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CELLULAR AND MOLECULAR IMMUNOLOGY



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Cellular and Molecular Immunology

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OVERVIEW OF INNATE IMMUNITY

The term *innate immunity* refers to defense mechanisms that are always present, ready to combat microbes and other offending agents. The innate immune system, which was introduced in Chapter 1, consists of many types of cells and soluble molecules in tissues and blood that constantly prevent microbes from invading and establishing infections. If microbes do establish a foothold, innate immune responses provide early defense, before adaptive immune responses can develop (see Fig. 1.1). In this chapter, we will describe in more detail the components, specificity, and antimicrobial mechanisms of innate immunity. Although the focus of much of the subsequent chapters is on the role of the adaptive immune response in host defense and disease, throughout we will also point out the impact of the innate immune system on adaptive immune responses and how the innate immune system contributes to protection against infections.

Functions and Reactions of Innate Immune Responses

Innate immunity serves several essential functions that protect us against microbes and tissue injury. The major components of the innate immune system are surface epithelia, which block the entry of microbes; tissue sentinel cells, including macrophages, dendritic cells, and mast cells, which detect microbes that have breached epithelia and initiate host responses; white blood cells (leukocytes), including neutrophils, macrophages derived from monocytes, natural killer cells, and other cells, which enter the tissues from the blood and eliminate microbes that have invaded through epithelia and also get rid of damaged host cells; and several types of plasma proteins, which combat microbes that have entered the circulation. We discuss the functions of each of these later in the chapter. Many other cell types, including epithelial cells and other tissue cells, also possess intrinsic mechanisms for defending themselves against microbes.

The functions of innate immune responses have some important general features.

- ***The innate immune system maintains physical and chemical defenses at epithelial barriers such as the skin and lining of the gastrointestinal and respiratory tracts, which block microbial entry.*** Microbes are able

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to colonize tissues only if they are capable of crossing epithelia. If these barriers are damaged or microbes are able to penetrate them, innate and adaptive immune responses are activated to provide the next lines of defense.

- ***Innate immune responses are the initial reactions to microbes that serve to prevent, control, or eliminate infection of the host by many pathogens.*** The importance of innate immunity in host defense is illustrated by clinical observations and experimental studies showing that deficiencies, inhibition, or elimination of any of several mechanisms of innate immunity increase susceptibility to infections, even when the adaptive immune system is intact and functional. Many pathogenic microbes have evolved strategies to resist innate immunity, and these strategies are crucial for the virulence of the microbes. Innate immune responses to such microbes may keep the infection in check until adaptive immune responses are activated. Adaptive immune responses typically are more potent and specialized, and therefore able to eliminate microbes that resist the defense mechanisms of innate immunity.
- ***Innate immunity eliminates damaged cells and initiates the process of tissue repair.*** These functions involve recognition and response to host molecules that are produced by, released from, or accumulate in stressed, damaged, and dead host cells. The injury that elicits these innate responses may occur as a result of infection or it may be sterile cell and tissue damage in the absence of infection.
- ***Innate immune responses stimulate adaptive immune responses and can influence the nature of the adaptive responses to make them optimally effective against different types of microbes.*** Thus, innate immunity not only serves defensive functions early after infection, but also provides the danger signals that alert the adaptive immune system to respond. Moreover, different components of the innate immune system often react in distinct ways to different microbes (e.g., extracellular bacteria versus intracellular viruses) and thereby influence the type of adaptive immune response that develops. We will return to this concept at the end of the chapter.
- ***The two major types of protective reactions of the innate immune system are inflammation and antiviral defense.*** Inflammation is the process by which circulating leukocytes and plasma proteins are brought into sites of infection in the tissues and are activated to destroy and eliminate the offending agents. Inflammation is also the major reaction to damaged or dead cells and to accumulations of abnormal substances in cells and tissues. Antiviral defense mechanisms prevent virus replication and promote killing of infected cells, thus eliminating reservoirs of viral infection without an inflammatory reaction (although inflammation also may contribute to defense against viruses).

Comparative Features of Innate and Adaptive Immunity

In order to understand how innate and adaptive immunity complement each other to protect against pathogens, it is instructive to highlight their important differences.

- Innate immune responses to a microbe are immediate and do not require prior exposure to the microbe. In other words, innate immune effector cells and molecules are either fully functional even before infection or are rapidly activated by microbes to prevent, control, or eliminate infections. In contrast, effective adaptive immune responses to a newly introduced microbe develop over several days as clones of antigen-specific lymphocytes undergo expansion and differentiate into functional effector cells.
- For most innate responses to microbes, there is no appreciable change in the quality or magnitude of the response upon repeated exposure—that is, there is little or no memory. In contrast, repeated exposure to a microbe enhances the rapidity, magnitude, and effectiveness of adaptive immune responses. There may be some exceptions to the rule that there is no memory in innate immunity. For instance, natural killer cell responses to certain viral infections are increased in magnitude upon subsequent exposure to the same virus. It is not clear how specific these enhanced responses are, which and how many viruses are capable of eliciting them, or if such responses contribute to increased protection against repeat infections.
- The innate immune response is activated by recognition of a relatively limited set of molecular structures that are either products of microbes or are expressed by injured or dead host cells. It is estimated that the innate immune system recognizes only about 1000 products of microbes and damaged cells. By contrast, the adaptive immune system potentially can recognize millions of different microbial antigens, and can also recognize nonmicrobial environmental antigens as well as self antigens that are normally present in healthy tissues.
- The receptors used by the innate and adaptive immune systems are fundamentally different in structure and extent of variation, accounting for the different specificities of these two types of host defense. The receptors of innate immunity are described in detail later in this chapter, and those of adaptive immunity in subsequent chapters.

Evolution of Innate Immunity

Innate immunity, the first line of defense against infections, is phylogenetically the oldest part of the immune system. It coevolved with microbes to protect all multicellular organisms from infections. Some components of the mammalian innate immune system are remarkably similar to components in plants and insects, suggesting that these appeared in common ancestors long ago in evolution. For example, peptides that are toxic to bacteria and fungi, called defensins, are found in plants and mammals and have essentially the same tertiary structure in both life forms. A family of receptors that we will discuss in detail later in this chapter, called Toll-like receptors, recognize pathogenic microbes and activate

antimicrobial defense mechanisms. Toll-like receptors are found in every life form in the evolutionary tree from insects up to mammals. The major signal transduction pathway that Toll-like receptors engage to activate cells, called the NF-κB pathway in mammals, also shows remarkable evolutionary conservation. In fact, most of the mechanisms of innate immune defense that we will discuss in this chapter appeared very early in evolution, when the first multicellular organisms evolved, about 750 million years ago. An adaptive immune system, in contrast, is clearly recognizable only in vertebrates that appeared about 350 to 500 million years ago.

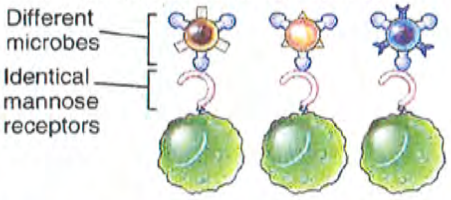
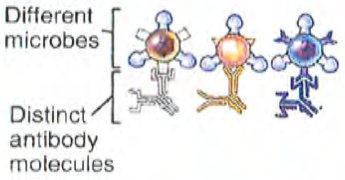
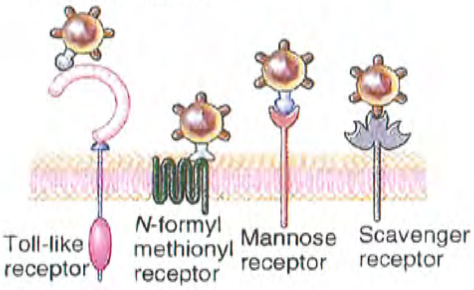
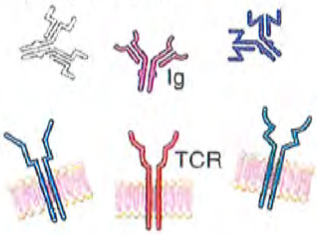
We begin our discussion of the innate immune system by describing how it recognizes microbes and damaged host cells. We will then proceed to the individual components of innate immunity and their functions in host defense.

RECOGNITION OF MICROBES AND DAMAGED SELF BY THE INNATE IMMUNE SYSTEM

The specificities of innate immune recognition have evolved to combat microbes and are different from the specificities of the adaptive immune system in several respects (Table 4.1).

The innate immune system recognizes molecular structures that are produced by microbial pathogens. The

TABLE 4.1 Specificity of Innate and Adaptive Immunity

	Innate Immunity	Adaptive Immunity
Specificity	For structures shared by classes of microbes (pathogen-associated molecular patterns) 	For structural detail of microbial molecules (antigens); may recognize nonmicrobial antigens 
Number of microbial molecules recognized	About 1000 molecular patterns (estimated)	$>10^7$ antigens
Receptors	Encoded in germline; limited diversity (pattern recognition receptors) 	Encoded by genes produced by somatic recombination of gene segments; greater diversity 
Number and types of receptors	<100 different types of invariant receptors	Only 2 types of receptors (Ig and TCR), with millions of variations of each
Distribution of receptors	Nonclonal: Identical receptors on all cells of the same lineage	Clonal: clones of lymphocytes with distinct specificities express different receptors
Genes encoding receptors	Germline encoded, in all cells	Formed by somatic recombination of gene segments only in B and T cells
Discrimination of self and nonself	Yes; healthy host cells are not recognized or they may express molecules that prevent innate immune reactions	Yes; based on elimination or inactivation of self-reactive lymphocytes; may be imperfect (giving rise to autoimmunity)

Ig, Immunoglobulin; *TCR*, T cell antigen receptor.

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microbial substances that stimulate innate immunity are often shared by classes of microbes and are called **pathogen-associated molecular patterns (PAMPs)**. Different types of microbes (e.g., viruses, gram-negative bacteria, gram-positive bacteria, fungi) express different PAMPs (Table 4.2). These structures include nucleic acids that are unique to or more abundant in microbes than in host cells, such as double-stranded RNA found in replicating viruses and unmethylated CpG DNA sequences found in bacteria; features of proteins that are found in microbes, such as initiation by *N*-formylmethionine, which is typical of bacterial proteins; and complex lipids and carbohydrates that are synthesized by microbes but not by mammalian cells, such as lipopolysaccharide (LPS) in gram-negative bacteria, lipoteichoic acid in gram-positive bacteria, and oligosaccharides with terminal mannose residues found in microbial but not in mammalian glycoproteins. Whereas the innate immune system has evolved to recognize only a limited number of molecules that are typical of different classes of microbes, the adaptive immune system is capable of recognizing many more and diverse foreign substances (antigens), which may be unique to different individual microbial species and may also be nonmicrobial in origin.

