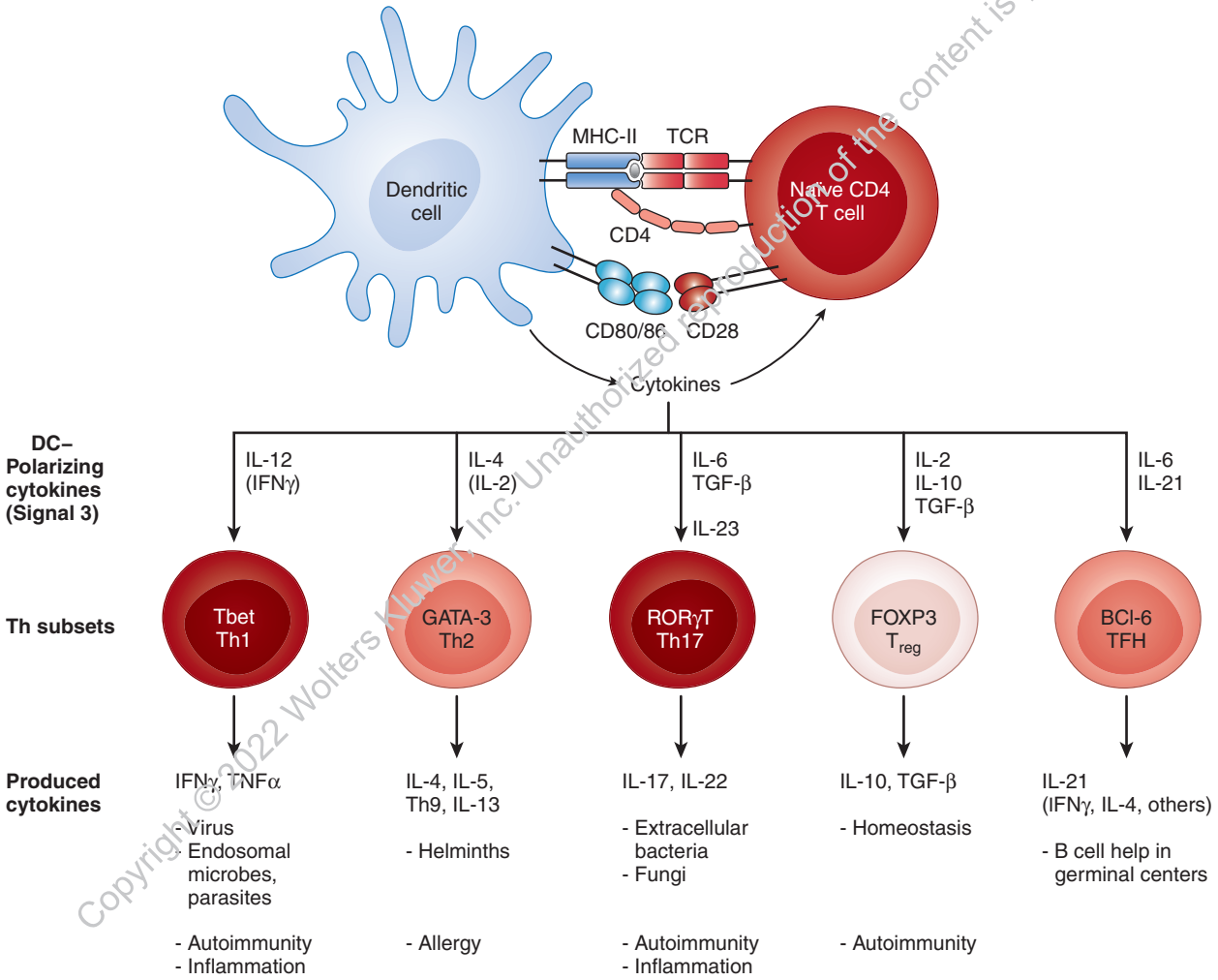


FIG. 1.5. T helper cell phenotypes and division of labor in the adaptive immune response. A DC (*light blue*) stimulates a naïve CD4 T cell (*red*) through its Signal 1 TCR, Signal 2 CD28, and DC-polarizing cytokines. Molecules produced by the DC are in light blue and by the T cell in red. The classic transcription factor for each Th cell is shown within the nucleus of the cell, and the classic cytokines produced, the physiological functions, and potential dysfunctions are shown below the cells. The Th types are colored by their extent of inflammation: Th1 and Th17 cells dark red; Th2 and TFH light red; T_{reg} whitish red.

differentiation, and survival of the T or B cells, differs for each cell type. In the case of the T cell, activation of the initial naïve, quiescent antigen-specific T cell requires DCs that have migrated to the SLO displaying pMHC on their surface, as described. However, in addition to the pMHC (which provides Signal 1 for T cells by engaging their TCR), any DC that was in the presence of a threat in the tissue also conveys the second signal. This second signal is provided by specific surface molecules upregulated on DCs that have been exposed to PAMPs or DAMPs in the tissue. These surface molecules (eg, B7 or CD80) are called costimulatory ligands and engage costimulatory receptors (eg, CD28) on T cells (Fig. 1.5). T cells which receive both Signal 1 and Signal 2 from DC become fully activated, while those that receive only Signal 1 are programmed to become inactivated (a process known as anergy) or die apoptotically. The teleological rationale here is that if the DCs were displaying pMHC without a costimulatory ligand, it likely acquired the antigen from a healthy uninfected tissue (that did not have PAMPs or DAMPs) and hence Signal 1 is a self-antigen against which an immune response would be autoimmune. So, Principle #2 (tolerance) kicks in.

In the case of B cells, the two-signal model operates slightly differently. Again, Signal 1 is still the BCR recognition of the specific target antigen. However, Signal 2 in this case must come from a helper T cell that is specific for the same pathogen or threat (Fig. 1.6). The fully activated T cell (following Signals 1 plus 2 from a DC) must interact with a Signal-1-activated B cell and provide a second signal in the form of surface molecules (eg, CD40L on the T cell and CD40 on the B cell) and cytokines (eg, IL-21) to fully induce B cell differentiation toward antibody secretion.

Activation of Helper T Cells

Migrating DCs from the site of infection settle into the T cell zone of the SLOs where circulating naïve T cells have an opportunity to sample pMHC with their TCR. CD4+ Th cells engaging pMHC-II and receiving costimulation then undergo a period of activation for about 32 hours before they enter the first cell division. Subsequently, the rapid cycle of cell divisions results in the generation of a clonal brigade of T cells. Helper T cells play a central role in driving and coordinating the subsequent immune response. Therefore, as discussed in Principle #4, these cells must choose one of many possible "weaponization" trajectories during this early period. This would match their eventual effector mechanisms with the nature of the original threat and the tissue affected. The information necessary for T cells to understand both threat and tissue comes from DCs as well (Fig. 1.5). While this communication of contextual information involves many different mechanisms (Chapters 12, 28) including some poorly understood, a major component is the category of cytokines made by DCs. For instance, faced with a bacterial infection (eg, *Mycobacterium leprae* which causes leprosy, Chapter 43), DCs secrete the cytokine IL-12, which in turn promotes the differentiation of Th cells to produce IFNγ. The IFNγ produced can activate macrophages to kill the intracellular bacterium. In contrast, if the DCs instruct T cells to differentiate into IL-4-producing effector cells, the bacterium will persist and form long-lasting lesions. Based on classic studies, it was suggested that early innate signals instruct Th cells to differentiate into one of many "classes" of effector cells which were named as Th1 (those producing IFNγ, TNF, etc), Th2 (producing IL-4, IL-5, IL-13, etc), Th17, and so on (Fig. 1.5). The

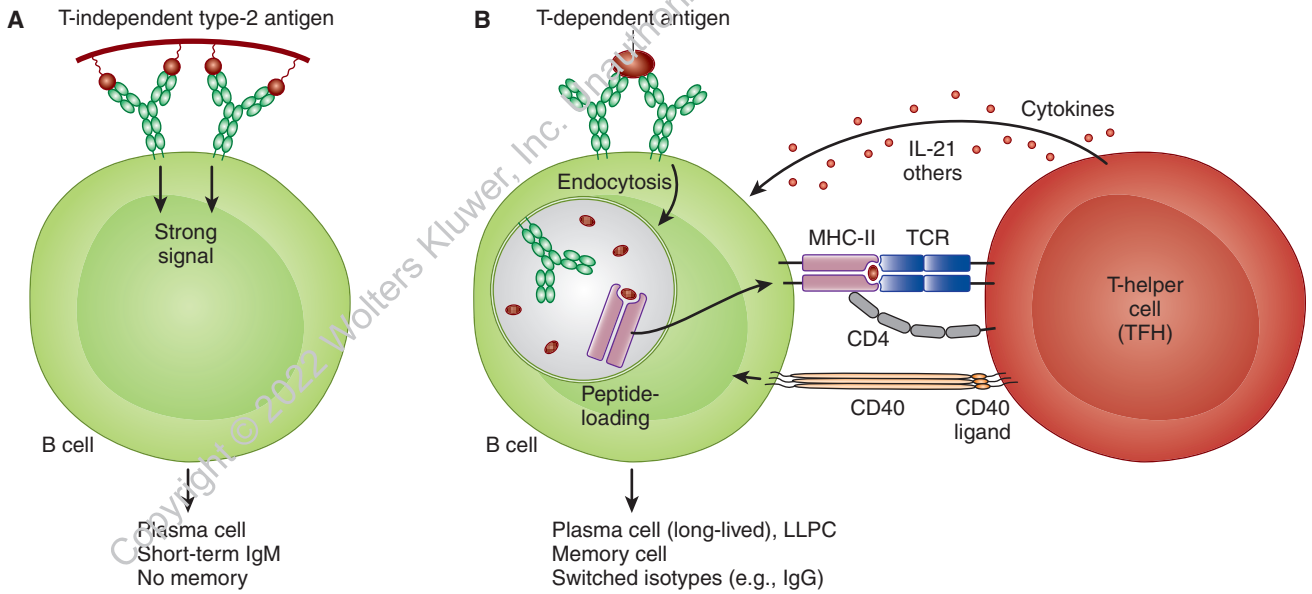


FIG. 1.6. Two forms of B-cell activation, T-independent and T-dependent. A: When a naïve B cell (*green*) is stimulated through its IgM BCR by an antigen (*dark red*) with repeating identical epitopes, the strong BCR signal can result in differentiation into short-term IgM-producing plasma cells. **B:** When a B cell (*green*) receives Signal 1 through its BCR with minimal cross-linking, it takes the antigen (*red*) into the cell where it is degraded. Peptides derived from the antigen bind to MHC class II in endosomes, which is then transported to the cell surface to activate primed TFH cells (red) that provide costimulatory signals in the form of CD40-Ligand (CD40L) and cytokines. These B cells undergo affinity maturation and class-switch recombination and go on to become long-lived plasma cells (LLPC) or memory cells.

innate-derived cytokines made by DC (eg, IL-12) that promote particular T cell classes were categorized as "Signal 3" or "innate differentiation cytokines." While these terminologies are still quite widely used, it is increasingly clear that Th classes are not as rigidly segregated in vivo as previous in vitro experiments had suggested. For instance, there are cases in which Th cells can differentiate to effectors secreting both IFNγ and IL-10, which seems to be a hybrid of Th1 and Th2. Further new classifications of effector Th states are still being described (Chapter 24) (Fig. 1.5). While a new consensus nomenclature to replace Th-class designations is yet to emerge, increasing availability for single cell measurements in vivo is likely to drive the field to adopt a new naming system.

In recent years, two major differentiation pathways of CD4 Th cell differentiation have gained special significance (Fig. 1.5). One is a subset of cells called T-follicular helper (TFH) cells, which play a critical role in providing Signal 2 for B cells as well as driving their further differentiation. A second is a subset known as regulatory T cells (T_{regs}) which seems to be primarily concerned with inhibiting or balancing aggressive immune responses. T_{regs} are identified by the expression of a transcription factor FoxP3, which is sufficient to drive their lineage differentiation (Chapter 29). Unlike all other Th cell classes, a significant fraction of them arises during development as predetermined T_{regs} (AKA "natural" T_{regs}) and are discussed further in the context of lymphocyte development below. But these can also arise in the periphery if naïve CD4+ T cells are stimulated by antigen in the presence of the cytokine transforming growth factor-beta (TGF-β) as Signal 3. The amelioratory role of FoxP3-expressing cells is underscored by patients defective in FOXP3, a congenital disease called immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX).

Activation of CTL (Chapters 34, 44, 49)

Antigen-specific naïve CD8 T cells in the SLOs are also activated by DCs, like the activation of naïve Th cells discussed above, that present the correct pMHC-I complex together with costimulation (Signal 2). This presents a conundrum, since we discussed above how CTLs are suited to killing infected cells or tumors. The problem is that if an epithelial cell were infected by a virus which does not infect DCs or had become a tumor, conventional DCs would be unable to present those antigens on MHC-I, since conventional MHC-I peptides are derived from antigens in the cells own endoplasmic reticulum (ER) (see below). The solution to this is satisfied by the existence of specialized DCs (called cDC1 or BATF3-dependent DC, Fig. 1.3) which can "cross-present" antigen. These DCs acquire external cell debris or pathogen particles from the infected tissues or tumors and siphon the phagocytosed material into the MHC-I pathway for processing, followed by activation of naïve CD8 T cells. In addition to Signals 1 and 2 from PRR-activated cross-presenting DC, CD8 T cells can also be further activated by interactions with activated Th cells. This form of interaction is also called T cell help (for CD8 T cells) and can be delivered by cytokines like IL-2 and/or cell surface molecules, like the CD40L-CD40T cell activation of B cells mentioned above.

Activation of B Cells

Like naïve T cells, resting B cells must undergo activation, proliferation, and differentiation before they secrete their clonally selected antibodies. Antigen-specific IgG antibodies of high affinity are the result of such clonal selection; this response requires "help" from T cells (Fig. 1.6). Antigen enters the B cell zone or B cell follicle in the lymph nodes via the lymphatics and enters conduits where it can provide Signal 1 to antigen-specific B cells or be transported to specialized cells in the follicle called follicular dendritic cells (FDCs). Antigens that are "tagged" with C′ C3 fragments as described above can be transferred from the lymph to the SLO B cell zone via specialized macrophages called subcapsular sinus macrophages. From there, noncognate (non-antigen-specific) B cells can transport the antigen through their C′ receptors to FDC. In the spleen, specialized macrophages in the highly vascularized marginal zone (MZ) transport antigens to the FDC in splenic B cell follicles. Antigen-specific B cells in any SLO detect native antigens displayed on the FDC, which provides Signal 1 via the BCR. Simultaneously to BCR recognition, C′ fragments on the antigen can bind to C′ receptors on the antigen-specific B cells and lower the threshold for activation by orders of magnitude. Signal 1 induces B cells to upregulate T cell-specific chemokine receptors that attract them to the borders of the T and B cell zones, where B cells can then interact with activated T cells, which themselves have upregulated B cell-specific chemokine receptors. B cells use their BCR to take up antigen, process it in lysosomes, and display peptides from the antigen on MHC-II molecules (Fig. 1.6). Importantly, all other noncognate B cells, unlike DC, are incapable of taking up antigen except through the BCR; this focused uptake mechanism ensures that only the antigen-specific B cells will engage antigen-specific T cells at the T-B zone border. Recognition of pMHC-II on the B cells by activated T cells provides cell surface Signal 2 to B cells (CD40, as described above) and cytokines such as IL-21. The B cell can either become a plasma cell, secreting mainly IgM, or move back into the follicle to initiate the so-called germinal center (GC) reaction that leads to affinity maturation.

This primary Ig repertoire is sufficiently large that most epitopes on foreign antigens will encounter B cells with a complementary BCR. Thus, if adequate T cell help is generated, antibody responses can be made to a wide array of antigens. Nonetheless, the antibody that is initially produced has a relatively low affinity for the antigen. This is partially compensated for by the fact that IgM, the antibody initially made in the primary response, is a pentamer, and via multivalent binding high avidities can be achieved even when individual combining sites have modest affinities (Chapter 21). Mammals have developed a specialized SLO structure called a germinal center (GC) for affinity maturation of the antibody response. After their interaction with T cells described above, some B cells move back into the follicle to initiate the GC reaction. The B cells rapidly divide and upregulate the enzyme activation-induced cytidine deaminase (AID), which is required for somatic hypermutation (SHM) of the antibody genes and for class-switch recombination (CSR, described below) (Chapter 22). During the SHM process, mutational

rates of 1/1000 base pairs/generation can be achieved. Thus, with each cell division approximately one mutation will occur in either the Ig heavy (H)- or light (L)-chain V region of an individual cell. Such a high rate of SHM creates an enormous increase in antibody diversity. Although most mutations will neither modify nor enhance affinity, some will increase it. Thus, some B cells emerge that can bind antigen more avidly than the initial responding cells. Because there is an active process of apoptosis in the GC from which B cells can be rescued by the binding of antigen on FDC with their BCR, cells with the most avid receptors have an advantage over other antigen-specific B cells and dominate the population of responding cells. Therefore, upon rechallenge, the affinity of the antibody produced will be greater than that in the initial response. As time after immunization elapses, the affinity of antibody produced increases. This results in immunized individuals with high-affinity antibodies that are more effective in protecting against microbial agents than the antibody initially produced. This process is also associated with CSR to other Ig isotypes, which is described below in the Nuts and Bolts of the Adaptive Immune System section. Following the GC reaction, plasma cells and memory cells are generated that provide substantial protection to reinfection.

B cells can also be stimulated in the absence of T cell help to produce antibodies, the so-called T-independent response (Fig. 1.6). When an antigen-specific B cell encounters an antigen with repeating identical epitopes, as are found on bacterial carbohydrates, the BCR can be extensively cross-linked and the strong Signal 1 alone can induce differentiation into plasma cells. Such stimulation results in the production of short-term protective IgM but does not induce a memory response.

The Effector Arms of the Primary Response

The initial activation, proliferation, and differentiation of T and B cells in the SLOs take 3 to 7 days (Fig. 1.1D). At this point, differentiated Th and CTL cells have altered the expression of chemokine receptors that now guide them from the SLO to the infection sites. As the cells are migrating out of SLO, they are fully armed to deliver effector functions (whether it be in secreting inflammatory cytokines like IFNγ or being able to kill other cells) but are not continuously making these effector products. The migration itself is also not well focused to just the initially threatened tissue sites, meaning that differentiated (but not actively secreting) effector T cells can be found in the circulation as well as in the healthy tissues. Two principles ensure that these cells deliver effector function maximally only at the relevant sites. First, although "casting a wide net" in terms of migrating out, the inflammatory changes in the infected tissues are quantitatively better at both recruiting more effector cells and at retaining them there. Second, and most importantly, effector T cells must reengage their target pMHC to deliver effector function, but at this point costimulatory molecules are not required for activation. For CD8 T cells, this means that any cells infected with the pathogen (or having mutant tumor proteins) would be the ones to display pMHC-I with the correct peptides. In the case of CD4 effector T cells, these could also be some infected cells which present pMHC-II in response to inflammatory stimuli. However, more often, the CD4s interact with macrophages or other innate cells, which present antigens from threats, as mentioned above. In this case, Th cells instruct the innate cells to either change their own activation status to fight the threat or alter the course of their ongoing response. Depending on the kind of pathogen involved, these instructions can be quite diverse (Fig. 1.5). For intracellular pathogens, effector Th-derived instructions can enhance the generation of ROS in macrophages, while for large parasites appropriately differentiated CD4s can instruct goblet cells to increase mucus production. T cells exploit and augment the abilities of the previous layers to effectively combat the threat.

There are two major mechanisms of CTL-mediated cytotoxicity (Chapter 31). One involves the production of perforin, a molecule that can insert into the membrane of target cells and promote their lysis. Perforin-mediated lysis depends on a series of enzymes produced by activated CTLs referred to as granzymes. Many active CTLs also express large amounts of the TNF superfamily member fas ligand on their surface. The binding of fas ligand with its receptor, fas, on the surface of the target cell initiates apoptosis in the target cell. CTL-mediated lysis is a major mechanism for the destruction of virus-infected cells (Chapter 44). If activated when a virus is in its eclipse phase, CTLs may help eliminate the virus with relatively limited cell destruction, as normally occurs as the killing mechanisms induce target cells to undergo noninflammatory programmed cell death (Chapter 7). However, vigorous CTL activity while a virus is widely disseminated may lead to substantial tissue injury. Thus, clinical disease may be caused by the destruction of tissue by CTLs rather than by the virus itself. One example is hepatitis B, in which much of the liver damage represents the attack of hepatitis B virus–specific CTLs on infected liver cells. CTLs are also the major mechanism involved in the destruction of tumor cells, as described below.

After activation, Ig-secreting plasma cells change their chemokine receptors and also move out of the SLO into bone marrow (BM) and other body sites (Fig. 1.1D). The effector arm of the B cell response is secreted antibodies which circulate in the blood and access most of the niches in the body including the site of infection. Antibodies can neutralize the pathogen by directly binding and blocking critical pathogen functions (such as invasion receptors, vital nutrient transporters). In addition, antibodies can also help macrophages and NK cells find the pathogen by using Fc receptors (FcR, Chapter 33).

The Secondary Immune Response and Immunological Memory (Chapters 26, 30)

The primary adaptive immune response takes about a week to fully engage and be maximally effective against a pathogen. While this is acceptable in many cases, if the invader causes a lot of pathology in this time or replicates too fast to overcome the slowly developing adaptive response, it can lead to severe morbidity or mortality. These limitations are circumvented if the pathogen invades a second time, due to the phenomenon of immunological memory. Memory refers to the ability of the system to respond faster and more robustly to the same pathogen

during a reexposure (Fig. 1.1E). There are five main processes which form the biological basis of memory. First, after the initial response, a small fraction of the T cells that expanded clonally to fight the pathogen are retained in the body. This is significant because in the primary infection, pathogen-specific naïve T cells are quite rare (a so-called **"precursor frequency"** as low as 1 in 100,000). Even if a few of these T cells are retained as memory T cells (between 10 and 100 per 100,000 total T cells), this represents a significant increase in the frequency of pathogen-specific T cells available to respond to the infection. Secondly, naïve T cells need extensive checks and balances for full activation, resulting in a 32-hour lag time before their first proliferation. Memory T cells can be recruited more rapidly and with lower doses of stimulating antigen. Third, a subset of initial effector T cells are retained in tissue sites as **"resident memory" T cells**, so that they can respond at the same time as the innate cells beneath the barrier get activated. This is a massive advantage for the secondary response, since it takes the primary response about a week to dispatch effector cells to the tissues. Fourth, plasma cells called long-lived plasma cells (LLPC) remain after the primary response stationed in the BM to constantly secrete high levels of the antibody that they made in the primary response. These LLPC-produced antibodies are the source of antipathogen antibodies detected in the blood months and sometimes even years after an initial infection. These offer a definitive advantage to the host, since they can rapidly neutralize or opsonize the pathogen attempting to reinfect the host at any tissue site. Finally, a subset of B cells that mirror memory T cells are called memory B cells (MBC). These also await pathogen reappearance and can be rapidly activated to provide protective immunity. The difference between LLPC and MBC is that the former continually secrete antibodies regardless of pathogen presence, while the MBC must be reactivated during reinfection before they can start secreting antibodies. Taken together, the primed adaptive system now can repel the repeat invader much more efficiently than the first time. In many cases, this means that the second attack only leads to subclinical infections where the pathogen can often be cleared without the patient even becoming symptomatic. In other cases, this response still clears the microbe faster, leading to less morbidity or mortality than occurs in a primary infection.

This is the basis of most of the licensed vaccines in use today (Chapter 40). Exposing the immune system to components (proteins) of the pathogen generates a combination of all five of the above. In this case, a vaccinated individual encountering the cognate pathogen for the first time can already mount a secondary immune response, rather than going through the slower and less-effective primary response. The great power of vaccines is illustrated by the elimination of smallpox from the world and by the complete control of polio in the western hemisphere.

NUTS AND BOLTS OF THE ADAPTIVE IMMUNE SYSTEM

Immunoglobulins

Igs are antigen-specific membrane receptors (B cell receptors [BCRs]) and secreted products of B cells. They are members of a large family of proteins designated the Ig supergene family (IgSF). Members of this family have sequence homology, a common gene organization (generally one exon encoding each domain), and similarities in three-dimensional structure of the IgSF domain, consisting of seven-to-nine β-pleated sheets organized into two opposing sheets in an open-face sandwich (Fig. 1.7) (Chapters 4, 21). Many of the cell surface proteins that participate in immunologic recognition processes, including the TCR and BCR, the signaling molecules associated with the antigen receptors, and many more are members IgSF family members. A lymphocyte may bear as many as 30 such members on its surface.

The Igs themselves consist of two H and two L chains (Fig. 1.8). The H and L chains are composed of a series of IgSF domains, each consisting of approximately 100 amino acids. There are two L chains in mammals, κ and λ, which have two IgSF domains; in other vertebrates there are as many as five subclasses of L chains (Chapter 4). The carboxy-terminal domain is essentially identical among L chains of a given type and is referred to as the constant (C) region. The amino-terminal domain varies among L chains and contributes to the binding site of antibody: because of its variability it is called the V region. V domain diversity is largely concentrated in three segments, designated the hypervariable (HV) or complementarity-determining regions (CDRs) (Chapter 21). The three CDRs contain the amino acids that are the L chain's contribution to the antibody-combining site (Fig. 1.7) and are interspersed among four regions with much lower variability, designated framework regions (FR).