TABLE 4.2 Examples of Pathogen-Associated Molecular Patterns and Damage-Associated Molecular Patterns

		Microbe Type
Pathogen-Associated Molecular Patterns		
Nucleic acids	ssRNA	Virus
	dsRNA	Virus
	CpG	Virus, bacteria
Proteins	Pilin	Bacteria
	Flagellin	Bacteria
Cell wall lipids	LPS	Gram-negative bacteria
	Lipoteichoic acid	Gram-positive bacteria
Carbohydrates	Mannan	Fungi, bacteria
	Glucans	Fungi
Damage-Associated Molecular Patterns		
Stress-induced proteins	HSPs	—
Crystals	Monosodium urate	—
Proteolytically cleaved extracellular matrix	Proteoglycan peptides	—
Mitochondria and mitochondrial components	Formylated peptides and ATP	—
Nuclear proteins	HMGB1, histones	—

ATP, Adenosine triphosphate; *CpG*, cytosine-guanine–rich oligonucleotide; *dsRNA*, double-stranded RNA; *HMGB1*, high-mobility group box 1; *HSP*, heat shock protein; *LPS*, lipopolysaccharide; *ssRNA*, single-stranded RNA.

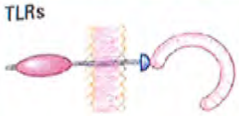
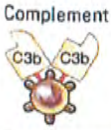
The innate immune system recognizes microbial products that are often essential for survival of the microbes. This evolutionary adaptation of innate immune recognition is important because it ensures that microbes cannot evade innate immunity by mutational loss of the molecules recognized by the host. An example of a target of innate immunity that is indispensable for microbes is double-stranded viral RNA, which is an essential intermediate in the life cycle of many viruses. Similarly, LPS and lipoteichoic acid are structural components of bacterial cell walls that are recognized by innate immune receptors; both are required for bacterial survival. In contrast, as we will see in Chapter 16, microbes may mutate or lose many of the antigens that are recognized by the adaptive immune system, thereby enabling evasion of host defense without compromising their own survival.

The innate immune system also recognizes endogenous molecules that are produced by or released from damaged and dying cells. These substances are called **damage-associated molecular patterns (DAMPs)** (see Table 4.2). DAMPs may be produced as a result of cell damage caused by infections, but they may also indicate sterile injury to cells caused by any of myriad reasons, such as chemical toxins, burns, trauma, or loss of blood supply. DAMPs are generally not released from cells dying by apoptosis. In some cases, endogenous molecules that are produced by healthy cells are released when the cells are damaged, and they then stimulate innate responses. These molecules are a subset of DAMPs and are often called **alarmins** because their presence outside cells alarms the immune system that something is causing cell death.

The innate immune system uses several types of cellular receptors, present in different locations in cells, and soluble molecules in the blood and mucosal secretions, to recognize PAMPs and DAMPs (Table 4.3). Cell-associated recognition molecules of the innate immune system are expressed by phagocytes (primarily macrophages and neutrophils), dendritic cells (DCs), epithelial cells that form the barrier interface between the body and the external environment, mast cells, and many other types of cells that reside in tissues. The cellular receptors for pathogen- and damage-associated molecular patterns are called **pattern recognition receptors**. They are expressed on the surface, in phagocytic vesicles, and in the cytosol of various cell types, all of which are locations where microbes may be present (Fig. 4.1). When these cell-associated pattern recognition receptors bind to PAMPs and DAMPs, they activate signal transduction pathways that promote the antimicrobial and proinflammatory functions of the cells in which they are expressed. In addition, there are many proteins present in the blood and extracellular fluids that recognize PAMPs (see Table 4.3). These soluble molecules are responsible for facilitating the clearance of microbes from the blood and extracellular fluids by enhancing uptake into phagocytes or by activating extracellular killing mechanisms.

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TABLE 4.3 Pattern Recognition Molecules of the Innate Immune System

Pattern Recognition Receptors	Location	Specific Examples	Ligands (PAMPs or DAMPs)
Cell-Associated			
TLRs 	Plasma membrane and endosomal membranes of DCs, phagocytes, B cells, endothelial cells, and many other cell types	TLRs 1–9	Various microbial molecules including bacterial LPS and peptidoglycans; viral nucleic acids
NLRs 	Cytosol of phagocytes, epithelial cells, and other cells	NOD1/2 NLRP family (inflammasomes)	Bacterial cell wall peptidoglycans Intracellular crystals (urate, silica); changes in cytosolic ATP and ion concentrations; lysosomal damage
RLRs 	Cytosol of phagocytes and other cells	RIG-1, MDA-5	Viral RNA
CDSs 	Cytosol of many cell types	AIM2; STING-associated CDSs	Bacterial and viral DNA
CLRs 	Plasma membranes of phagocytes	Mannose receptor DC-sign Dectin-1, Dectin-2	Microbial surface carbohydrates with terminal mannose and fructose Glucans present in fungal and bacterial cell walls
Scavenger receptors 	Plasma membranes of phagocytes	CD36	Microbial diacylglycerides
N-Formyl met-leu-phe receptors 	Plasma membranes of phagocytes	FPR and FPRL1	Peptides containing *N*-formylmethionyl residues
Soluble			
Pentraxins 	Plasma	C-reactive protein	Microbial phosphorylcholine and phosphatidylethanolamine
Collectins 	Plasma Alveoli	Mannose-binding lectin Surfactant proteins SP-A and SP-D	Carbohydrates with terminal mannose and fructose Various microbial structures
Ficolins 	Plasma	Ficolin	*N*-acetylglucosamine and lipoteichoic acid components of the cell walls of gram-positive bacteria
Complement 	Plasma	Various complement proteins	Microbial surfaces

AIM2, Absent in melanoma; *CDSs*, cytosolic DNA sensors; *CLRs*, C-type lectin–like receptors; *DAMP*, damage-associated molecular pattern; *DC*, dendritic cells; *MDA*, melanoma differentiation-associated gene; *NLRs*, NOD-like receptors; *NOD*, nucleotide oligomerization domain; *PAMP*, pathogen-associated molecular pattern; *RLRs*, RIG-like receptors; *SP-D*, surfactant protein D; *STING*, stimulator of IFN genes; *TLRs*, toll-like receptors.

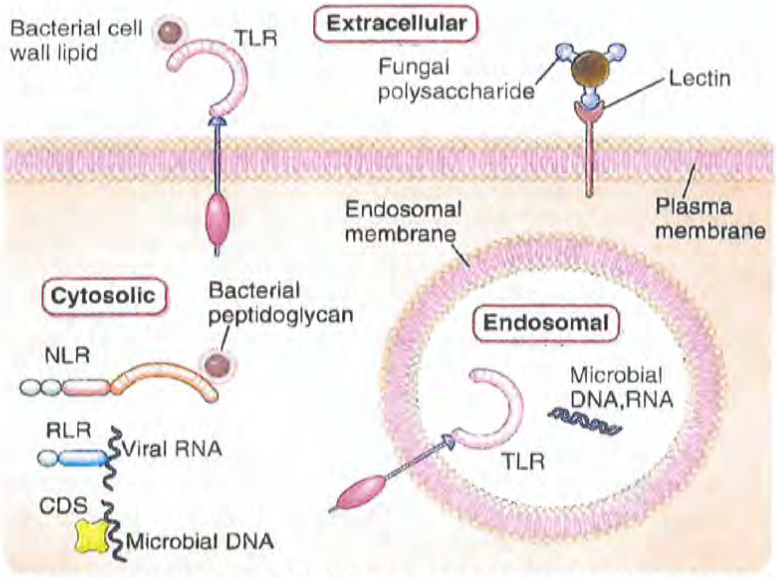


FIGURE 4.1 Cellular locations of pattern recognition receptors of the innate immune system. Some pattern recognition molecules, including members of the TLR family (see Fig. 4.2) and lectin receptors, are expressed on the cell surface, where they may bind extracellular pathogen-associated molecular patterns. Other TLRs are expressed on endosomal membranes and recognize nucleic acids of microbes that have been phagocytosed by cells. Cells also contain cytosolic sensors of microbial infection, including the NLRs, RLRs, and CDS. Only selected examples of microbial PAMPs recognized by these receptors are shown. Cytosolic receptors that recognize products of damaged cells (DAMPs) as well as some microbes are shown in Fig. 4.4. *CDS*, cytosolic DNA sensor; *NLR*, NOD-like receptor; *RLR*, RIG-like receptor; *TLR*, Toll-like receptor.

The receptors of the innate immune system are encoded by inherited (germline) genes, whereas the genes encoding receptors of adaptive immunity are generated by somatic recombination of gene segments in the precursors of mature lymphocytes. As a result, the diversity of innate immune system receptors and the range of their specificities are small compared with those of B and T cells of the adaptive immune system. It is estimated that innate immune recognition is mediated by about 100 different receptors belonging to a few protein families, whereas in the adaptive immune system there are only two families of receptors (immunoglobulins and T cell receptors) that produce millions of variations that recognize a vast number of antigens. Furthermore, whereas the adaptive immune system can distinguish between antigens of different microbes of the same class and even different antigens of one microbe, innate immunity can distinguish only classes of microbes, or only damaged cells from healthy cells, but not particular species of microbes or cell types.

The innate immune system does not react against normal, healthy cells and tissues. This characteristic is, of course, essential for the health of the organism. The failure to recognize healthy self is due to three main mechanisms: normal cells do not produce ligands for innate immune receptors, these receptors are located in cellular compartments where they do not encounter host molecules they could recognize, and regulatory proteins expressed by normal cells prevent activation of various components of innate immunity. We will discuss examples of such regulation later in this chapter.

With this introduction, we can proceed to a discussion of the large variety of molecules in the body that are capable of recognizing PAMPs and DAMPs, focusing on their specificity, location, and functions. We will begin with cell-associated molecules expressed on membranes or in the cytosol of cells. The soluble recognition and effector molecules of innate immunity, found in the blood and extracellular fluids, are described later.