IgH chains are of several classes determined by their constant regions (μ, δ, γ [of which there are several subclasses], α, and ε) (Fig. 1.9). An assembled Ig molecule, consisting of one or more units of two identical H and L chains, derives its name from the C regions. Thus, there are IgM, IgD, IgG, IgA, and IgE antibodies. The H chains each consist of a single amino-terminal V region and three or four C regions. In many H chains, a hinge region separates the first and second C regions and conveys flexibility to the molecule, allowing the two identical combining sites of a single unit to move in relation to one another from nearly 0 to 180° (Fig. 1.8). Such divalent binding to a single antigenic structure and inherent flexibility results in a great gain in energy of interaction (Chapter 21). The H chain V region, like that of the L chain, contains three CDRs lining the combining site of the antibody and four FR. Thus, the antibody-combining sites are an amalgam of six CDRs, three each for VH and VL.

The C region of each H chain class conveys the functional attributes to the antibodies. Among the distinct biologic functions of each class of antibody are the following:

- IgM are potent neutralizers of microbes and activators of the C′ system (Chapters 4, 13).
- IgA are secreted into a variety of bodily fluids and are principally responsible for immunity and homeostasis at mucosal surfaces (Chapter 38).
- IgE are bound by specific receptors (FcεRI) on basophils and mast cells. When cross-linked by antigen, these IgE/FcεRI complexes cause the cells to release a set of mediators

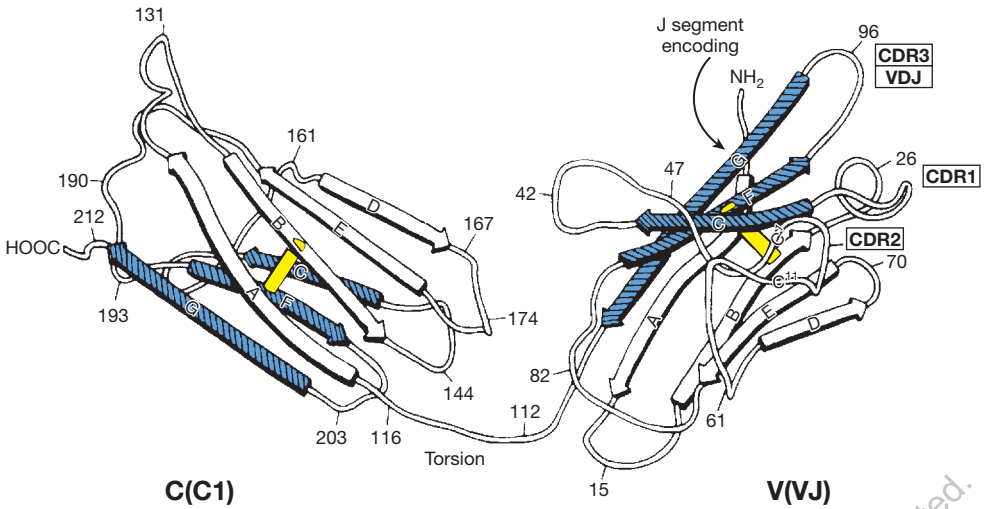


FIG. 1.7. IgSF V and C domains in an Ig L chain or TCR chain. V domains are made up of nine beta strands (A, B, C, C′, C″, D, E, F, G) forming two sheets (A, B, D, E and C, C′, C″, F, G). CDRs are found on the loops between strands B and C (CDR1), strands C′ and C″ (CDR2), and between strands F and G (CDR3), which is generated by the RAG-mediated rearrangement events. This V domain is called a VJ domain because its G strand has a conserved Gly-X-Gly motif involved in V domain dimerization in Ig and TCR. Dimerization occurs via the C, F, G sheet interface for V domains. The C domain is compact, with the "C1 domain" found in Ig, TCR, and MHC class I and class II being most compact. The strands and sheets are the same as in the V domain except C′ and C″ strands are not present in C domains. There is torsion in the loop connecting the V and C domains such that the C-C dimer interface is formed between the A, B, D, E sheet interface. Intradomian disulfide bonds shown in yellow. Note that the 'D' in CDR3 is only found in IgH and TCRB/TCRD.

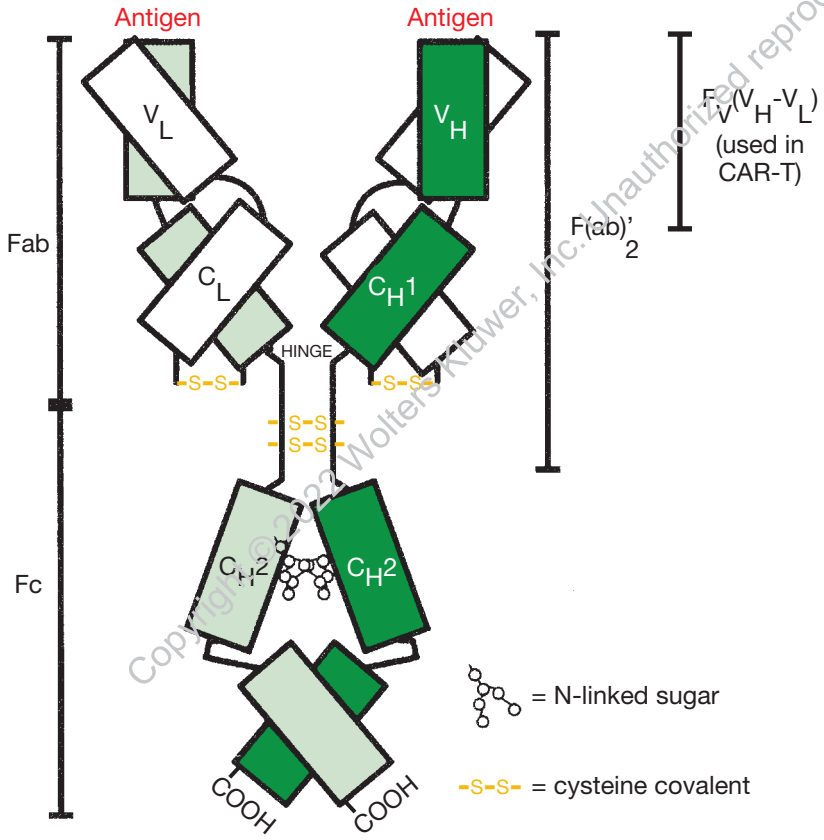


FIG. 1.8. IgG antibody molecule. An IgG molecule is made up of a "dimer of dimers," with a heavy chain (*H, green*) and light chain (*L, white*) making up each dimer. IgG H chains have an N-terminal V domain and three C domains, while L chains have and N-terminal V domain and one C domain. The four chains are covalently associated with interchain disulfide bonds as indicated (*yellow*). Fc, fragment crystallizable for effector functions; Fab, fragment-antigen binding made up of the N-terminal two domains of the H chain and the L chain; $F(ab)'_2$, fragment-antigen-binding-2, made up of two disulfide-linked Fabs that retain the bivalent combining sites; FV, fragment-variable that only contains the N-terminal V domains of VH and VL, often used in therapeutic antibodies or chimeric antigen receptors on T cells (CAR-T). The Asn-linked sugar on the CH2 domain is vital for Fc effector functions (Chapter 33). The hinge provides flexibility for antigen binding by the two Fabs.

responsible for allergic inflammatory responses (Chapters 16, 33, 47).
- IgD act as membrane receptors for antigen and are involved in mucosal inflammation (Chapters 4, 21, 26).
- IgG antibodies, made up of four subclasses in humans, are expressed at the highest levels in the serum (Chapter 21). They mediate a wide range of functions including transplacental passage, opsonization of antigens through binding of antigen/antibody complexes to specialized FcR on macrophages and other cell types, and C′ fixation (Chapters 13, 21, 33).

IgD, IgG, and IgE antibodies consist of a single unit of two H and L chains. IgM antibodies are constructed of five or six such units, although they consist of a single unit when they act as BCR. IgA antibodies may consist of one or two units. IgM and dimeric IgA contain an additional polypeptide chain, the J chain, which is important for the polymerization process during Ig biosynthesis in plasma cells. In addition, secreted IgA contains a secretory piece, that is derived from the receptor for polymeric IgA and IgM (J chain-dependent), which is required for the transport of IgA and IgM through the epithelial cells lining the lumen of lung and intestinal mucosae.

Each of the distinct Igs can exist as secreted antibodies made by plasma cells and as BCR on naïve (IgM/IgD-expressing) or memory (IgG-, IgA-, IgE-, and sometimes IgM-expressing) B cells (Fig. 1.9). Secreted antibodies and BCR of the same class have identical structures except for differences in their carboxy-terminal regions. Membrane Igs possess a hydrophobic transmembrane region and a cytoplasmic tail, both of which are lacking in the secretory form. Secreted IgM and IgA have an evolutionarily conserved C-terminal region required for J chain association.

Immunoglobulin Genetics (Chapters 4, 22)

The IgH chain gene of a mature lymphocyte is derived from a set of genetic elements that are separated from one another in the germline (Fig. 1.9). The V gene is composed of three types of genetic elements: *V*, *D*, and *J*. More than 100 V_H elements exist in humans, 9 *D* elements, and 6 J_H elements.

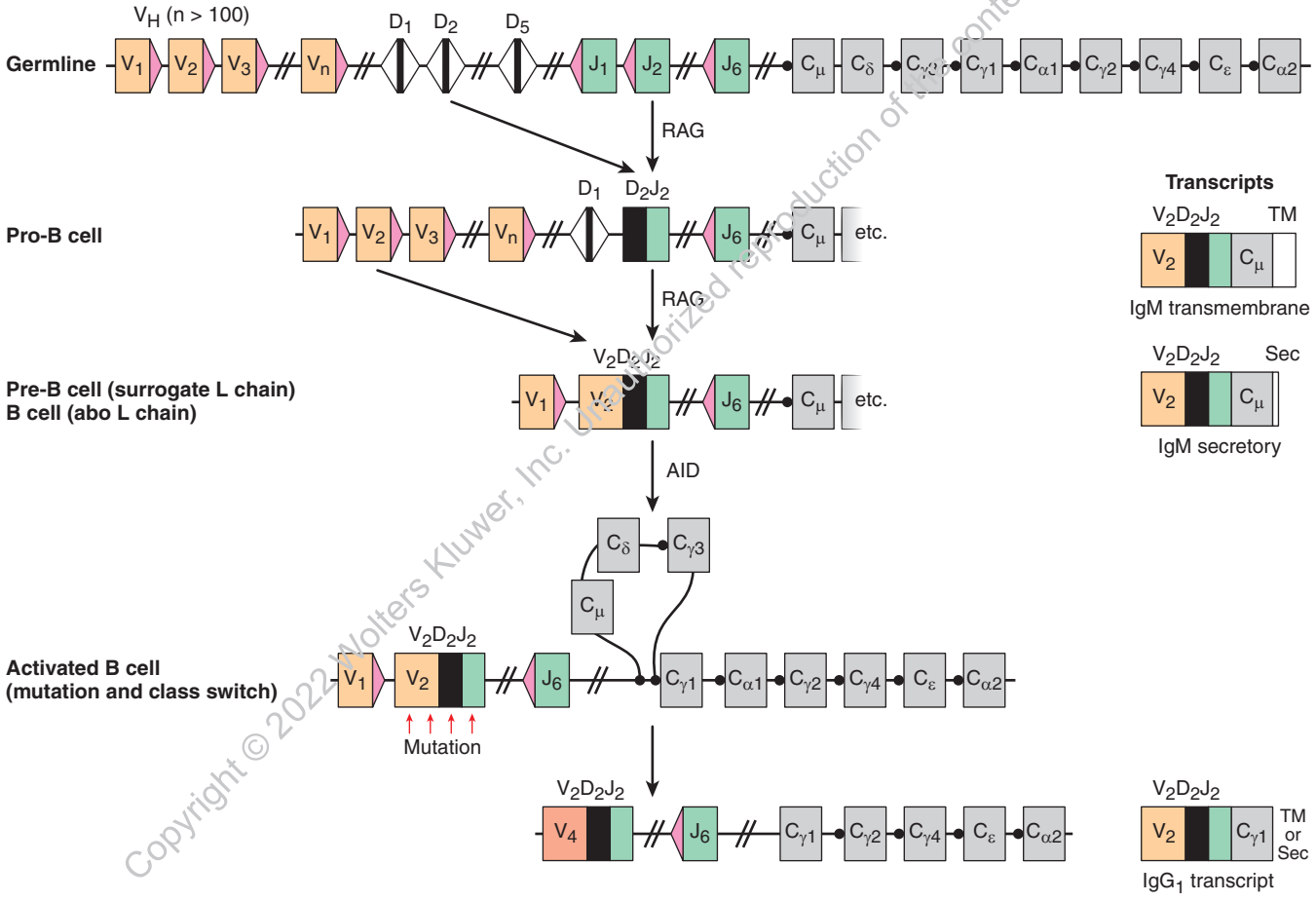


FIG. 1.9. The IgH locus, rearrangement, and class-switch recombination. V (*orange*), D (*black*), J (*green*), and C (*gray*) gene segments at the human IgH locus. RSS are shown as triangles flanking V, D, and J segments: pink triangle 23 bp spacer and white triangle 12 bp spacer. In a developing B cell in the bone marrow, first D joins to J and then V to that DJ in a RAG-dependent fashion. This permits production of an IgM molecule that associates with a surrogate light chain. Light chain rearrangement commences (not shown) and IgM BCRs are then produced by transitional and then mature B cells in the follicles of secondary lymphoid tissues. When B cells are activated in a T-dependent fashion, they upregulate AID which is required for somatic hypermutation (arrows in the V gene) and class-switch recombination, here, showing a switch from IgM to IgG1. IgH transcripts can either encode transmembrane or secretory IgH via alternative splicing.

An H-chain $V_H DJ_H$ gene is created by the joining of one of the *D* elements on a given chromosome to one of the J_H elements on that chromosome, usually with the excision of the intervening DNA. The recombination-activating genes (RAG) are required for this process, recognizing recombination signal sequences (RSS) at the 3′ end of V segments, the 5′ end of J segments, and both sides of the D elements. This is followed by a second rearrangement event in which one of the V_H elements is joined with the assembled DJ_H element to create the V region ($V_H DJ_H$) gene (Fig. 1.9). Although the choice of the V_H, *D*, and J_H elements that are assembled is not entirely random, the combinatorial process allows the creation of a very large number of distinct H chain V-region genes. Additional diversity is created by the imprecision of the joining events and by the deletion of nucleotides, and especially by the addition of new, untemplated nucleotides between *D* and J_H and between V_H and *D*, forming "N regions" in these areas. This greatly increases the diversity of distinct IgH chains that can be generated from the relatively modest amount of genetic information present in the germline. The assembly of L-chain genes follows similar rules, but L chains are assembled only from V_L and J_L elements. Although there is junctional diversity, N regions are found at lower levels in L chains. Additional diversity is provided by the existence of two classes of L chains, κ and λ. An Ig molecule is assembled by the pairing of an IgH chain with an IgL chain. Although this process is not completely random, it allows the formation of an exceedingly large number of distinct Ig molecules having distinct specificities. The regulation of V(D)J rearrangement is discussed below in the B Cell Development section.

The RAG genes were acquired by vertebrates 500 million years ago from a microbial transposon that entered the genome (Chapter 4). The enzymes as well as the RSS directing rearrangement invaded a germline IgSF exon between the parts encoding the F and G stands (Fig. 1.7). The gene became thus inactivated and could only be stitched back together by the action of the RAG enzymes. This transposition event is believed to have been an innovative force in the emergence of adaptive immunity as almost all features of adaptive immunity described in this volume are found in all extant jawed vertebrates (starting with sharks), that is, the RAG transposon initiated a "Big Bang" of adaptive immunity. The active enzymatic fragment of RAG1, called transib, which is required to cleave DNA at the RSS/rearranging segment to initiate rearrangement, is found in transposons inserted into genomes throughout the animal kingdom.

Jawless vertebrates (lamprey and hagfish) have antigen receptors called variable lymphocyte receptors (VLRs) based on an entirely different gene family, the leucine-rich repeats (LRR). These receptors are also somatically generated by enzymes related to the AID and are clonally expressed. Proof of the existence of two sets of rearranging receptors in the vertebrates was one of the most exciting findings in basic immunology in the past 15 years (Chapter 4).

Class Switching (Chapters 22, 26)

An individual B cell continues to express the same IgH-chain V region as it matures, but it can switch its IgH-chain C region (Fig. 1.9). Thus, a cell that expresses receptors of the IgM and IgD classes may differentiate into a cell that expresses IgG, IgA, or IgE BCR and then into a cell secreting antibody of the same class that it expressed on the cell surface. This process allows the production of antibodies capable of mediating the distinct biologic functions described above yet retaining the same antigen-combining specificity. When linked with the process of affinity maturation of antibodies, Ig CSR provides antibodies of extremely high efficacy in preventing reinfection with microbes or in rapidly eliminating pathogens. The coupled phenomena of CSR and affinity maturation account for the high degree of effectiveness of antibodies produced in secondary immune responses.

The CSR process involves a genetic recombination event between specialized switch (S) regions, containing repetitive sequences and AID target sites, located upstream of each C region gene (except the δ C region). Thus, the S region upstream of the μ C_H region gene (Sμ) recombines with an S region upstream of a more 3′ isotype, such as Sγ1, to create a chimeric Sμ/Sγ1 region and in the deletion of the intervening DNA (Fig. 1.9). The genes encoding the C regions of all other Ig isotype genes are located 3′ of the Cμ and Cδ genes.

The induction of the switching process is dependent on the action of a specialized set of B-cell stimulants. Of these, the most widely studied are CD40L (CD154), expressed on the surface of activated T cells (Fig. 1.6), and TLR ligands, such as bacterial LPS. The targeting of the C region that will be expressed after CSR is determined by cytokines. For example, IL-4 determines that switch events in the human and mouse will be to the ε C region and to the γ4 (human) C region. IFN-γ determines switching to γ1/γ3, and TGF-β determines switching to α.

As already noted, both CSR and SHM depend upon AID. Humans lacking AID fail to undergo both processes, and in humans this causes an immunodeficiency called hyper-IgM syndrome.

T Cell Receptors

The TCR is a disulfide-linked heterodimer (Fig. 1.10) whose constituent chains (α and β, or γ and δ) are IgSF members. The TCR is associated with a set of transmembrane proteins, collectively designated the CD3 complex, which plays an indispensable role in signal transduction. The CD3 complex consists of γ, δ (note that the CD3 γ and δ chains and the TCR γ and δ chains are distinct polypeptides that, unfortunately, have similar designations), and ε chains and is associated with a homodimer of two ζ chains. CD3 γ, δ, and ε consist of extracellular domains that are IgSF members. The cytosolic domains of CD3 γ, δ, and ε and of ζ and η contain one or more copies of the immunoreceptor tyrosine–based activation motif (ITAM) (D/ExxYxxLxxxxxxxYxxL/I) that is found in a variety of chains associated with immune recognition receptors, most notably the BCR, NK cell receptors, and FcR. This motif is important in the signal-transduction process and provides a site through which protein tyrosine kinases can interact with these chains to propagate signaling events (Chapter 24).

The TCR chains are organized much like Ig chains, with N-terminal V domains and C-terminal C domains.

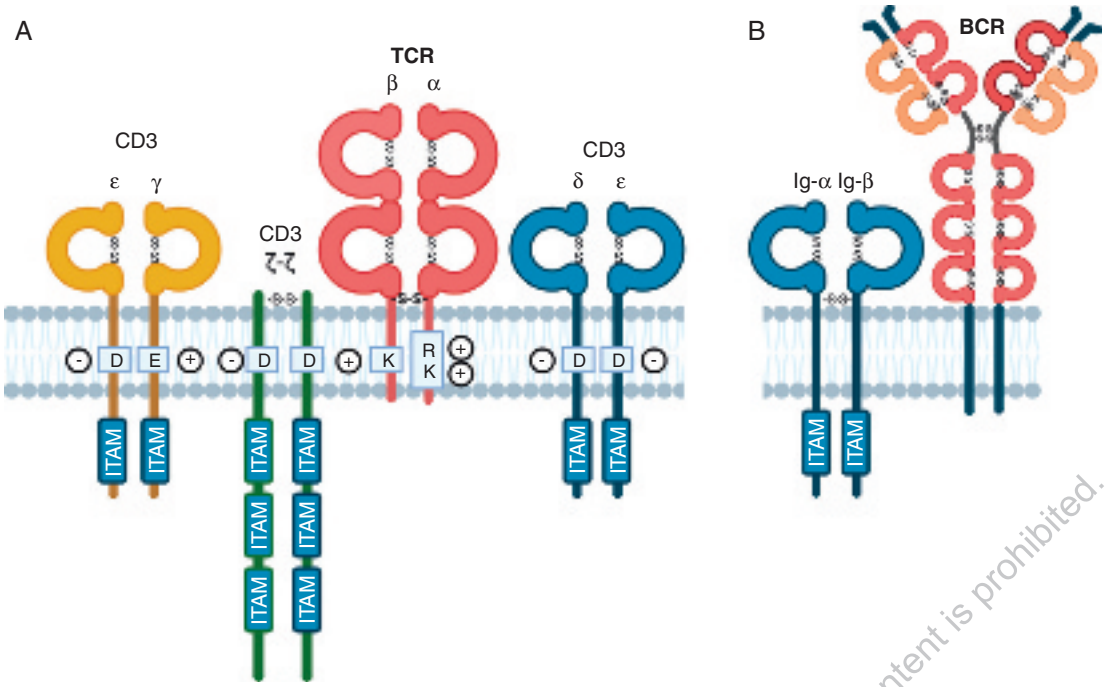


FIG. 1.10. The T cell receptor-CD3 complex. A: The TCR is made up two chains (α and β, red polypeptides) which have characteristic extracellular Ig folds that are stabilized by disulfide bonds (Chapter 23). The TCR does not have a cytosolic signaling domain. In order to couple extracellular ligand (peptide-MHC [pMHC] complexes) recognition to intracellular signaling, it constitutively associates with six polypeptides of the CD3 family—which exist as disulfide-linked dimers of CD3ε-CD3γ, CD3ε-CD3δ, CD3ζ-CD3ζ. The TCR-CD3 complex is stabilized by electrostatic interactions in the membrane-spanning region with the positive charges on Lysine (K) and Arginine (R) in the TCR α and β chains associating with the aspartic acid (D) on the CD3 molecules. In addition, glutamic acid residue (E) on the CD3γ chain stabilizes the extended lattice like interactions to form the holocomplex. The immunoreceptor tyrosine–based activation motifs (ITAMs) on the CD3 chains are marked as blue boxes. A total of 10 ITAMs are therefore associated with the TCR complex, the largest number for any receptor unit. The current consensus model for how pMHC can trigger the TCR are discussed in Chapters 23, 24. **B:** Signaling through the BCR uses the same strategy as through the TCR but with different associated molecules (Chapter 24).

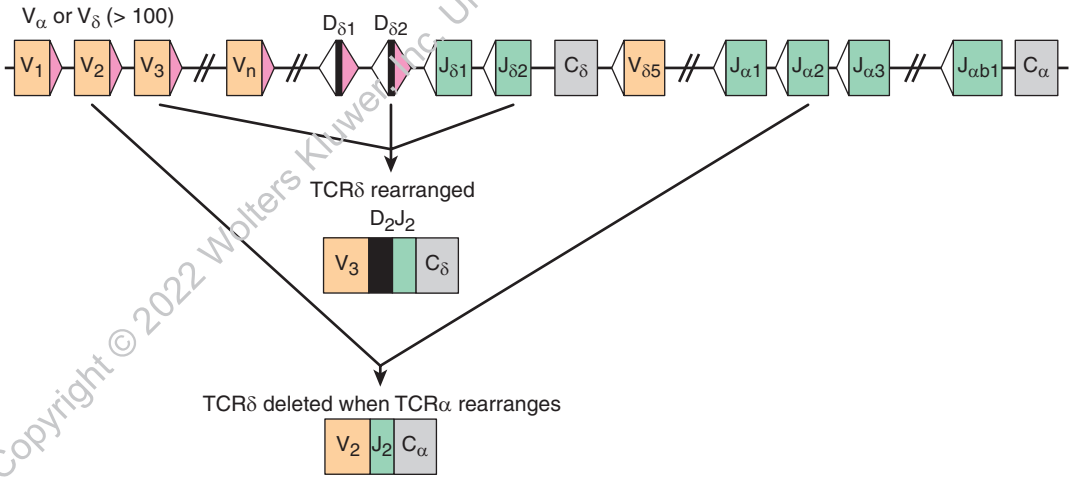


FIG. 1.11. T cell receptor α and δ Loci. The TCRδ locus is embedded in the TCRα locus. V segments (*orange*) for the two TCRs are admixed at the 5′ end of the locus. Only TCRδ has D segments (*black*) and an inverted V segment (Vδ5). J segments for both loci are in green, and the C gene segments in gray. RSS are shown as triangles flanking V, D, and J segments, pink triangle 23 bp spacer (*pink*) and white triangle 12 bp spacer. A functional TCRδ gene is produced upon VDJ rearrangement as shown. When a Vα segment joins to a Jα, then the entire TCRδ locus is deleted. Note the large number of Jα segments that can be used for receptor editing during thymus differentiation.

Furthermore, the same basic RAG-dependent rearrangement mechanisms are used to assemble the TCR V genes. Like IgH, the V region of the TCR β chain is encoded by a gene constructed from V_β, D, and J_β segments separated in the germline (Fig. 1.11 for TCR α and δ). Although the relative numbers of TCR rearranging gene segments differ from that for the IgH V region elements, the strategies for creation of a very large number of distinct genes by combinatorial assembly are the same. N-region addition further diversifies the genes and their encoded products. TCRβ has fewer *V* genes than IgH but much more diversity centered on the *D/J* region, which encodes CDR3. The α chain follows similar principles, except that like IgL chains it does not have *D* gene segments (Fig. 1.11).