CELL-ASSOCIATED PATTERN RECOGNITION RECEPTORS AND SENSORS OF INNATE IMMUNITY

Most cell types express pattern recognition receptors and therefore are capable of participating in innate immune responses. Phagocytes, especially macrophages, and DCs express the widest variety and greatest number of these receptors. This is in keeping with the fundamental role of phagocytes in detecting microbes and damaged cells and ingesting them for destruction, and the role of DCs in reacting to microbes in ways that elicit inflammation and subsequent adaptive immunity. Pattern recognition receptors are linked to intracellular signal transduction pathways that activate various cellular responses, including the production of molecules that promote inflammation and molecules that destroy microbes.

We will organize our discussion around several distinct classes of cellular pattern recognition receptors that differ in their structure and specificity for various types of microbes.

Toll-Like Receptors

Toll-like receptors (TLRs) are an evolutionarily conserved family of pattern recognition receptors expressed on many cell types that recognize products of a wide variety of microbes, as well as molecules expressed or released by stressed and dying cells. *Toll* was discovered as a Drosophila gene involved in establishing the dorsal-ventral axis during development of the fruit fly, but subsequently it was discovered that the Toll protein also mediated antimicrobial responses in these organisms. Furthermore, the cytoplasmic domain of Toll was found to be similar to the cytoplasmic region of the receptor for the innate immune cytokine interleukin-1 (IL-1). These discoveries led to the identification of mammalian homologues of Toll, which were named Toll-like receptors. There are nine different functional TLRs in humans, named TLR1 through TLR9 (Fig. 4.2).

The TLRs are type I integral membrane glycoproteins that contain leucine-rich repeats flanked by characteristic cysteine-rich motifs in their extracellular regions, which are involved in ligand binding, and a Toll/IL-1 receptor (TIR) domain in their cytoplasmic tails, which is essential for signaling. TIR domains are also found in the cytoplasmic tails of the receptors for the cytokines IL-1 and IL-18,

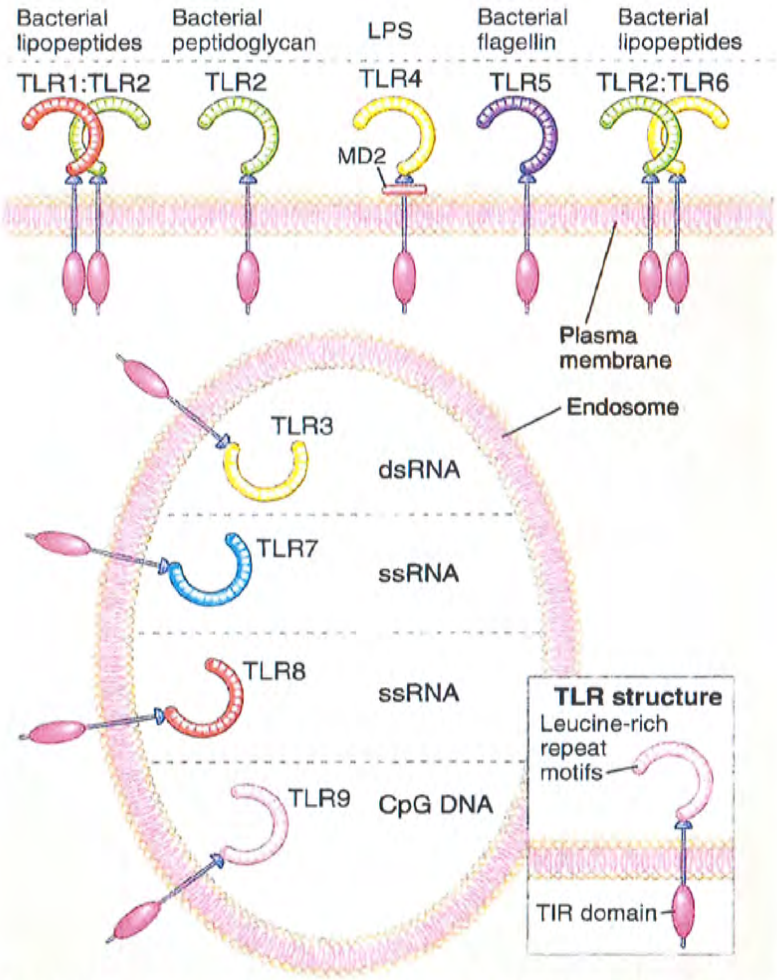


FIGURE 4.2 Structure, location, and specificities of mammalian TLRs. Note that some TLRs are expressed on the cell surface and others in endosomes. TLRs may form homodimers or heterodimers. *TIR*, Toll IL-1 receptor; *LPS*, lipopolysaccharide; *dsRNA*, double stranded RNA; *ssRNA*, single stranded RNA.

and similar signaling pathways are engaged by TLRs, IL-1, and IL-18.

Mammalian TLRs are involved in responses to a wide variety of molecules that are expressed by microbes but not by healthy mammalian cells. The ligands that the different TLRs recognize are structurally diverse and include products of all classes of microorganisms (see Fig. 4.2). Examples of bacterial products that bind to TLRs are the bacterial cell wall constituents LPS and lipoteichoic acid, mentioned earlier, and flagellin, a protein subunit component of the flagella of motile bacteria. Examples of nucleic acids that are TLR ligands are double-stranded RNAs, which make up the genomes of some viruses and are generated during the life cycle of most viruses and are distinguished from host dsRNAs by RNA editing and endosomal location; single-stranded RNAs, which are distinguished from cellular cytoplasmic single-stranded RNA transcripts by their location within endosomes and by their high guanosine and uridine content; and unmethylated CpG dinucleotides, which are common in prokaryotes but rare in vertebrate genomes.

TLRs are also involved in responses to endogenous molecules whose expression or location indicates cell damage. Examples of host molecules that engage TLRs include heat shock proteins (HSPs), which are chaperones induced in response to various cell stresses, and high-mobility group box 1 (HMGB1), an abundant DNA-binding protein involved in transcription and DNA repair. Both HSPs and HMGB1 are normally intracellular but may become extracellular when released from injured or dying cells. From their extracellular location, they activate TLR2 and TLR4 signaling in DCs, macrophages, and other cell types.

The structural basis of TLR specificities resides in the multiple extracellular leucine-rich modules of these receptors, which bind directly to PAMPs or to adaptor molecules that bind the PAMPs. There are between 16 and 28 leucine-rich repeats in TLRs. Each of these modules is composed of 20 to 30 amino acids that include conserved LxxLxLxxN motifs (where L is leucine, x is any amino acid, and N is asparagine) and amino acid residues that vary between different TLRs. The ligand-binding variable residues of the modules are on the convex surface formed by α helices and β turns or loops. These repeats contribute to the ability of some TLRs to bind hydrophobic molecules such as bacterial LPS. Ligand binding to the leucine-rich domains causes physical interactions between TLR molecules and the formation of TLR dimers. The repertoire of specificities of the TLR system is extended by the ability of TLRs to heterodimerize with one another. For example, dimers of TLR2 and TLR6 are required for responses to peptidoglycan.

Specificities of the TLRs are also influenced by various non-TLR accessory molecules. This is best defined for the TLR4 response to LPS. LPS first binds to soluble LPS-binding protein in the blood or extracellular fluid, and this complex serves to facilitate delivery of the LPS to the surface of the responding cell. An extracellular protein called MD2 (myeloid differentiation protein 2) binds to the lipid A component of LPS, forming a complex that then interacts with TLR4 and initiates signaling. Another protein called CD14 is also required for efficient LPS-induced signaling. CD14 is expressed by most cells (except endothelial cells) as a soluble protein or as a glycophosphatidylinositol-linked membrane protein. Both CD14 and MD2 can also associate with other TLRs. Thus, different combinations of accessory molecules in TLR complexes may serve to broaden the range of microbial products that can induce innate immune responses.

TLRs are found on the cell surface and on intracellular membranes and are thus able to recognize microbes in different cellular locations (see Fig. 4.2). TLRs 1, 2, 4, 5, and 6 are expressed on the plasma membrane, where they recognize various PAMPs in the extracellular environment. Some of the most potent microbial stimuli for innate immune responses bind to these plasma membrane TLRs, such as bacterial LPS and lipoteichoic acid, which are recognized by TLRs 4 and 2, respectively. In contrast, TLRs 3, 7, 8, and 9 are mainly expressed inside cells on endoplasmic reticulum and endosomal membranes, where they detect several different microbial nucleic acids. These include double-stranded RNA, which binds to TLR3; single-stranded RNA, which binds to TLR7 and TLR8; and unmethylated CpG motifs in DNA, which bind to TLR9. Single- and double-stranded RNA are not unique to microbes, but their location in endosomes likely reflects origin from microbes. This is because host cell RNA is not normally present in endosomes, but microbial RNA may end up in endosomes of neutrophils, macrophages, or DCs when the microbes are phagocytosed by these cells. Enzymatic digestion of the microbes within endosomes will release their nucleic acids so these are able to bind TLRs in the endosomal membrane. Thus, the endosomal TLRs may distinguish nucleic acids of normal cells from microbial nucleic acids on the basis of the cellular location of these molecules. A protein in the endoplasmic reticulum called UNC-93B is required for the endosomal localization and proper function of TLRs 3, 7, 8, and 9. Genetic deficiency in UNC-93B leads to susceptibility to certain viral infections, especially herpes simplex virus encephalitis, demonstrating the importance of the endosomal location of TLRs for innate defense against viruses.

TLR recognition of microbial ligands results in the activation of several signaling pathways and ultimately transcription factors, which induce the expression of genes whose products are important for inflammatory and antiviral responses (Fig. 4.3). The signaling pathways are initiated by ligand binding to the TLR at the cell surface or in the endoplasmic reticulum or endosomes, leading to dimerization of the TLR proteins. Ligand-induced TLR dimerization is predicted to bring the TIR domains of the cytoplasmic tails of each protein close to one another. This is followed by recruitment of TIR domain–containing adaptor proteins, which facilitate the recruitment and activation of various protein kinases, leading to the activation of different transcription factors. The major transcription factors that are activated by TLR signaling pathways are nuclear factor κB (NF-κB), activation protein 1 (AP-1), interferon response factor 3 (IRF3), and IRF7. NF-κB and AP-1 stimulate the expression of genes encoding many of the molecules required for inflammatory responses, including inflammatory cytokines (e.g. tumor necrosis factor [TNF] and IL-1),

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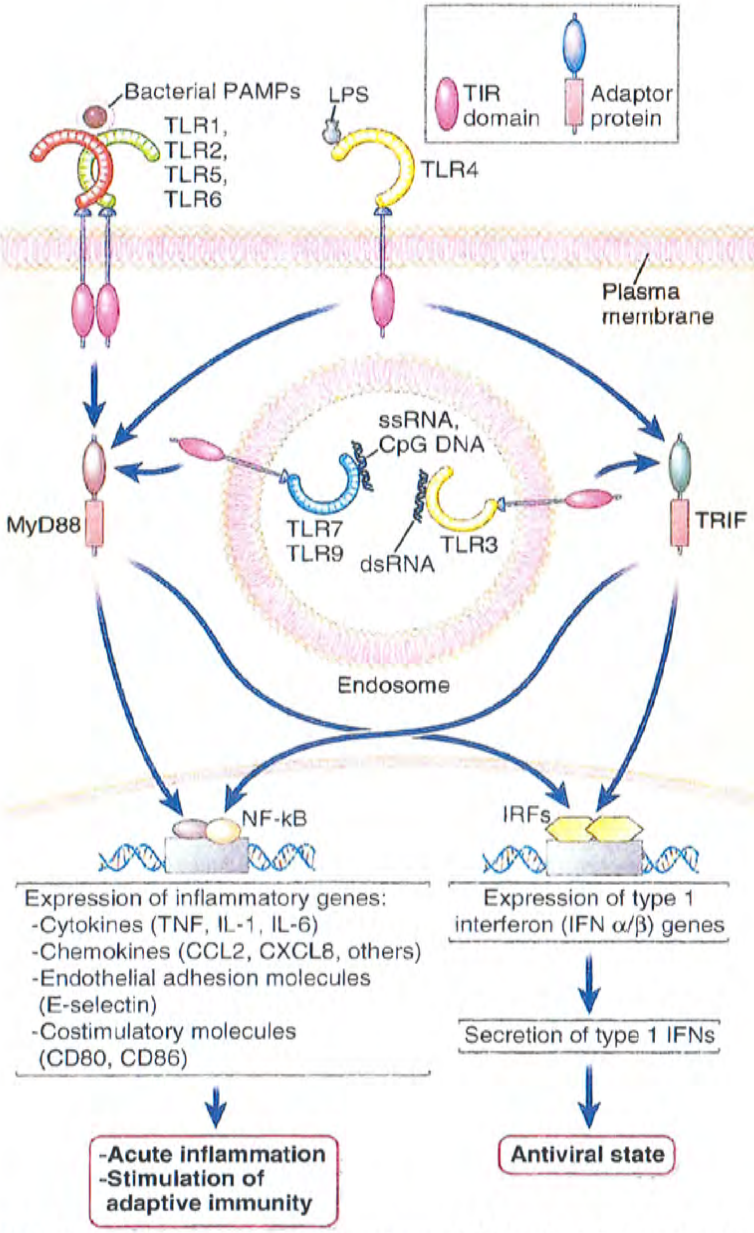


FIGURE 4.3 Signaling pathways and functions of TLRs. TLRs 1, 2, 5, and 6 use the adaptor protein MyD88 and activate the transcription factors NF-κB and AP-1. TLR3 uses the adaptor protein TRIF and activates the IRF3 and IRF7 transcription factors. TLR4 can activate both pathways. TLRs 7 and 9 in the endosome use MyD88 and activate both NF-κB and IRF7. *IFN*, interferon; *IRFs*, interferon regulatory factors; *NF-κB*, nuclear factor kappa B.

chemokines (e.g., CCL2 and CXCL8), and endothelial adhesion molecules (e.g., E-selectin) (discussed later). IRF3 and IRF7 promote production of type I interferons (IFN-α and IFN-β), which are important for antiviral innate immune responses.

Different combinations of adaptors and signaling intermediates are used by different TLRs, accounting for the common and unique downstream effects of the TLRs. For example, cell surface TLRs that engage the adaptor MyD88 lead to NF-κB activation, and TLR

signaling that uses the adaptor called TRIF (TIR domain-containing adaptor inducing IFN-β) leads to IRF3 activation. All TLRs except TLR3 signal through MyD88 and are therefore capable of activating NF-κB and inducing an inflammatory response. TLR3 signals through TRIF and therefore activates IRF3, which stimulates production of type I interferons. TLR4 signals through both MyD88 and TRIF and is able to induce both types of responses. The endosomal TLRs 7 and 9, which are most highly expressed in plasmacytoid DCs (see Chapter 6), signal through a MyD88-dependent, TRIF-independent pathway that activates both NF-κB and IRFs. Therefore, TLR7 and TLR9, like TLR4, induce both inflammatory and antiviral responses. We will discuss the details of NF-κB activation in Chapter 7.

Cytosolic Receptors for Pathogen-Associated Molecular Patterns and Damage-Associated Molecular Patterns

In addition to the membrane-bound TLRs, which sense pathogens outside cells or in endosomes, the innate immune system has evolved to equip cells with pattern recognition receptors that detect infection or cell damage in the cytosol (see Fig. 4.1 and Table 4.3). Three major classes of these cytosolic receptors are NOD-like receptors, RIG (retinoic acid-inducible gene)-like receptors, and cytosolic DNA sensors. These cytosolic receptors, similar to the TLRs, are linked to signal transduction pathways that promote inflammation or type I interferon production. The ability of the innate immune system to detect infection in the cytosol is important because parts of the normal life cycles of some microbes, such as viral gene translation and viral particle assembly, take place in the cytosol. Some bacteria and parasites have mechanisms that enable them to escape from phagocytic vesicles into the cytosol. Microbes can produce toxins that create pores in host cell plasma membranes, including endosomal membranes, through which microbial molecules can enter the cytosol. These pores also can result in changes in the concentration of endogenous molecules, such as ions, in the cytoplasm, which are reliable signs of infection and damage and are detected by the cytosolic receptors.

NOD-Like Receptors: NOD1 and NOD2

NOD-like receptors (NLRs) are a family of more than 20 different cytosolic proteins, some of which recognize PAMPs and DAMPs and recruit other proteins to form signaling complexes that promote inflammation. Typical NLRs include a C-terminal leucine-rich repeat domain that senses the presence of ligand, a central NOD (nucleotide oligomerization domain, also called NACHT) domain required for NLR proteins to bind to one another and form oligomers; and an N-terminal effector domain, which recruits other proteins to form signaling complexes (Fig. 4.4). There are three NLR subfamilies that serve as innate immune receptors, each using a different effector domain to initiate signaling. These include NLRB, which uses the BIR (baculovirus inhibition of apoptosis protein repeat) effector domain; NLRC, which uses CARDs (caspase recruitment and activation domains); and NLRP, which uses Pyrin domains (so called because they are found in proteins involved in causing fever) (see Fig. 4.4). NLRs are found in a wide variety of cell types, although some have restricted cellular expression. Some of the best studied NLRs are found in immune and inflammatory cells and in barrier epithelial cells. We will discuss two NLR sensors of bacterial PAMPs here, named NOD1 and NOD2, and other NLRs later, when we discuss inflammasomes.

NOD1 and NOD2, members of the NLRC subfamily, are expressed in the cytosol of several cell types, including mucosal epithelial cells and phagocytes, and they respond to bacterial cell wall peptidoglycans. NOD2 is highly expressed in intestinal Paneth cells in the small bowel, where it stimulates expression of antimicrobial substances called defensins in response to pathogens. NOD1 recognizes a glycosylated tripeptide called DAP (diaminopimelic acid), derived mainly from gram-negative bacterial peptidoglycan, whereas NOD2 recognizes a distinct molecule called MDP (muramyl dipeptide), derived from both gram-negative and gram-positive peptidoglycans. These peptides are released from intracellular or extracellular bacteria; in the latter case, their presence in the cytosol requires specialized bacterial mechanisms of delivery of the peptides into host cells. These mechanisms include type III and type IV secretion systems, which have evolved in pathogenic bacteria as a means of delivering toxins into host cells. When oligomers of NODs recognize their peptide ligands, including bacterial toxins, a conformational change occurs that allows the CARD effector domains of the NOD proteins to recruit multiple copies of the kinase RIP2, forming a signaling complex that has been called the NOD signalosome. The RIP2 kinases in these complexes activate NF-κB, which stimulates production of cytokines and other molecules involved in inflammation, similar to TLRs that signal through MyD88, discussed earlier. Both NOD1 and NOD2 appear to be important in innate immune responses to bacterial pathogens in the gastrointestinal tract, such as *Helicobacter pylori* and *Listeria monocytogenes*.

Certain *NOD2* gene polymorphisms increase the risk for an inflammatory disease of the bowel called Crohn's disease. A possible explanation for this association is that the disease-associated NOD2 variants are defective in their ability to sense microbial products, resulting in defective innate responses against commensal and pathogenic organisms in the intestine. If these organisms gain access to the intestinal wall, they may trigger chronic inflammation. Also, gain-of-function mutations of NOD2 that cause increased NOD signaling and NF-κB activation lead to a systemic inflammatory disease called Blau syndrome.