The genes for TCR γ and δ chains are assembled in a similar manner, with only TCRδ having D segments (Fig. 1.11). The TCRδ D and J segments are located between the Vα/Vδ segments and the Jα/Cα segments, so that when TCR Vα genes are assembled during thymic differentiation the entire TCRδ locus is deleted.

The MHC and Antigen Presentation

As described above, the MHC governs T cell recognition of antigen-derived peptides bound to specialized grooves in MHC-I and MHC-II proteins, and they were first recognized because of the dominant role that MHC proteins play in transplantation immunity (Chapters 19, 48). The genetic basis of transplantation rejection between mice of distinct inbred strains is mediated by multiple genetic regions, but one region was shown to play the dominant role. Differences at this region alone would cause prompt (or acute) graft rejection, whereas any other individual difference usually resulted in a slow (or chronic) rejection. For this reason, the genetic region responsible for acute graft rejection was termed the *major histocompatibility complex*. Unfortunately, for 40 years the polymorphic MHC's role in transplantation obscured its true function: focusing the T cell recognition of microbial infection.

An MHC exists in all jawed vertebrates (Chapter 4). Its defining features are the transplantation antigens that it encodes, MHC-I and MHC-II molecules. MHC-I/II genes show a remarkable degree of polymorphism. The MHC also includes other genes, such as those for the C′ components C2, BF, and C4 as well as the cytokines TNFα and lymphotoxin in the so-called MHC class III region. In addition, genes encoding the proteasome subunits (LMP [low molecular mass polypeptide]) and ER transporters (TAP [transporter associated with antigen processing]), which produce and transport peptides for binding to class I, are also encoded in the MHC.

Class I MHC Molecules

Class I MHC molecules (MHC-I) are transmembrane glycoproteins expressed on most cells. They consist of an α chain of approximately 45,000 daltons noncovalently associated with β2-microglobulin, a 12,000-dalton molecule (Fig. 1.12A). The gene for the α chain is MHC-encoded, whereas that for β2-microglobulin is not. The membrane-proximal domain of the α chain and β2-microglobulin are IgSF members. The two membrane-distal domains form a groove called the

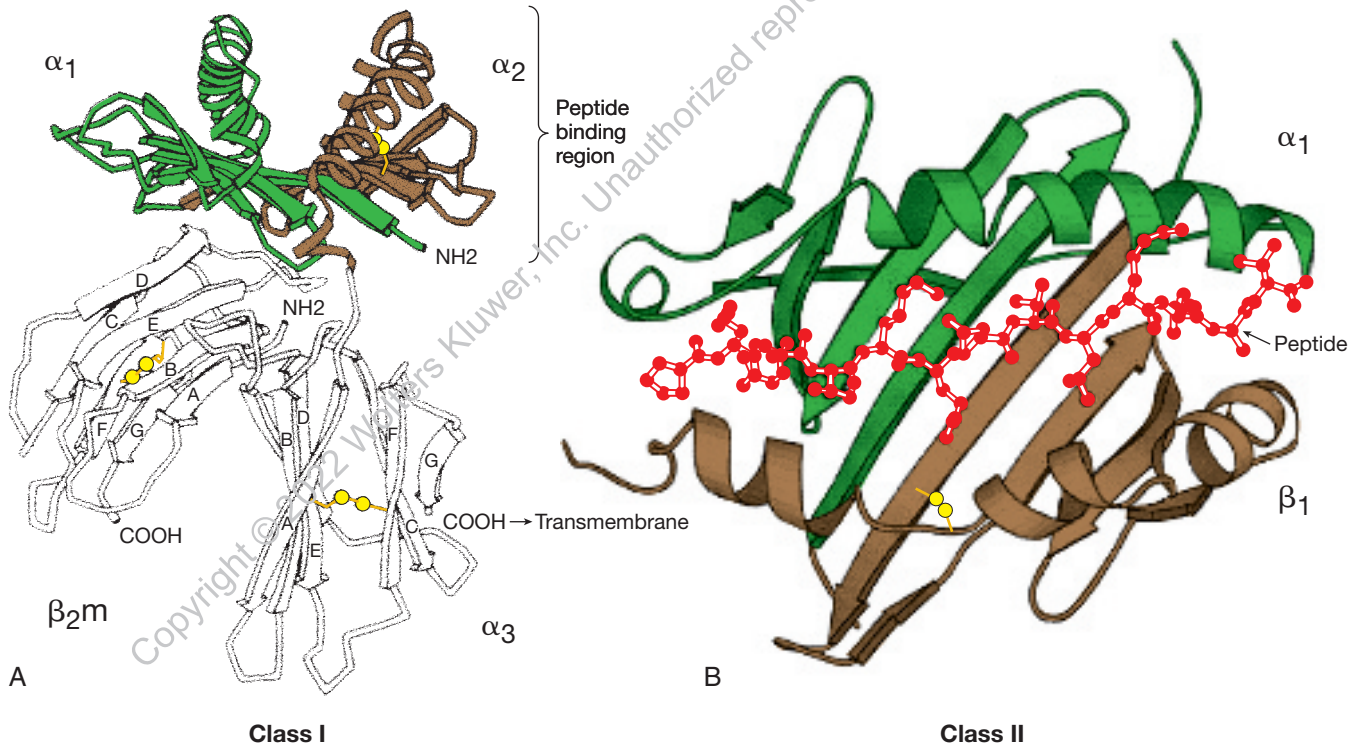


FIG. 1.12. MHC proteins. A: A class I molecule (MHC-I) in all vertebrates is composed of two N-terminal domains that make up the peptide-binding region (*green and brown*) and two C-terminal C1 domains that are members of the IgSF superfamily (Fig. 1.7). Intradomain disulfide bonds in yellow. **B:** Bird's-eye view of an MHC-II PBR with bound peptide. Each domain is made up of four contiguous beta strands topped by an alpha helix (α1 domain in green and α2 domain in light brown). The peptide (*red*) is bound as a beta strand and protrudes from both ends of the groove; for MHC-I the peptide is locked in at both ends of the groove (not shown). Intradomain disulfide bond in yellow.

peptide-binding region (PBR) made up of a β sheet floor topped with two α helices. The α chain is highly polymorphic with the diversity found in the PBR, specifically those amino acids that bind the antigen-derived peptides and contact sites for the TCR.

In the human, three loci encode classical class I molecules (or class Ia) and are designated human leukocyte antigen (HLA)-A, HLA-B, and HLA-C. HLA-A and HLA-B are highly polymorphic, and this high level of polymorphism is found in most vertebrate classical class I genes (Chapter 4). In addition, there is a second set of nonpolymorphic genes that encode class I–like molecules called nonclassical or class Ib molecules. One of the earliest of these to be discovered (M3) has antigen-presenting activity for formylated peptides, specialized to present prokaryotic antigens to CTL. The class Ib molecule CD1 has antigen-presenting function for glycolipids, providing a mechanism for generating T cells with special properties specific for such molecules (Chapter 36, see below). Another class Ib molecule, MR1, presents microbial metabolites to another subset of so-called "innate T cells" called mucosal-associated innate T cells (MAITs) (Chapter 37, see below).

Class II MHC Molecules

Class II MHC molecules (MHC-II) are also heterodimeric membrane glycoproteins whose chains are designated α and β, both encoded in the MHC (Fig. 1.12B). In the human MHC, there are three major sets of class II molecules, encoded in the DR, DQ, and DP regions of the HLA complex. The membrane-proximal domains (α2 and β2) of each chain are IgSF members, and the α1 and β1 domains form the PBR like that described for class I above. Class II genes are also polymorphic with most the diversity again in the PBR. The overall conformation of class II MHC molecules is quite like that of class I molecules. One distinct difference between MHC-I and MHC-II lies in the size of the peptides bound. Class I molecules bind to the N- and C-termini of peptides, locking them in the groove so that the peptide size ranges only from 8 to 10 amino acids. Class II molecules permit peptides to overhang on both ends, the size ranging from 11 to 22 amino acids (Fig. 1.12B).

MHC-II molecules have a restricted tissue distribution compared to MHC-I molecules. MHC-II molecules are found on B cells, DCs, epidermal Langerhans cells, macrophages, thymic epithelial cells, and in human, activated T cells. Levels of MHC-II expression are regulated in many cell types by IFNs. Indeed, IFNs can even induce expression of MHC-II on many cell types that normally lack them. IFNs also potently upregulate the expression of class I MHC molecules.

Antigen Presentation

The function of class I and class II MHC molecules is to bind and present antigen-derived peptides to T cells, whose receptors can recognize the peptide/MHC complex (pMHC) that is generated, as mentioned above (Chapter 20). There are two major types of antigen-processing pathways which are specialized to the distinct classes of pathogens that the T cell system must confront (Fig. 1.13). Extracellular bacteria and proteins may enter APCs by endocytosis or phagocytosis, enhanced by opsonization. Antigens within endosomes or lysosomes are fragmented in these organelles, and peptides derived from the antigen are loaded onto MHC-II molecules as these proteins traverse the vesicular compartments in which the peptides were formed. The loading of peptide stabilizes the MHC-II molecule, and the acidic pH of the compartments in which loading occurs facilitates the loading process. Once the peptide-loaded MHC-II molecules attain neutral pH, such as at the cell surface, the pMHC complex is stable. Peptide dissociation from such class II molecules is very slow, with a half-life measured in hours. The pMHC-II is recognized by CD4 T cells having complementary TCR. The tropism of CD4 T cells to the recognition of p/MHC-II complexes is partly due to the affinity of the CD4 molecule for monomorphic determinants on an MHC-II IgSF domain, focusing CD4 cells on those cells that normally express MHC-II molecules, namely DCs, B cells, and macrophages.

T cells also can recognize proteins that are produced within the cell (Fig. 1.13). The major pathogens recognized by this means are viruses and other obligate intracellular (nonendosomal/nonlysosomal) microbes that have infected cells (Chapter 44). In addition, proteins that are unique to tumors, such as mutant oncogenes, are overexpressed in tumors also can be recognized by T cells (Chapter 49). Endogenously produced proteins are fragmented in the cytosol by specialized proteases in the proteasome specific to adaptive immunity (immunoproteasomes). The resultant peptides are transported into the ER by the TAP where they are available for loading into MHC-I molecules. In contrast to the loading of MHC-II molecules, which is facilitated by the acid pH of the loading environment, the loading of class I molecules is controlled by interaction of the class I α chain with β2-m and several chaperones and catalysts called the "class I-loading complex" (Chapter 20). The peptide-loaded MHC-I molecule is stable and transported to the cell surface via the so-called default pathway. In contrast to peptide-loaded class II molecules that are recognized by CD4 T cells, peptide-loaded MHC-I molecules are recognized by CD8 T cells, in which the CD8 molecule plays a role in TCR recognition, like CD4 mentioned above. This form of antigen processing and presentation can be performed by virtually all cells because MHC-I molecules are universally expressed. This makes sense since any cell in the body can become virally infected or transformed.

Although the specialization of MHC-I to bind and present endogenously produced peptides and of class II molecules to bind and present peptides derived from exogenous antigens is generally correct, there are exceptions having physiologic importance. Particularly important is the capacity of some DCs (cDC1 or BATF3-dependent DC, Fig. 1.3) to load peptides from exogenous antigens into MHC-I molecules, allowing sensitization of naïve CD8 T cells to the antigens of pathogens that infect cells other than DCs. This "cross-priming" and "cross-presentation" was described above in the context of an immune response (Chapter 34).