Cytosolic DNA Sensors and the STING Pathway

Cytosolic DNA sensors (CDSs) are molecules that detect microbial double-stranded (ds) DNA in the cytosol and activate signaling pathways that initiate antimicrobial responses, including type I interferon production and autophagy. DNA may be released into the cytosol from various intracellular microbes. The ability of the innate immune system to distinguish and react to microbial

Subfamily	Examples	Typical domain structure	Activating stimuli	Function
NLRA	CIITA		IFN-γ	Class II MHC expression
NLRB	NAIP		Flagellin	Control of *Legionella pneumophila* infection
NLRC	NOD1, NOD2, NLRC3-5		DAP (NOD1)	NF-κB activation
			MDP (NOD2)	NF-κB activation, autophagy, type 1 interferon production
			Flagellin (NLRC4)	Caspase 1 activation, cell death
NLRP	NLRPs, 1-10		Extracellular ATP, alum, asbestos, bacterial toxins, silica, sodium urate, ROS, reduced cytosolic K+ (NLRP3)	Caspase 1 activation
			Lipopeptides (NLRP7)	Caspase 1 activation

FIGURE 4.4 NLRs involved in innate immunity. Members of the NLR family that perform immune functions can be assigned to one of four subfamilies: NLRA, NLRB, NLRC, and NLRP, each with a different N-terminal effector domain. NLRA, better known as CIITA, is a transcription factor that has an N-terminal transactivating (TA) domain required for class II MHC gene expression. NLRB has a beculovirus inhibition of apoptosis protein repeat (BIR) domain, of unknown function. NLRC members have an N-terminal caspase recruitment and activation domain (CARD), which is involved in caspase-1 activation. NLRP members have a pyrin (PYD) domain, which also activates caspase-1. All NLRs contain a central NOD or NACHT (NAIP, CIITA, HET-E, and TP1) domain involved in nucleotide binding, and C-terminal leucine-rich repeat domains involved in ligand recognition. Some of the principal functions, and activating ligands of NLRs, are shown. *DAP*, Diaminopimelic acid; *LRR*, leucine rich repeat; *MDP*, muramyl dipeptide; *NOD*, nucleotide oligomerization domain.

DNA and not host DNA is partly related to the location of most of the DNA sensors in the cytosol, where microbial DNA but not mammalian DNA may be present.

The STING (stimulator of IFN genes) pathway is an important mechanism of dsDNA-induced activation of type 1 interferon responses (Fig. 4.5). In this pathway, cytosolic dsDNA, usually of microbial origin, activates the enzyme cGAS (cyclic GMP-AMP synthase), which generates a signaling molecule called cGAMP (cyclic GMP-AMP). STING is an endoplasmic reticulum–localized transmembrane adaptor protein that binds to cGAMP and activates the TBK1 kinase, which phosphorylates and activates the IRF3 transcription factor, leading to type 1 IFN gene expression. STING also responds to other cytosolic DNA sensors besides cGAS, including DAI (DNA-dependent activator of IFN-regulatory factors) and IFI16 (interferon inducible protein 16). In addition to inducing IFN production, STING stimulates autophagy, a mechanism by which cells degrade their own organelles, such as mitochondria, by sequestering them within membrane-bound vesicles and fusing the vesicles with lysosomes. In innate immunity, autophagy is a mechanism to deliver cytosolic microbes to the lysosome, where they are killed by proteolytic enzymes. In adaptive immunity, autophagy is one of several mechanisms for generating microbial peptides for presentation to T cells.

Some cytosolic DNA sensors may work through STING independent pathways.

- RNA polymerase 3 binds and transcribes AT-rich microbial dsDNA into an RNA-containing-triphosphate moiety, which then activates the RIG-I pathway leading to type I interferon expression, as described later.
- AIM2 (absent in melanoma-2) is another cytosolic sensor that recognizes cytosolic dsDNA and forms an enzyme complex called an inflammasome, which proteolytically generates a biologically active inflammatory cytokine IL-1β from an inactive precursor. Inflammasomes are also formed with other innate immune sensors and are described later.

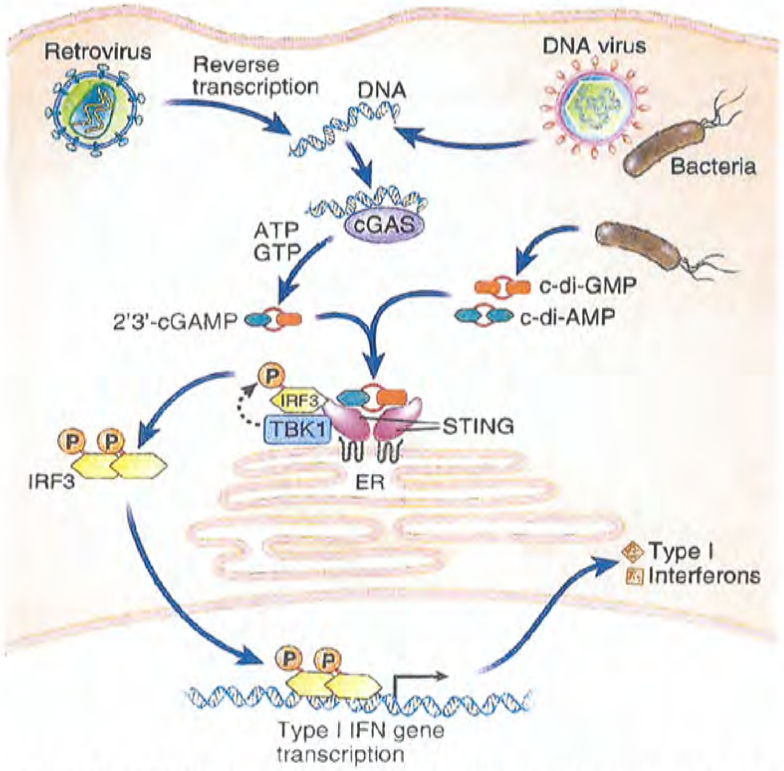


FIGURE 4.5 The STING cytosolic DNA sensing pathway. Cytoplasmic microbial DNA activates the enzyme cGAS, which catalyzes the synthesis of cyclic GMP-AMP (cGAMP) from ATP and GTP. cGAMP binds to STING in the endoplasmic reticulum membrane, and then STING recruits and activates the kinase TBK1, which phosphorylates IRF3. Phospo-IRF3 moves to the nucleus, where it induces type I IFN gene expression. The bacterial second messenger molecules cyclic di-GMP (c-di-GMP) and cyclic di-AMP (c-di-AMP) are directly sensed by STING. *cGAS*, cyclic GMP-AMP synthase; *IFN*, interferon; *IRF3*, interferon response factor 3.

RIG-Like Receptors

RIG-like receptors (RLRs) are cytosolic sensors of viral RNA that respond by inducing the production of the antiviral type I interferons. This family of receptors is named after *RIG* (retinoid-inducible gene). RLRs can recognize double-stranded RNA and RNA-DNA heteroduplexes, which include the genomes of RNA viruses and RNA transcripts of RNA and DNA viruses. The two best characterized RLRs are RIG-I and MDA5 (melanoma differentiation-associated gene 5). Both of these proteins contain two N-terminal caspase recruitment domains that interact with other signaling proteins, an RNA-helicase domain, and a C-terminal domain, the latter two being involved in RNA recognition. RIG-I and MDA5 recognize different sets of viral RNAs that are characteristic of distinct viruses and not typical of mammalian RNA. For example, MDA5 recognizes long dsRNA (1 to 6 kb), which is longer than dsRNA that may be formed transiently in normal cells, and RIG-I will only recognize RNA with a 5′ triphosphate moiety, which is not present in mammalian host cell cytosolic RNA because of addition of a 7-methylguanosine cap or removal of the 5′ triphosphate. RLRs are expressed in a wide variety of cell types, including bone marrow–derived leukocytes and various tissue cells. Therefore, these receptors enable the many cell types that are susceptible to infection by RNA viruses to mount innate immune responses to these viruses.

On binding viral dsRNA, the RLRs are recruited to the outer mitochondrial membrane by the MAVS (mitochondrial antiviral-signaling) protein, leading to the formation of filaments by a prion-like mechanism. This initiates signaling events that lead to phosphorylation and activation of IRF3 and IRF7, as well as NF-κB, and these transcription factors induce production of type I interferons. MDA5 and RIG-I not only induce type I IFN production but also directly inhibit viral replication by inhibiting viral RNA-protein interactions.

Inflammasomes

Inflammasomes are multiprotein complexes that form in the cytosol in response to cytosolic PAMPs and DAMPs, whose function is to generate active forms of the

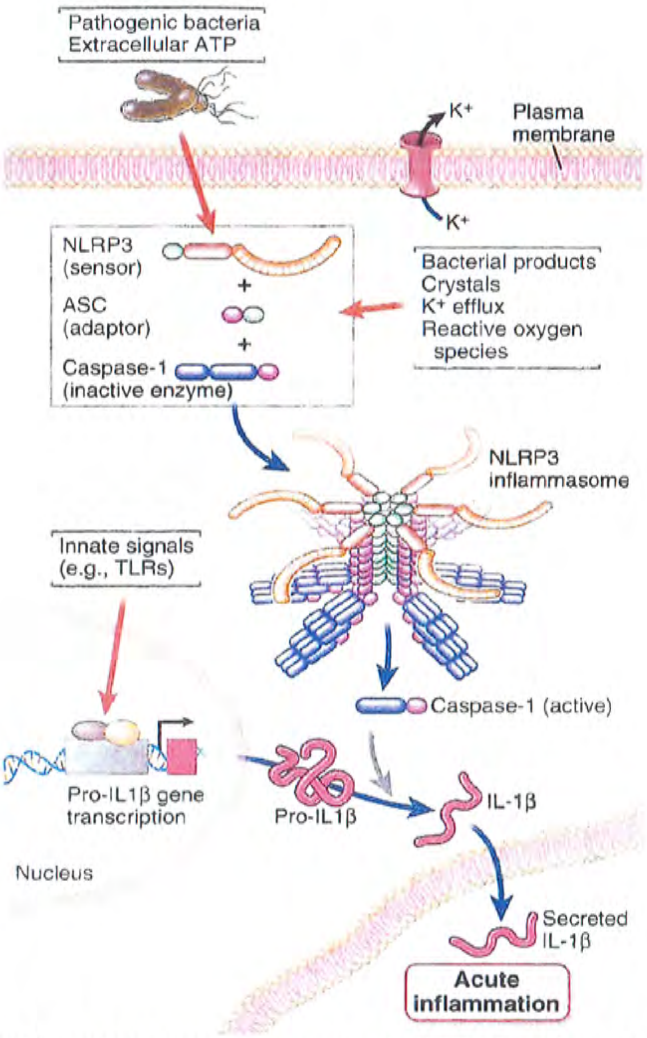


FIGURE 4.6 The inflammasome. The activation of the NLRP3 inflammasome, which processes pro–IL-1 to active IL-1, is shown. Inflammasomes with other NLRP proteins function in a similar way. Various PAMPs or DAMPs induce pro–IL-1β expression through pattern recognition receptor signaling. *ASC*, Apoptosis-associated speck-like protein containing a CARD; *IL-1*, interleukin-1.

inflammatory cytokines IL-1β and IL-18 (Fig. 4.6). These two homologous cytokines are expressed as inactive precursors, which must be proteolytically cleaved by the enzyme caspase-1 to become active cytokines that are released from the cell and promote inflammatory responses. Inflammasomes are composed of oligomers of a sensor, caspase-1, and an adaptor that links the two, and these oligomeric complexes only form when the sensors respond to PAMPs, DAMPs, or changes in the cell indicating the presence of infection or damage. Thus, inflammation mediated by IL-1β and IL-18 occurs when there are PAMPS or DAMPS in the cytosol, indicating infection or cell injury.

Inflammasomes can form with several different sensor proteins. NLR family sensors found in different inflammasomes include NLRB, NLRC4, and at least six NLRP proteins (see Fig. 4.4). Sensors that are not in the NLR family but are used by inflammasomes include members of the AIM2 family, including AIM2 and IFI16, which we discussed earlier as DNA sensors. These proteins contain a DNA sensing domain and a pyrin domain. Pyrin is another non-NLR sensor protein that contains an N-terminal pyrin domain and participates in the formation of an inflammasome. The gene encoding pyrin is mutated in familial Mediterranean fever, as discussed later.

The formation of the inflammasome is induced either when sensor proteins in the cytosol directly recognize microbial products or, probably more commonly, when sensors detect changes in the amount of endogenous molecules or ions in the cytosol which indirectly indicate the presence of infection or cell damage. In response to the PAMPs or indirect signals, the sensors become able to bind other proteins through homotypic interactions between shared structural domains, thereby forming the inflammasome complex. For example, after binding of a ligand, multiple identical NLRP3 proteins interact to form an oligomer, and each NLRP3 protein in the oligomer binds an adaptor protein called ASC (apoptosis-associated speck-like protein containing a CARD). The binding of ASC to sensors such as NLRP proteins causes it to undergo a conformational alteration that triggers conformational changes of other ASC molecules in the cytosol by a prion-like self-propagating mechanism. This results in the formation of filaments of ASC that can cluster and recruit an inactive precursor of caspase-1, called pro-caspase-1. The recruitment and consequent clustering of pro-caspase-1 proteins result in the generation of active caspase-1. Caspases are proteases with cysteine residues in their active site that cleave substrate proteins at aspartate residues. Although several other caspases participate in a form of cell death called apoptosis (see Chapter 15), the main function of caspase-1 is to cleave the inactive cytoplasmic precursor forms of IL-1β and IL-18. Caspase-1 cleavage generates active forms of these cytokines, which then leave the cell and perform various proinflammatory functions. IL-1β lacks a signal peptide required for the secretion of most proteins from cells. The exact mechanism of its release from cells after it is processed is unclear. We will describe the action of IL-1β and IL-18 and the inflammatory response in detail later in this chapter. Suffice it to say here that the inflammation induced by IL-1 serves a protective function by eliminating the microbes and damaged cells that incited the formation of the inflammasome.

Inflammasome activation is induced by a wide variety of cytoplasmic stimuli that are often associated with infections and cell stress, including microbial products, environmentally or endogenously derived crystals, and reduction in cytosolic potassium ion (K^+) concentrations (see Fig. 4.6). NLRC4 recognizes cytosolic flagellin and components of the type III secretion system of bacteria. NLRP1 recognizes the anthrax lethal toxin. NLRP3 senses many DAMPS and PAMPS, including uric acid crystals, aluminum hydroxide crystals used in vaccine adjuvants, adenosine triphosphate (ATP) released from mitochondria, silica, bacterial products, bacterial toxins produced by streptococci and staphylococci, bacterial DNA-RNA hybrids, and the influenza virus. NLRP12 senses PAMPS from Yersinia species and is required for control of Yersinia. Pyrin responds to numerous bacterial toxins that all mediate post-translational modification of endogenous Rho family GTPases.

The mechanism by which such varied molecules activate the same NLR sensors is unclear. The structural diversity of the agents that activate these sensors suggests that they do not directly bind to NLR proteins but may act by inducing a shared set of changes in endogenous cytoplasmic conditions that activate the NLRs. Reduced cytoplasm potassium ion concentrations may be one such common mechanism, because reductions in cellular K^+ induced by some bacterial pore-forming toxins can activate inflammasomes, and many of the other known inflammasome activators, such as extracellular ATP, cause increased K^+ efflux from cells. Another common mechanism implicated in inflammasome activation is the generation of reactive oxygen species (ROS), which are toxic free radicals of oxygen that are often produced during cell injury. Inflammasome-activating crystals may work by damaging lysosomal membranes, thereby releasing ROS into the cytosol, where they are detected by the sensors that stimulate inflammasome formation.

Inflammasome activation also causes an inflammatory form of programmed cell death of macrophages and DCs (but not of neutrophils and most other cell types) called **pyroptosis**, characterized by swelling of cells, loss of plasma membrane integrity, and release of inflammatory mediators including IL-1β, IL-18, TNF, IL-6, and IL-8. Pyroptosis also results in the death of certain microbes that gain access to the cytosol. A key feature of pyroptosis is caspase-mediated cleavage of a protein called gasdermin D, which leads to formation of membrane pores. Pyroptosis may be induced by activation of either the canonical inflammasomes that use caspase-1, described above, or a noncanonical inflammasome that uses a different caspase (caspase-11 in rodents, caspase-4 or caspase-5 in humans). Bacterial LPS in the cytosol can directly bind to caspase-11, leading to inflammasome activation and pyroptosis. The amplification of inflammation provided by pyropotosis enhances bacterial clearance but also may contribute to septic shock, a severe systemic reaction to inflammatory cytokines. The absence of caspase-11 in genetically engineered mice protects them from LPS induced septic shock.

The discovery that some crystalline substances are potent inflammasome activators has changed our understanding of certain inflammatory diseases. **Gout** is a painful inflammatory condition of the joints that has long been known to be associated with deposition of monosodium urate crystals in joints. Experimental evidence suggests that when these crystals are phagocytosed, they damage the lysosomal membranes of the cells, and this leads to activation of inflammasomes and subsequent inflammation. Based on these findings, IL-1 antagonists have been used to effectively treat cases of severe gout that are resistant to conventional antiinflammatory drugs. Similarly, pseudogout is caused by deposition of calcium pyrophosphate crystals and inflammasome activation. Occupational inhalation of silica and asbestos can cause chronic inflammatory and fibrotic disease of the lung, and there is also interest in the potential of blocking the inflammasome or IL-1 to treat these diseases.

Dysregulated activation of the inflammasome due to autosomal gain-of-function mutations in one or another of its component proteins leads to inappropriately triggered and excess IL-1 production. The result is recurrent attacks of fever and localized inflammation, most commonly in the skin, joints, and abdominal cavity. These disorders are called **autoinflammatory syndromes**

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because they are characterized by spontaneous inflammation without an overt inciting trigger. The longest studied of these diseases is familial Mediterranean fever, caused by mutation of the *MEFV* gene, which encodes pyrin. Autoinflammatory diseases caused by mutations in NLRP3 (also known as cryopyrin) are called cryopyrin-associated periodic syndromes (CAPS). Patients with CAPS can be successfully treated with IL-1 antagonists, as predicted from the pathogenesis of the syndromes. Such diseases are distinct from *autoimmune disorders*, which are disorders of adaptive immunity caused by antibodies and/or T cells reactive with self antigens.

A great deal of interest in the inflammasome has recently been generated by findings that it may be activated by excessive amounts of endogenous substances deposited in tissues in the setting of various diseases. These substances include cholesterol crystals within macrophages in atherosclerosis, free fatty acids and lipids in adipose tissue in obesity-associated metabolic syndrome and type 2 diabetes, and β-amyloid in Alzheimer's disease. In all these situations, activation of the inflammasome leads to production of IL-1 and inflammation, which may contribute to the pathogenesis of the diseases. Such findings have spurred clinical trials to alleviate some of these diseases using IL-1 antagonists.

Other Cell-Associated Pattern Recognition Receptors

Several other types of plasma membrane and cytoplasmic receptors transmit activating signals similar to TLRs that promote inflammatory responses and enhance killing of microbes, or mainly participate in the uptake of microbes into phagocytes (see Table 4.3).

C-Type Lectin Receptors for Microbial Carbohydrates

Cellular receptors that recognize carbohydrates on the surface of microbes facilitate the phagocytosis of the microbes and the secretion of cytokines that promote inflammation and subsequent adaptive immune responses (Table 4.4). These receptors belong to the C-type lectin family, so called because they bind carbohydrates (hence *lectins*) in a Ca^{++}-dependent manner (hence *C-type*). The C-type lectin receptors are also called CLRs to parallel the nomenclature of TLRs and other receptors. These receptors are integral membrane proteins found on the surfaces of macrophages, DCs, and some tissue cells. Other lectins are soluble proteins in the blood and extracellular fluids (discussed later). All of these molecules contain a conserved carbohydrate recognition domain. There are several types of plasma membrane C-type lectins with specificities for different carbohydrates, including mannose, glucose, N-acetylglucosamine, and β-glucans. In general, these cell surface lectins recognize carbohydrate structures found on the cell walls of microorganisms but not mammalian cells. Some of these C-type lectin receptors function in the phagocytosis of microbes, and others have signaling functions that induce protective responses of host cells to microbes.