Cytokines

Many of the functions of immune cells are mediated through cytokines (Chapters 9, 10, 12). These proteins can be divided

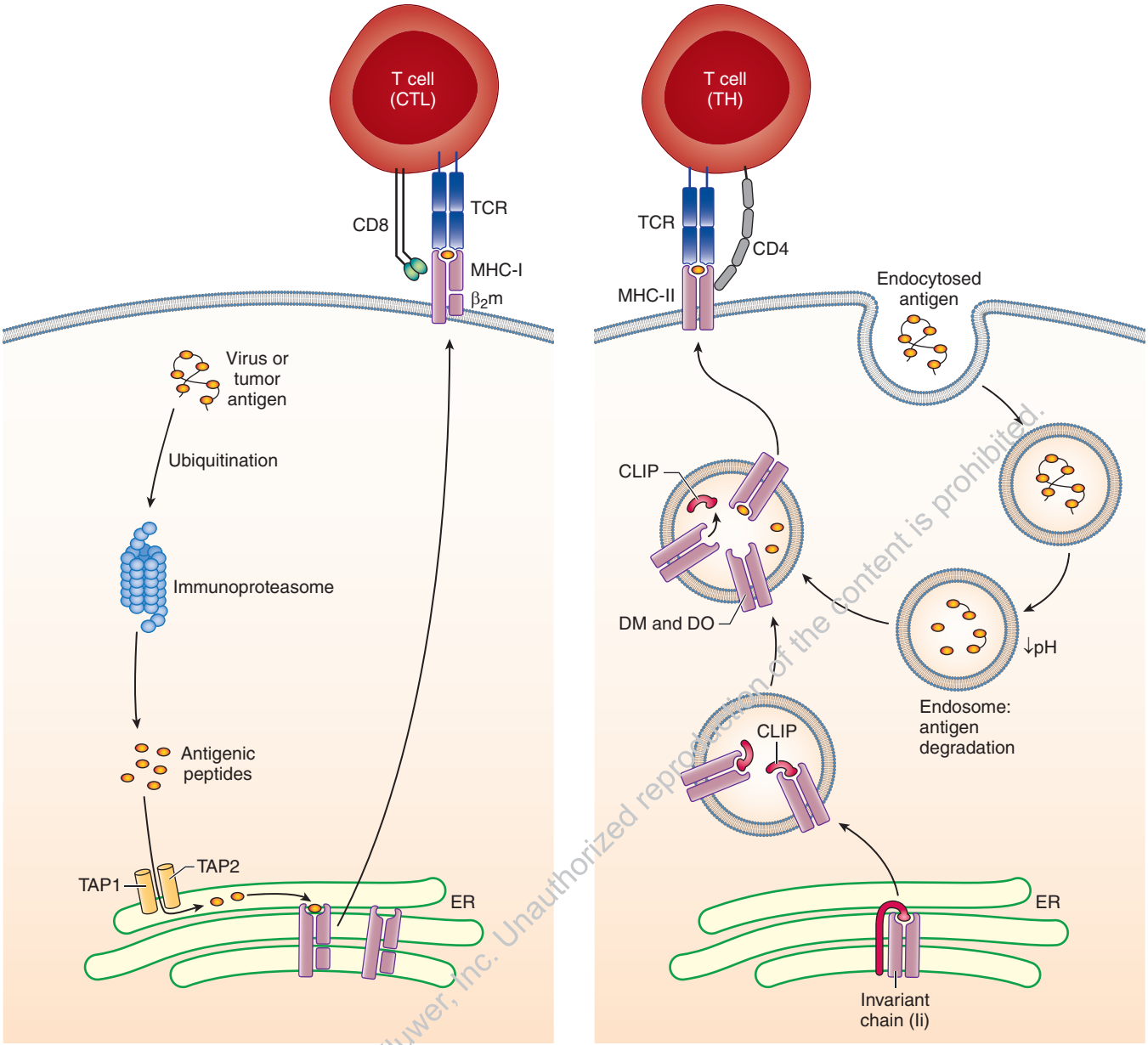


FIG. 1.13. Antigen presentation by MHC-I and MHC-II. A: Antigen (*orange*) in the cytosol misfolds and is conveyed to the immunoproteasome (*light blue*) after ubiquitination and then transported into the endoplasmic reticulum (ER) by the TAP (transporter associated with antigen processing) dimer (*light orange*). Peptides bind to class I molecules (*purple*) in the ER lumen. The class I molecules so-stabilized are transported to the cell surface where they are recognized by CD8 T cells (*red*). **B:** Antigens (*orange*) taken up into the endosome in antigen-presenting cells are degraded by cathepsins and other lysosomal proteases. MHC class II (*purple*) is deflected from the default pathway by the invariant chain (Ii) into endosomes containing the peptides (*orange*). CLIP, the last piece of Ii still attached to MHC class II, is removed from the MHC-II PBR which then binds peptides and moves to the cell surface where it is recognized by CD4 T cells (*red*). DM and DO class II molecules are involved in the removal of CLIP and selection of optimal peptides to bind class II.

into several families. Type I cytokines or hematopoeitins encompass many of the interleukins (ie, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-11, IL-12, IL-13, IL-15, IL-21, IL-23, and IL-27), as well as several hematopoietic growth factors. Type II cytokines include the IFNs and IL-10. The TNF superfamily molecules make up a very large family of regulatory molecules, the most recognizable being TNFα, lymphotoxin, CD40, and Fas ligand. The IgSF-based IL-1 family includes IL-1, IL-18, IL-33, IL-36, and IL-37. Chemokines (Chapter 11) are a large family of molecules that play critical roles in a wide variety of immune and inflammatory functions. IL-17 and its congeners (IL-17A-F), constitute a structurally unique set of cytokines.

Many of the cytokines are T cell products (Fig. 1.5), but most cytokines are not constitutive products of the T cell.

Rather, they are produced following T cell activation, usually resulting from presentation of antigen to T cells by APCs in concert with the action of costimulatory molecules (Fig. 1.5). Although cytokines are produced in small quantities, they are potent, binding to their receptors with equilibrium constants of approximately 10^{10} M^{-1}. In some instances, especially in adaptive immunity, cytokines are directionally secreted into the immunological synapse formed between a T cell and an APC; indeed, many cytokines have limited action at a distance from the cell that produced them. This is particularly true of many of the type I cytokines. However, other cytokines act by diffusion through extracellular fluids and blood to target cells distant from the producers. Among these are cytokines that have proinflammatory effects described above, such as IL-1, IL-6, and TNFα, and the chemokines that play important roles in regulating the migration of leukocytes described in Fig. 1.1.

Chemokines (Chapter 11)

Chemotactic cytokines (chemokines) have a variety of functions, the most dramatic of which is the regulation of leukocyte migration. They are critical dynamic organizers of cell distribution in the immune and inflammatory responses that use seven transmembrane-spanning, G-protein coupled receptors. We described the classical chemokine-directed migration above in the inflammation section, but other chemokines direct "every" type of cellular movement in the immune system such as migration of thymocytes to and through the thymus, placement of naïve lymphocytes in their correct positions within SLO, and positioning of effector lymphocytes throughout the body, to name just a few.

Chemokines are subdivided based on the number and positioning of their highly conserved cysteines. Among chemokines with four conserved cysteines, the cysteines are adjacent in one large group (the CC chemokines), whereas in a second large group they are separated by one amino acid (CXC chemokines). There are also rare chemokines in which the cysteines are separated by three amino acids (CX3C) or in which there are only two conserved cysteines (C chemokines). Individual chemokines may signal through more than one chemokine receptor, and individual receptors may interact with more than one chemokine, producing a complex set of chemokine/chemokine receptor pairs and providing opportunities for exceedingly fine regulation of cellular functions.

HEMATOPOIETIC CELL DEVELOPMENT AND INNATE LYMPHOCYTES

A striking feature of the immune system, with profound consequences to clinical immunology, is that the entire cellular catalog of innate, adaptive, and red blood cells can be generated from a single stem cell, known as the hematopoietic pluripotent stem cells (HSCs) (Fig. 1.3). These cells reside in the BM of adults and from there continuously generate all the numerous circulating lineages in healthy people. Indeed, this allows us to clinically transfer an entire immune system (for example, to transplant a healthy immune system into a person with an immunodeficiency) between persons by just isolating and injecting HSCs from donor to recipient. The process of development of each of the mature blood cell lineages (eg, RBC, macrophage, T cell, B cell) proceeds in multiple bifurcating steps from the HSC. Along the way dedicated progenitor cells form which can give rise to some but not other lineages. For instance, one of the earliest bifurcations results in the HSC generating common lymphoid progenitors (CLPs) and common myeloid progenitors (CMPs). CLPs can further differentiate along intermediate steps to arrive at mature ILCs, NK cells, T cells, or B cells, but not other lineages. At the same time CMPs can further differentiate into megakaryocyte-erythroid precursors (MkEPs) which can produce mature megakaryocytes and RBCs, or into granulocyte and monocyte precursors (GMPs) which can generate neutrophils, macrophages, etc. This overall framework has multiple additional branches and stable intermediates, which are discussed for each cell type in specific chapters.

The mature HSC found in the adult itself originates during embryonic development from endothelial cells in the aorta/gonad/mesonephros (AGM) regions of the vertebrate embryo. These transdifferentiated early HSCs then migrate to the fetal liver before birth and expand considerably there. The migration of these fetal liver HSCs to the BM during circulation also results in their enrichment in the cord blood. In addition to HSCs, another source of a distinct subset of mature immune cells (especially those that seed distinct tissue niches near barrier sites) is an earlier wave of cells developing from progenitors in the yolk sac. While most of these cells get replaced over time with HSC-derived cells, some lineages (notably tissue macrophage subsets and B1 cells) persist from the yolk-sac lineage into adulthood.

B Cell Development (Chapter 25)

B-lymphocytes derive from lymphoid progenitor cells, which in turn are derived from HSC (Fig. 1.3). A detailed picture has been obtained of the molecular mechanisms through which committed early members of the B lineage develop into mature B cells. These events occur in the fetal liver and adult BM. Interaction with specialized stromal cells and their products, including cytokines such as interleukin (IL)-7 and BAFF, is critical to the normal regulation of this process.

B cell development requires commitment to the B lineage and repression of differentiation to cells of other lineages, especially T cells. In pro-B cells and pre-B cells, the genetic segments that encode the BCRs are assembled. As described above, the genetic elements encoding the variable portions of Ig H and L chains are not contiguous in germline DNA nor in the DNA of nonlymphoid cells (Chapter 22) (Fig. 1.9). In pro- and pre-B cells, these genetic elements are rearranged to construct expressible V-region genes in a RAG-dependent fashion. The differentiation process is controlled at several steps by checks that determine whether prior steps have been successfully completed. These checks depend on the surface expression of appropriately constructed Ig or precursor-Ig molecules. The IgH V, D, and J elements rearrange first, and

the H-chain V region is expressed along with the μ C-region gene to make the IgH chain of IgM associated with a surrogate light chain, VpreB/λ5. The V region of the surrogate chain is a complete germline V domain, not generated by V-J rearrangement. The surrogate chain "checks" whether the newly generated H chain can interact with typical L chains in a mature B cell. The successful completion of the process of IgH gene rearrangement and the expression of the resultant IgM on the cell surface marks the transition between the pre-B and B cell states, and the cell undergoes a proliferation stage. After proliferation, the developing B cell reexpresses RAGs and commencement of L chain gene rearrangement occurs, eventually resulting in an IgM BCR at the cell surface and the cell moves out of the BM as a "transitional B cell." B cells complete their maturation process by expressing on its surface a second class of Ig composed of the same L chain and the same V (VDJ) region but of a different H chain C region, the aforementioned IgD. Thus, through an alternative splicing mechanism, mature naïve B cells express both IgM and IgD surface molecules that share the same V region.

There are several checkpoints during B cell development to ensure a functioning, self-tolerant B cell population. First, if rearrangement of the H chain gene fails to produce a functioning H chain, either because the gene is out-of-frame, or the protein cannot associate with the surrogate L chain, the cell will die. Second, the L chain V-J rearrangement may be out-of-frame, or the L chain cannot associate with the H chain because of its sequence; in this case the L chain V segments upstream and J segments downstream of the rearranged V-J can undergo "receptor editing," generating a new V-J and deleting the previous one. Furthermore, if the nascent IgM is autoreactive, receptor editing can also occur in an attempt to produce a non-self-reactive BCR. These checks on tolerance are important because up to two-thirds of newly generated BCR are self-reactive. After the B cell satisfies these BM checkpoints and moves to the periphery, the transitional B cells are also tolerance-susceptible and will die when coming in contact with self-antigens of the body. Finally, mature IgM/IgD-positive B cells move into the B cell follicles of spleen, lymph node, or mucosal SLO and can respond to antigen as described above (Fig. 1.6).

B1 and Marginal Zone B Cells, AKA Innate B Cells (Chapter 25)

B cells consist of at least three distinct populations: conventional or follicular B cells (or B2 cells), B1 cells, and MZ B cells. B1 cells were initially recognized because a large subpopulation uniquely expresses the cell-surface protein CD5. In the adult mouse, B1 B cells are found in relatively high frequency in the peritoneal cavity and some mucosal surfaces cavity but at low levels in SLO. B1 cells undergo self-renewal throughout life, and their development is under different rules than conventional B cells, as they are derived from an early precursor derived from the yolk sac, not the AGM. B1 cells are not IL-7-dependent and do not use the surrogate L chain during their development. Their differentiation is positively selected by the BCR, which has a repertoire skewed toward recognition of conserved epitopes on pathogens, like the PAMP paradigm. Interestingly, expression of a B1 BCR in conventional B cells induces a change to a B1 phenotype, for example, capable of responding rapidly to antigen in a T-independent fashion.

MZ B cells are localized in the highly vascularized and antigen-filtering splenic MZ that surrounds the white pulp. Like B1 B cells, MZ B cells express a repertoire biased toward bacterial cell wall constituents and senescent self components. MZ and B1 cells respond very rapidly to antigenic challenge, often independently of T cells. Uniquely, among all populations of B cells, MZ B cells are dependent on Notch-2 signaling for their development.

B1 cells and MZ B cells are responsible for the secretion of the majority of serum IgM that exists in nonimmunized mice, often referred to as natural IgM. Among the antibodies found in such natural IgM are molecules that can combine with phosphatidylcholine (a component of pneumococcal cell walls) and with LPS and influenza virus. B1 B cells also produce autoantibodies, although they are generally of low affinity and not pathogenic. There is evidence that B1 B cells are important in resistance to several pathogens and may have a significant role in mucosal immunity.