- One of the most studied membrane C-type lectins is the **mannose receptor** (CD206), which is involved in phagocytosis of microbes. This receptor recognizes certain terminal sugars on microbial surface carbohydrates, including D-mannose, L-fucose, and *N*-acetyl-D-glucosamine. These terminal sugars are often present on the surface of microorganisms, whereas eukaryotic

TABLE 4.4 C-Type Lectin Receptors

	Mannose Receptor (CD 206)	Dectin-1 (CD369)	Dectin-2 and Mincle	DC-Sign (CD209)	Langerin (CD207)
Ligand	Terminal mannose and fucose on microbial cell surfaces	β-glucan on fugal cell surfaces	High mannose on fungal hyphae and bacteria	Terminal mannose and fucose on microbial cell surfaces	Terminal mannose on microbial cell surfaces
Signaling	Uncertain	ITAM/SYK/CARD9 pathway of NF-κB activation	ITAM/SYK/CARD9 pathway of NF-κB activation	Uncertain	Uncertain
Cellular expression	Macrophages	Dendritic cells	Dendritic cells	Dendritic cells, macrophages and sinusoidal endothelial cells	Dendritic cells in epithelial barriers
Function	Phagocytosis; antifungal immunity	Inflammation and antigen presentation; Th17 differentiation; antifungal immunity	Inflammation and antigen presentation; Th17 differentiation; antifungal and mycobacterial immunity	Adhesion; hepatitis C virus and HIV-1 infection	Phagocytosis, antigen presentation

DC-SIGN, Dendritic cell-specific intercellular adhesion molecule 3 (ICAM-3)-grabbing nonintegrin; *ITAM*, Immunoreceptor tyrosine-based activation motif; *Mincle*, Macrophage inducible Ca^{++} dependent lectin.

cell surface carbohydrates are most often terminated by galactose and sialic acid. Thus, the terminal sugars on microbes can be considered PAMPs. Mannose receptors may not have any intrinsic signaling functions and are thought to bind microbes as the first step in their ingestion by macrophages and DCs.

- **Dectins** (DC-associated C-type lectins) include several different CLRs encoded within a gene cluster on human chromosome 12, which also includes genes encoding natural killer cell receptors (discussed later). Dectins are expressed on DCs and macrophages, and play important roles in antifungal immunity, as well as in responses to certain bacteria. Dectin-1 (CD369) binds β-glucan, which is a major cell wall component of many fungal species, and this CLR is required for immunity to various pathogenic fungal species, including candida, aspergillus, and pneumocystis. Dectin-2 and mincle are two dectins that recognize high-mannose oligosaccharides on the hyphal form of some fungi and bacteria. In response to binding of their ligands, these dectins induce signaling events in DCs and macrophages that activate a variety of immune functions. The cytoplasmic tail of dectin-1 has an immunoreceptor tyrosine-based activation motif (ITAM) which engages tyrosine kinases, which transduce signals leading to gene transcription. Dectin-2 and mincle rely on an associated ITAM-bearing signaling partner protein called FcRγ. ITAMs are used by many different cell-activating receptors in the immune system, and their mechanism of signaling will be discussed in Chapter 7. In response to ligand binding to dectin-1, dectin-2, or mincle, DCs produce cytokines and other proteins that promote inflammation and enhance adaptive immune responses. Some of the induced cytokines promote the development of a type of effector T cell called Th17, which is particularly effective in defense against fungal and some bacterial infections (see Chapter 10).
- **Langerin** (CD207) and **DC-SIGN** (CD209) are two other CLRs expressed on DCs, both of which bind mannose and have roles in innate and adaptive immune responses to various microbes. Langerin is expressed by epidermal Langerhans cells and other subsets of dendritic cells in skin and other epithelial barriers. DC-SIGN is expressed on the majority of DCs as well as on macrophages and sinusoidal endothelial cells. DC-SIGN binds to envelope glycoproteins of the hepatitis C virus and HIV-1 and may play a pathogenic role in disseminating infection by these viruses.

Scavenger Receptors

Scavenger receptors comprise a structurally and functionally diverse collection of cell surface proteins that were originally grouped on the basis of the common characteristic of mediating the uptake of oxidized lipoproteins into cells. Some of these scavenger receptors, including SR-A and CD36, are expressed on macrophages and mediate the phagocytosis of microorganisms. In addition, CD36 functions as a coreceptor in TLR2/6 recognition and response to bacterially derived lipoteichoic acid and diacylated lipopeptides. There is a wide range of molecular structures that bind to each scavenger receptor, including LPS, lipoteichoic acid, nucleic acids, β-glucan, and proteins. The significance of scavenger receptors in innate immunity is highlighted by increased susceptibility to infection in gene knockout mice lacking these receptors and by the observation that several microbial pathogens express virulence factors that block scavenger receptor–mediated recognition and phagocytosis.

Formyl-Peptide Receptors

The **formyl peptide receptor-1** (FPR1), expressed on leukocytes, recognizes bacterial peptides containing *N*-formylmethionyl residues and stimulates directed movement of the cells. Because all bacterial proteins and few mammalian proteins (only those synthesized within mitochondria) are initiated by *N*-formylmethionine, FPR1 enables phagocytes to detect and respond preferentially to bacterial proteins. The bacterial peptide ligands that bind this receptor are some of the most potent known chemoattractants for leukocytes. Chemoattractants include several types of diffusible molecules, often produced at sites of infection, that bind to specific receptors on cells and direct their movement toward the source of the chemoattractant. Other chemoattractants, such as the chemokines discussed in Chapter 3, are made by host cells. FPR1 and all other chemoattractant receptors belong to the seven-transmembrane, guanosine triphosphate (GTP)–binding (G) protein–coupled receptor (GPCR) superfamily. These receptors initiate intracellular responses through associated trimeric G proteins (see Chapter 7). The G proteins stimulate many types of cellular responses, including cytoskeletal changes that are responsible for the increased cell motility.

CELLULAR COMPONENTS OF THE INNATE IMMUNE SYSTEM

The cells of the innate immune system serve as barriers against infections and as sentinels to detect microbes and damaged cells in tissues and perform functions that are essential for defense against microorganisms. Several cell types express the various pattern recognition receptors we have just discussed, and after recognizing PAMPs and DAMPs, the cells respond by producing inflammatory cytokines and antiviral proteins; other cells kill microbes or infected cells. In addition, some of the cells of innate immunity are critical for stimulating subsequent adaptive immune responses.

Epithelial Barriers

Intact epithelial surfaces form physical barriers between microbes in the external environment and host tissue, and epithelial cells produce antimicrobial chemicals that further impede the entry of microbes (Fig. 4.7). The main interfaces between the environment and the mammalian host are the skin and the mucosal surfaces of the gastrointestinal, respiratory, and genitourinary tracts. These interfaces are lined by continuous layers of specialized epithelial cells that serve many physiologic functions, including preventing the entry of microbes. Loss of the integrity of these epithelial layers by trauma or other

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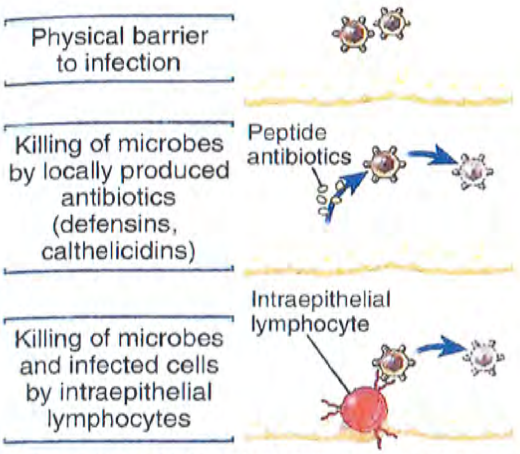


FIGURE 4.7 Epithelial barriers. Epithelia at the portals of entry of microbes provide physical barriers, produce antimicrobial substances, and harbor intraepithelial lymphocytes that are believed to kill microbes and infected cells.

reasons predisposes an individual to infections. We will summarize the main features of innate defense by epithelial barriers here and discuss epithelial barrier immunity in greater detail in Chapter 14.

The protective function of barrier epithelia is in large part physical. The epithelial cells form tight junctions with one another, blocking passage of microbes between the cells. In the skin, the outer layer of keratin, which accumulates as keratinocytes on the surface die, serves to block microbial penetration into deeper layers of the epidermis. Mucus, a viscous secretion containing glycoproteins called mucins, is produced by respiratory, gastrointestinal, and urogenital epithelial cells and physically impairs microbial invasion. The function of these barriers is enhanced by ciliary action in the bronchial tree and peristalsis in the gut, which facilitate elimination of microbes. Although these physical properties alone are very important in host defense, other epithelial defense mechanisms have evolved to complement the mechanical barrier.

Epithelial cells as well as some leukocytes produce peptides that have antimicrobial properties. Two structurally distinct families of antimicrobial peptides are the defensins and the cathelicidins.

- **Defensins** are small peptides, 29 to 34 amino acids long, that contain both cationic and hydrophobic regions and three intrachain disulfide bonds. Two families of human defensins, named α and β, are distinguished by the location of these bonds. Defensins are produced by epithelial cells of mucosal surfaces and by granule-containing leukocytes, including neutrophils, natural killer cells, and cytotoxic T lymphocytes. The set of defensin molecules produced differs between different cell types. Paneth cells within the crypts of the small bowel are a major producer of α-defensins. Paneth cell defensins are sometimes called crypticidins; their function is to limit the amount of lumenal microbes near the epithelial barrier. Defensins are also produced elsewhere in the colon, in respiratory mucosal cells, and in the skin. Some defensins are constitutively produced by some cell types, but their secretion may be enhanced by cytokines and microbial products. In other cells, defensins are produced only in response to cytokines and microbial products. The protective actions of the defensins include both direct toxicity to microbes, including bacteria, fungi, and enveloped viruses, and the activation of cells involved in the inflammatory response to microbes. Defensins kill microbes by a variety of mechanisms, many of which depend on their ability to insert into and disrupt functions of microbial membranes.
- **Cathelicidin**, produced by neutrophils and barrier epithelial cells in the skin, gastrointestinal tract, and respiratory tract, is synthesized as an 18-kD two-domain precursor protein and is proteolytically cleaved into two peptides, each with protective functions. Both precursor synthesis and proteolytic cleavage may be stimulated by inflammatory cytokines and microbial products. The active cathelicidins protect against infections by multiple mechanisms, including direct toxicity to a broad range of microorganisms and the activation of various responses in leukocytes and other cell types that promote eradication of microbes. The C-terminal fragment, called LL-37, can bind and neutralize LPS, the toxic component of the outer wall of gram-negative bacteria that is recognized by TLR4.