T Cell Development (Chapter 27)

Upon entry into the thymus, T cell precursors do not express TCR, the CD3 complex, or the CD4 or CD8 molecules (Fig. 1.14A). Because these cells lack both CD4 and CD8, they are called double-negative (DN) T cells, which simultaneously rearrange TCR β, γ, and δ genes; if the cells have productive rearrangement of γ and δ and a functional receptor is generated, this provides a strong signal for the cell to develop into a γ/δ T cell that expresses neither CD4 nor CD8. This is a rare event after birth in many mammalian species, where most T cells are of the conventional αβ type. However, γ/δ cells are numerous early in fetal life and express TCR with specific V regions and no N-region diversity (Chapters 23, 35).

DN thymocytes that have rearranged their TCRβ genes express the protein with a surrogate receptor called Pre-Tα, which performs the same function as the surrogate chain in B cells. The cells undergo a proliferative phase and then express both CD4+ and CD8+ (double-positive [DP] cells) on their surface. The DP cells then commence rearrangement of the TCRα chain genes. Once TCR chains are expressed, these cells undergo two selection processes within the thymus, positive and negative selection. These events result in the deletion of potentially autoreactive (capable of high-affinity binding of self-peptide/self-MHC complexes) cells and the enrichment of T cells biased toward the recognition of peptides in association with self-MHC molecules. **Positive selection** occurs when T cells with receptors with "intermediate affinity" for self-peptides bound to self-MHC molecules expressed by the cortical epithelium are selected, thus forming the bias of the T cell repertoire for foreign peptides associated with self-MHC molecules (Figs. 1.14A and B). The capacity of the TCR and CD4 (or CD8) to bind to an MHC-II (or an MHC-I) molecule on the thymic epithelium leads either to the differentiation of DP thymocytes into CD4+ (or CD8+) single-positive (SP)

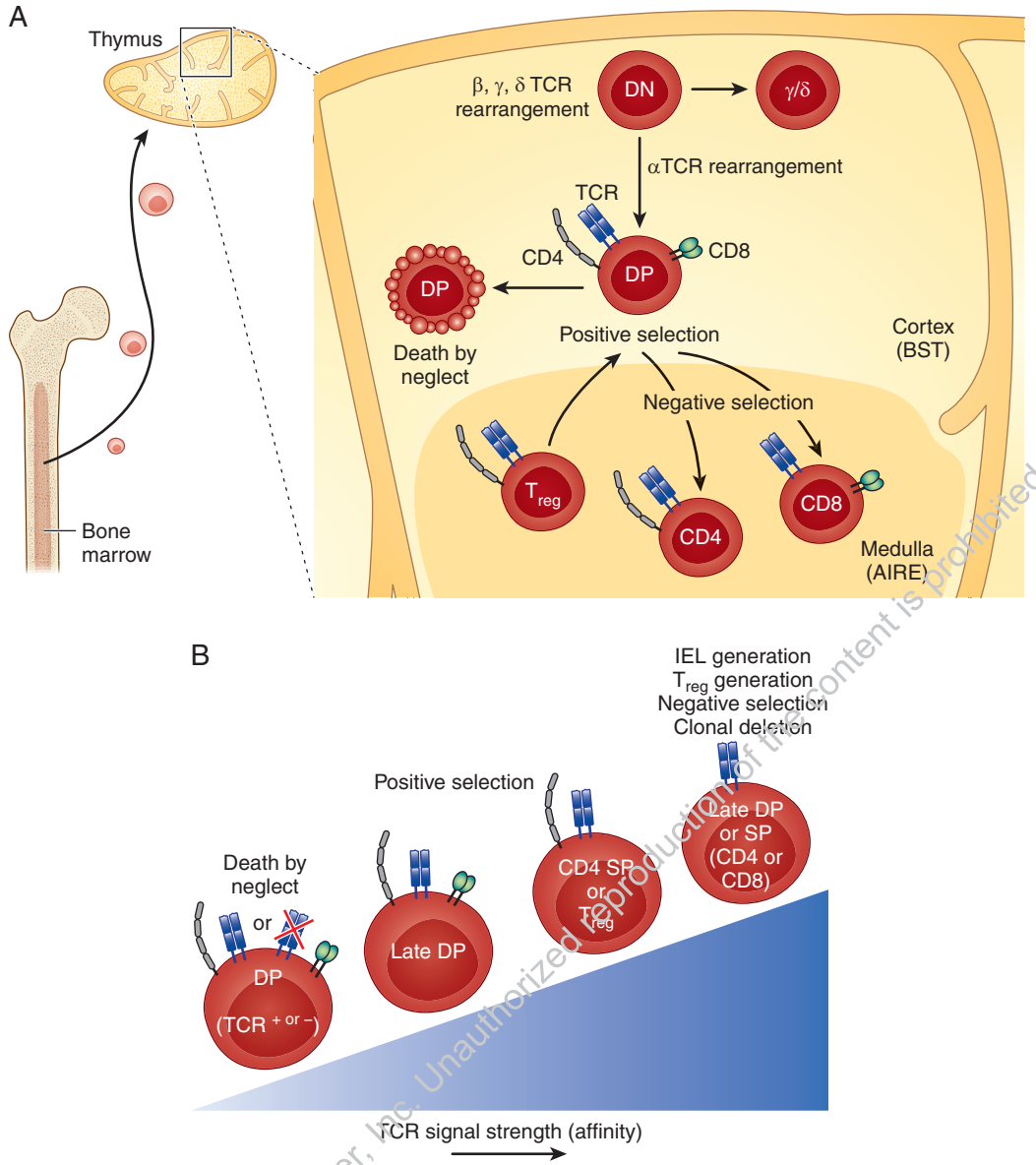


FIG. 1.14. T cell development. **A:** Differentiation of cells in the thymus progresses from the thymic cortex to medulla. Precursor cells (light red) move from the bone marrow to the thymus, where they are first found in the subcapsular region as double-negative cells (no CD4 or CD8, red). DN cells simultaneously rearrange β, γ, and δ genes. If the γ and δ rearrangements are productive, γδ T cells are generated. If they are nonproductive and TCRβ rearrangements are productive, then the cells undergo a proliferative phase followed by expression of CD4 and CD8 (DP), and TCRα rearrangement commences. If the TCR can be positively selected on the cortical epithelium, then the cells become single-positive (SP) CD4 or CD8; if not, then the cells are programmed to die. If the positively selected cells bind to MHC/peptide complexes too avidly at the corticomedullary junction or the medulla the cells undergo apoptosis, referred to as negative selection, or may be recruited as T_{reg} cells or intraepithelial T cells. **B:** The affinity of TCR binding to the thymic epithelium results in different T cell fates: Death by neglect if there is no interaction or weak interaction; positive selection of cells with intermediate affinity; and negative selection (clonal deletion) or selection of T_{regs} or intraepithelial lymphocytes (IEL) with high avidity interactions.

T cells or to the selection of cells that have "stochastically" differentiated down the CD4 (or CD8) pathway. T cells that receive no signal from the TCR are eliminated in the thymic cortex by apoptosis, called "death by neglect," much like death of B cells in the GC failing to recognize antigen on FDC.

Negative selection is the deletion of cells with high-affinity TCR for complexes of self-peptides associated with self-MHC molecules on APC at the thymic cortical-medullary junction or in the medulla on the epithelium (Fig. 1.14A). This is a major mechanism by which the T cell compartment develops immunologic unresponsiveness to self-antigens (Chapters 27, 32). T cells that are negatively selected are deleted through apoptosis. Medullary epithelial cells express a molecule called AIRE (Autoimmune Regulatory Element) that induces

expression of self-molecules expressed throughout the body, AKA tissue-restricted antigens (TRAs) such as insulin (pancreas-specific) or C-reactive protein (liver-specific). Mutations of AIRE result in an autoimmune disease called autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED).

Regulatory T Cells (Chapter 29)

T cells can diminish as well as help immune responses, and the cells that mediate such inhibitory effects are called regulatory T cells (T_{regs}). T_{regs} typically express Foxp3, CD25, and the IL-2Rα chain. These cells inhibit both CD4 and CD8 T cell responses to their cognate antigens. How their inhibitory functions are mediated remains controversial. In some instances, cell/cell contact is required for suppression, whereas T_{reg} cytokines like IL-10 and TGF-β may inhibit others.

T_{regs} are critical in autoimmune diseases. In the absence of T_{regs}, conventional T cells cause several autoimmune conditions, including autoimmune gastritis, diabetes mellitus, adrenal insufficiency, and inflammatory bowel disease. T_{regs} normally recognize autoantigens and their responses result in the suppression of conventional T cell responses. Whether the receptor repertoire of T_{regs} and the conventional T cells are the same has not been fully determined, although T_{regs} may derive from a thymic CD4 T-cell population with relatively high affinity for self-antigen (Fig. 1.14B). As mentioned above, iT_{regs} can be derived in the periphery from naïve CD4 T cell populations when naïve cells are stimulated on DC by their cognate ligands in the presence of TGF-β and IL-2 (Fig. 1.5).

Innate Lymphoid Cells and NK Cells (Chapter 18)

NK cells play an important role in the immune system. Indeed, in mice that lack mature T and B cells the NK system still provides these animals a substantial measure of protection against infection. NK cells lack TCR (or Ig) but are closely related to T cells in their functions. They have a set of activating receptors (associated with ITAM-containing receptors) that allow them to recognize features associated with virally infected cells or tumor cells, such as "SOS molecules" that are only expressed at the cell surface in times of stress. They also express receptors with inhibitory cytosolic domains, such as ITIM (immunoreceptor tyrosine–based inhibitory motif) for MHC molecules that prevent NK lytic activity. Thus, virally infected cells or tumor cells that escape the surveillance of CTL by downregulating expression of MHC-I molecules become targets for efficient killing by NK cells because the cytotoxic activity of the latter cells is no longer inhibited by the recognition of particular MHC- I alleles, a phenomenon known as "missing self."

NK cells also express FcγRIII (CD16). Antibody-coated cells can be recognized by NK cells, and such cells can then be lysed (Chapter 33). This process is referred to as antibody-dependent cellular cytotoxicity (ADCC). NK cells are efficient producers of IFN-γ in response to recognition of virally infected cells and tumor cells, cross-linkage of FcγRIII, and stimulation by the cytokines IL-12 and IL-18.

Since the previous *FI* edition, a new subclass of lymphocytes came into prominence called innate lymphoid cells (ILCs) (Fig. 1.3) (Chapter 18). They were discovered over 20 years ago as lymphoid tissue-inducer cells (LTI) that organize SLO generation (for all tissues except the spleen). ILC types (ILC1-4) mimic the different Th cell types described above in the types of cytokines secreted. However, like NK cells (a type of ILC), ILCs do not have receptors generated by RAG-mediated rearrangement; instead, ILCs are activated by cytokines in the tissue in which they reside. ILCs have been best studied in mucosal tissues such as the lung and intestine in responses to parasites, but this is just the tip of the iceberg. ILCs' roles in infection and homeostasis will continue to be a fertile area of research.

Gamma Delta T Cells (Chapters 23, 35)

γ/δ T cells were discovered in the early 1980s as a complete surprise, with no underlying biology. A breakthrough in the 1990s was the realization that γ/δ T cells were not MHC-restricted and likely recognized antigen more like antibodies, as detailed above. For many years these cells were known for their innate phenotypes, including a major subset in human blood that recognizes isopentenyl pyrophosphate (IPP, and other such intermediates from microbes), and for waves of γ/δ cells in mice that are generated during fetal life and peripheralized to the skin and mucosal surfaces. Recently, members of the butyrophilin family have been shown to participate in the recognition of IPP and of other unknown antigens. Some γ/δ crystal structures show that the δV CDR3 recognizes the "true" antigen, suggesting that γ/δ T cells can also have adaptive characteristics. It has been suggested that a large proportion of T cells could have both innate (rapid fire) and adaptive (large TCR repertoire) characteristics, so-called "adaptate" T cells.

Natural Killer-T Cells (Chapter 36) and Mucosal-Associated Innate T Cells (Chapter 37)

The first "innate αβ T cells" to be discovered, natural killer-T (NKT) cells, recognize the class Ib molecule CD1, which does not present peptides like classical MHC-I but rather lipid or glycolipid antigens. These T cells are defined by their restricted TCRα usage and positive selection on CD1 expressed by thymocytes, not the thymic epithelium. NKT cells respond rapidly when confronted by pathogens, and different NKT subsets produce cytokines that can fight pathogens and skew adaptive responses. The first NKT cells discovered were shown to make IL-4, but subsets of cells secreting different cytokines are apparent, like conventional CD4 T cell subsets (Fig. 1.5), but these phenotypes are imprinted during thymus differentiation rather than in response to foreign antigens.

The class Ib molecule MR1 was discovered 20 years ago with no known functions for nearly a decade. The innate-like MAIT T cells were found to use MR1 as a restricting element, predominantly in mucosal tissues. The basic TCR usage, positive selection, and rapid activation are like NKT cells. Recently MR1 was shown to present microbial metabolites to MAITs, a substantial breakthrough in immunology.

Currently, we could think of the class I system as a trifecta: (1) the polymorphic class I molecules presenting peptides to conventional CD8 cells; (2) the CD1 molecules presenting lipid antigens to innate T cells; and (3) MR1 molecules presenting bacterial "PAMPs" to MAITs.

CLINICAL IMMUNOLOGY

Immunological Tolerance and Autoimmunity (Chapters 32, 46)

One of the central problems facing the immune system is how to mount highly effective immune responses to threats posed by potentially pathogenic agents while ignoring the host's own tissues. This is further complicated by the need to respond to some self (albeit with mutations) cells as in tumors. The mechanisms ensuring the lack of response to healthy self-antigens are complex and involve a series of strategies. Chief among them is elimination or inactivation of cells capable of self-reactivity. The encounter of immature, naïve B cells with antigens with repetitive epitopes capable of cross-linking membrane Ig can lead to elimination of the B cells, particularly if no T-cell help is provided simultaneously. This elimination of potentially self-reactive cells is often referred to as clonal elimination. As described above, many self-reactive cells, rather than dying upon encounter with self-antigens, undergo a further round of Ig gene rearrangement called receptor editing described above, which allows a self-reactive cell to substitute a new receptor and therefore avoid elimination.

There are many self-antigens that are not encountered by the developing B-cell population or that do not have the capacity to cross-link BCR sufficiently to elicit the receptor editing/clonal elimination process. Such cells, even when mature, may nonetheless be inactivated through a process that involves cross-linking of receptors without the administration of critical costimulatory signals. These inactivated cells may be retained in the body but are unresponsive to antigen and are referred to as anergic. When removed from the presence of the anergy-inducing stimulus, anergic cells may regain responsiveness.

The immune system can discriminate between antigenic determinants expressed on foreign substances, such as pathogenic microbes, and potential antigenic determinants expressed by the tissues of the host. The ability of the system to forego full-blown immune responses to self-antigens is referred to as *immunologic tolerance*, a complex concatenation of processes. Perhaps the most important element is the active destruction or inactivation of self-reactive cells. The encounter of antigens (such as self-antigens) in the absence of cues from the innate immune system may fail to engender a response, may lead to a minimal response, or may lead to immune cellular inactivation through a process referred to as *anergy*. Finally, the T_{regs} described above actively *suppress* responses against self-antigens. Indeed, mutations in the key transcription factor *FOXP3,* which determines T regulatory cells, lead to severe multiorgan autoimmunity (IPEX syndrome). The critical necessity to control self-reactivity is clearly shown by this multilayered system that involves elimination, inactivation, and suppression.

Failure to establish immunologic tolerance or unusual presentations of self-antigens can give rise to tissue-damaging immune responses against host molecules, resulting in autoimmune diseases (Chapter 46). Autoimmune diseases or those with major autoimmune components include systemic lupus erythematosus (SLE), rheumatoid arthritis, insulin-dependent diabetes mellitus, multiple sclerosis, myasthenia gravis, and inflammatory bowel disease. Successful therapeutic modulation of the (auto)immune response is a reality using inhibitors of antigen-receptor signaling (Chapter 24), cytokines, or transfer of special cell types.

Primary Immunodeficiency (Chapter 50)

Mutations in genes that control the development or function of different immune cells can cause different primary (congenital or hereditary) immunodeficiencies (PID). As discussed above, all mature immune cells develop in adults from HSCs. Different regulatory genes are critical for each step in HSC development and deleterious mutations in any of these can result in the absence of mature cells derived from these precursors. For instance, both B cells and T cells require VDJ recombination to generate their receptors. Without functional receptors, the precursors to B and T cells cannot achieve their next developmental step. As a result, when critical enzymes (eg, the RAGs) are mutated in people, they fail to make both B and T cells, resulting in SCID. Similarly, mutations in receptors for key cytokines that instruct development of a joint precursor for T and NK cells can lead to SCID through absence of these critical cells. In each case, patients are susceptible to a range of infections that are normally kept at bay by these functional cells. However, the fact that these cells can continuously develop from HSCs offers a clear therapeutic approach—the transfer of healthy BM cells (derived from matched donors) intravenously to patients allows these to take over and repopulate the mature immune cell compartments.

Tumor Immunity (Chapter 49)

In healthy individuals, constant exposure to mutagens (as simple as sunlight), viral infections, errors in DNA replication, etc generate somatic mutations every day. While the propensity of such mutations to transform into tumors varies with genetic background, in most cases, the immune system can police these aberrations and effectively eliminate them. Recognition of tumors presents a particular challenge to the immune receptors which (see Principle #2) must maintain tolerance of self-tissues. Especially in the case of nonviral tumors (where there is no infectious etiology), identification is challenging. The solutions appear to be manifold. In the case of innate immune cells, dedicated sensors for DNA (such as STING) released from necrotic tumor cells or receptors for stress-associated molecules (such as Rae1) allow a variety of effector cells to be turned on. Typically, T cells (primarily CD8s, but also CD4s) activated by tumor-associated

antigens (TAA) or tumor-specific (neo)antigens (TSA) form the effector response. While these processes operate quite well in acute antitumor responses, immunological control of established tumors is challenging. For the most part, the immune system then begins to treat the slow-growing tumor as another self-tissue. Both immune termination signals (such as negative-regulatory checkpoints on T cells including PD1) and tolerance associated players (illustrated by the expansion of FOXP3+ regulatory T cells for instance) are now recruited to curtail robust antitumor effector responses. Over the past decade, several years of previous work defining the basic immunobiology of these processes has begun to bear clinical dividends in the form of promising immunotherapies.

First, it has become possible to sequence expressed genes from tumors and identify the mutations to detect those self-peptides that can bind to self-MHC allelic products and thus serve as targets for CD8 cells. Antigen-specific CD8 cells can be transferred into the patient or specific TCRs can be transduced into CTL before adoptive transfer. For virally induced tumors, T cells for common HLA alleles (eg, HLA-A02) can be taken "off the shelf" for adoptive transfer. Second, for hematological tumors (especially of B cells), chimeric antigen receptor (CAR)-T cells have been a major breakthrough. By combining the V regions of antibodies specific for the B cell markers CD19 or CD20 with TCR signaling machinery (Chapter 24) (hence the chimeric nature of the construct), transduced T cells can kill the tumor cells after adoptive transfer (Fig. 1.15). Of course, normal B cells are also killed off with this protocol, but patients can be treated with passive transfer of Igs to protect them, even for a lifetime. There is no end in sight in the mixing and matching of BCR/TCR/signaling components to perfect the CAR-T technique, making it applicable to different tumor types. Third, with the identification of the plethora of costimulatory molecules of either the activating or inhibitory type (Chapter 24), reagents that block certain inhibitory molecules, like CTLA4 and PD1, have been shown to unleash T cells specific for tumor-specific antigens, especially efficacious for highly mutable tumors like melanoma and renal cancer. Fourth, the tumor microenvironment has been scrutinized for suppressive cells (myeloid and lymphoid) and molecular mediators that inhibit T cell responses. Tumors can be made "hot" by blocking these suppressive mechanisms. There are other advances in this field (discussed in Chapter 49), but we just highlight those that have been successful in the clinic. It should be emphasized that *none of these breakthroughs* was in common usage in 2012, when the previous edition of *IPI* was published.

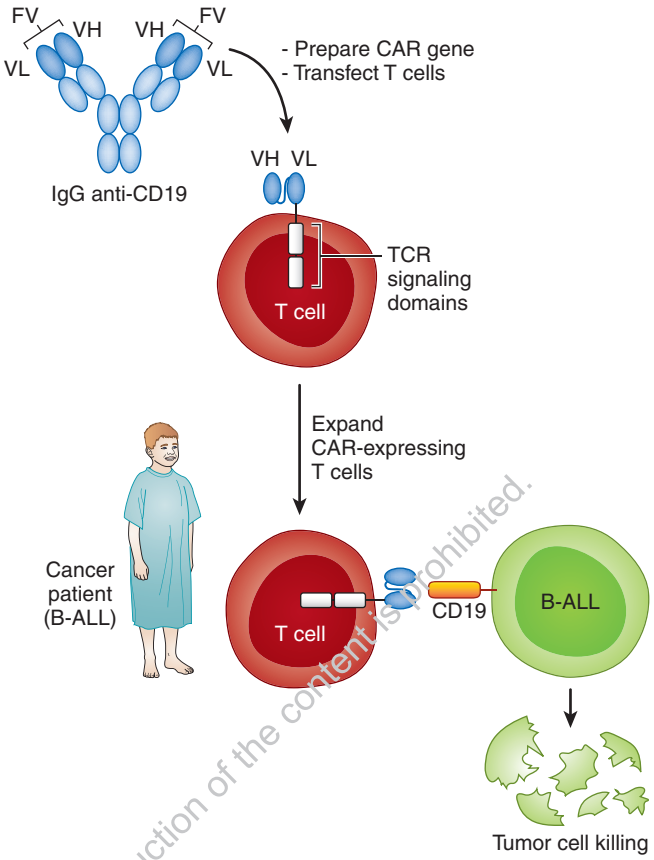


FIG. 1.15 Generation and use of CAR-T cells in the clinic. Chimeric antigen receptors (CAR) are generated with FV (VH/VL in *blue*, Fig. 1.7) from a monoclonal antibody with TCR signaling motifs from CD3 and costimulatory molecules (*white rectangles*, and see Fig. 1.10). The chimeric gene is transfected into the patient's T cells (*red*), which are then transferred to the patient. The CAR-T cells recognize the surface antigen on the cancer cells (CD19 in orange on B cell-acute lymphocytic leukemia [ALL] in this case, *green*) and kill them.

Transplantation (Chapter 48)

The field of transplantation has a special historical significance to immunology, since the pivotal efforts in the area by Gorer, Medawar, Brent, Billingham, Hasek, and Owen in the 1940s and 1950s identified many of the key phenomena which then took years of discovery to explain. This phase of discovery laid the foundations of much of cellular immunology as we know today. The identification of MHC alleles, the appreciation of their polymorphisms, and their significance in graft rejection have allowed for much of the success in modern clinical organ transplantation. In the 1970s, development of the immunosuppressive drug cyclosporin by Borel, which targets antigen-receptor signaling in T cells, allowed transplants to be performed even when the donor and recipient were not fully matched (the next-generation cyclosporin-related drug is Tacrolimus, Chapter 24). With increasing appreciation of other cell types and processes involved in tolerating transplants, newer approaches are now in laboratory evaluation. Chapter 48 discusses BM transplants from the same solid organ donors to generate tolerance via blood cell chimerism, as well as ways to block costimulatory pathways or the priming of donor-specific T cells in SLO. While not surmounting the rejection puzzle, the problem of a short supply of organs such as hearts for transplantation may be circumvented by xenotransplantation. Over the past 20 years, the miniature swine genome has been modified to overcome the acute and chronic rejection episodes that occur when these organs are transplanted into humans. The promise of using embryonic stem cells for differentiation into solid organs is also an area of intense study. Finally, the possibility of inhibiting innate pathways in transplanted tissue to block

activation of adaptive immunity is an active area of research. Before the next edition of FI in 10 years or so, like what has occurred in the tumor field, there will likely be significant breakthroughs in generating antigen-specific tolerance in the area of transplantation.

NEW TOPICS MATURING SINCE THE LAST EDITION

Thirty-seven of the chapters in FI2022 update contemporary advances of the same fields as in 2012, but 21 of those chapters have new authors, such as the chapter on the history of immunology (Chapter 2). The other chapters written by the same authors as in 2012 have been thoroughly updated. Twelve chapters cover new subfields, with a large focus on evolution of the immune system (Chapters 3-5) and innate lymphocytes, now covered in six chapters (Chapters 18, 35-37, and in Chapters 23, 25 in subsections). Other entirely new chapters cover the fields of Immunometabolism (Chapter 6) and Neuroimmunology (Chapter 39). The latter field has begun to make inroads into understanding how the immune system patrols neural tissues which were once considered privileged, but also how these two complex organ systems (the nervous and the immune systems) regulate each other. The ability of the mother to tolerate a fetus (which can bear many antigens encoded by the paternal genome which are "foreign" for the mother's immune system) remains one of the most intriguing puzzles of Immunology. The field of Maternal-Fetal Immunology has experienced major breakthroughs in the past decade and is presented in a new chapter (Chapter 41).

CONCLUSION

This introductory chapter has attempted to provide the reader with an appreciation of the overall organization of the immune system with its key cellular and molecular components. The immune system is highly complex, capable of a wide range of effector functions, and it has potent but only partially understood regulatory processes. The immune system is the key to the development of prevention and treatment of a broad range of diseases. Indeed, in addition to providing basic and conceptual knowledge of immunity, this edition also examines immunity to infectious agents and the immunologic mechanisms in disease. The introductory material provided here should orient the uninitiated reader in understanding the immunologic mechanisms brought into play in a wide range of clinical conditions.