Barrier epithelia contain certain types of lymphocytes, including intraepithelial T lymphocytes, which recognize and respond to commonly encountered microbes. Intraepithelial T lymphocytes are present in the epidermis of the skin and in mucosal epithelia. Various subsets of intraepithelial lymphocytes are present in different proportions, depending on species and tissue location. These subsets are distinguished mainly by the type of T cell antigen receptors (TCRs) they express. Some intraepithelial T lymphocytes express the conventional αβ form of TCR, which is present on most T cells in lymphoid tissues and the circulation. Other T cells in epithelia express a form of antigen receptor called the γδ TCR that may recognize peptide and nonpeptide antigens. Although most T lymphocytes are mediators of adaptive immunity, a common characteristic of intraepithelial T cells is the limited diversity of their antigen receptors, compared with most T cells in the adaptive immune system. These intraepithelial T lymphocytes are believed to recognize a small number of commonly encountered microbial structures, a typical feature of innate pattern recognition receptors we have described. It is also possible that these lymphocytes are activated not by antigen recognition but by cytokines and other molecules produced by epithelial cells in response to stress. Intraepithelial lymphocytes may function in host defense by secreting cytokines, activating phagocytes, and killing infected cells.

Phagocytes

Cells that have specialized phagocytic functions, primarily macrophages and neutrophils, are the first line of

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defense against microbes that breach epithelial barriers. We introduced these cell types in Chapter 2, and we will discuss many other details of their functions in the context of the inflammatory response later in this chapter. The essential role that phagocytes play in innate immune defense against microbes is demonstrated by the high rate of lethal bacterial and fungal infections in patients with low blood neutrophil counts caused by bone marrow cancers or chemotherapy and irradiation for cancer (which destroys immature cells in the bone marrow), and in patients with inherited deficiencies in the functions of neutrophils and macrophages. Some macrophages are always present in most tissues and function as sentinels of infection, while other phagocytes, including monocytes and neutrophils, are recruited into infected tissues in response to microbes or signals generated by the sentinel cells.

Dendritic Cells

Dendritic cells (DCs) rapidly and efficiently detect invading microbes because of their location in tissues and their expression of numerous pattern recognition receptors for PAMPs and DAMPs. DCs express more different types of TLRs and cytoplasmic pattern recognition receptors than any other cell type, making them the most versatile sensors of PAMPs and DAMPs among all cell types in the body. In response to invading microbes, they secrete inflammatory cytokines that promote recruitment of additional leukocytes from the blood. The plasmacytoid subset of DCs is a major source of the antiviral cytokines, type I interferons, produced in response to viral infections. This feature of plasmacytoid DCs is due in part to the fact that these cells express abundant amounts of the endosomal nucleic-acid specific TLRs (TLRs 3, 7, 8, 9), as well as cytosolic RNA and DNA sensors, all of which recognize viral nucleic acids inside cells. We will discuss the antiviral actions of type I interferons in more detail later in the chapter.

The reactions of DCs to microbes in the early innate response enhance the ability of the DCs to activate T cells in the subsequent adaptive immune response. In addition, depending on the nature of the microbe that induces the innate response, a DC will direct naive T cell differentiation into distinct types of effector cells, such as IFN-γ–producing Th1 cells or IL-17–producing Th17 cells. These features of DCs will be discussed later in this chapter and in Chapters 6 and 10.

Cytokine-Producing Innate Lymphoid Cells

Innate lymphoid cells (ILCs), which were introduced in Chapter 2, are bone marrow–derived cells with lymphocyte morphology that were discovered as cells that produced cytokines similar to those made by T cells but lacked TCRs. We call them "lymphoid cells," not "lymphocytes," because they do not express clonally distributed diverse antigen receptors like the T lymphocytes they otherwise resemble. There are different subsets of ILCs that arise from the same common lymphoid precursor that gives rise to B and T cells, but the precise steps in ILC development are not fully understood, especially in humans. It is clear that during their development, there are branch points giving rise to three different "helper" subsets of ILCs, which function mainly by secreting different types of cytokines, similar to $CD4^+$ helper T cell subsets, and a separate branch giving rise to natural killer (NK) cells, which function as cytotoxic effectors in addition to secreting the cytokine interferon-γ, similar to CD8+ cytotoxic T lymphocytes. We will describe cytokine-producing ILC subsets in this section and NK cells in the following section.

Three subsets of innate lymphoid cells, called ILC1, ILC2, and ILC3, produce different cytokines and express different transcription factors, analogous to the Th1, Th2, and Th17 subsets of $CD4^+$ T lymphocytes (Fig. 4.8). The cytokines each subset produces determine the roles of these cells in defense, and the transcription factors

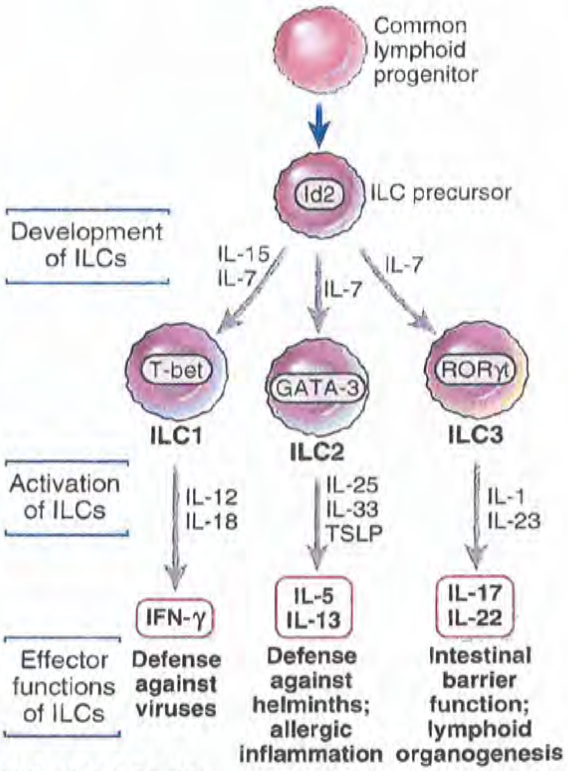


FIGURE 4.8 Cytokine producing innate lymphoid cell subsets. The three major subsets of cytokine producing innate lymphoid cells (ILCs) develop from the common lymphoid progenitor that also gives rise to B and T lymphocytes, and NK cells (not shown). A common ILC precursor identified by the Id2 transcription factor differentiates into three major subsets of cytokine-producing ILCs. Each differentiated subset is distinguished by expression of distinct transcription factors and by cytokines produced when activated, as indicated. The cytokines that drive differentiation into ILC1, 2, or 3 subsets, as well as the cytokines that activate ILCs to produce their own subset-specific cytokines, are shown. The major known functions of the ILCs are also indicated. The cytokines indicated in bold are discussed in Chapter 10, in the context of T cell responses. The functions of the other cytokines mentioned in the figure are summarized later in this chapter (see Table 4.5), and all these cytokines are listed in Appendix I.

are required for differentiation and function of each of the three subsets. ILC1s produce IFN-γ and express the transcription factor T-bet, like Th1 cells. ILC2s produce IL-5, IL-9, and IL-13, and express the transcription factor GATA-3, like Th2 cells. ILC3s produce IL-22 and/or IL-17 and express the transcription factor RORγt, like Th17 cells. Because ILCs do not express T cell receptors, they must be activated by different mechanisms than helper T cells to produce these cytokines. The best defined stimuli for ILC cytokine production are other cytokines, released in the context of innate responses to infections and tissue damage; each ILC subset is activated by different cytokines (see Fig. 4.8).

ILC subsets may participate in host defense against distinct pathogens and also may be involved in inflammatory disorders. ILC1s are likely important for defense against intracellular microbes. ILC2s are important for defense against helminthic parasites, and they also may contribute to allergic diseases. ILC3s are found at mucosal sites and participate in defense against extracellular fungi and bacteria, as well as in maintaining the integrity of epithelial barriers. Lymphoid tissue–inducer (LTi) cells are a subtype of ILC3s, which, in addition to secreting IL-17 and IL-22, also express the membrane molecule lymphotoxin-α and secrete TNF, both of which are required for the normal development of lymphoid organs (see Chapter 2).

The contribution of ILCs to host defense has been difficult to establish because it has not been possible to selectively eliminate these cells or their cytokines without impacting the analogous T lymphocytes as well. The feature of ILCs that makes them potentially important for early host defense is that they are always resident in epithelial barrier tissues, poised to react against microbes that breach those barriers. In contrast, T cells circulate through secondary lymphoid organs and migrate into tissues only after they are activated and differentiate into effector cells, a process that may take several days after encounter with a microbe. It is, therefore, possible that ILCs are early responders to microbes that colonize tissues, and over time this role is assumed by differentiated effector T cells, which are more specific and produce larger amounts of cytokines.

Natural Killer Cells

NK cells, often considered the first known ILC, are cytotoxic cells that play important roles in innate immune responses, mainly against viruses and intracellular bacteria. The "natural killer" designation derives from the fact that their major function is killing infected cells, similar to the adaptive immune system's killer cells, the cytotoxic T lymphocytes (CTLs), and they are ready to do so once they develop, without further differentiation (hence natural). NK cells also secrete IFN-γ and are sometimes referred to as a type of ILC1. Unlike the cytokine-producing ILCs discussed previously, which are found in peripheral tissues but are rare in the blood and lymphoid organs, NK cells constitute 5% to 15% of the mononuclear cells in the blood and spleen. They are rare in other lymphoid organs but are more abundant in certain organs such as the liver and placenta. NK cells in the blood appear as large lymphocytes with numerous cytoplasmic granules. NK cells do not express diverse, clonally distributed antigen receptors typical of B and T cells. Rather, they use germline DNA-encoded receptors (discussed later) to distinguish pathogen-infected cells from healthy cells. They can be identified in the blood by expression of CD56 and the absence of the T cell marker CD3. Most human blood NK cells also express CD16, which is involved in recognition of antibody-coated cells.

Functions of Natural Killer Cells

The effector functions of NK cells are to kill infected cells and to produce IFN-γ, which activates macrophages to destroy phagocytosed microbes (Fig. 4.9). The mechanism of NK cell–mediated cytotoxicity is essentially the same as that of $CD8^+$ CTLs, which we will describe in detail in Chapter 11. NK cells, like CTLs, have granules that contain proteins that mediate killing of target cells. When NK cells are activated, granule exocytosis releases these proteins adjacent to the target cells. One NK cell granule protein, called **perforin**, facilitates the entry of other granule proteins, called **granzymes**, into the cytosol of target cells. The granzymes are proteolytic enzymes that initiate a sequence of signaling events that cause death of the target cells by apoptosis. By killing cells infected by viruses and intracellular bacteria, NK cells eliminate reservoirs of infection. Early in the course of a viral infection, NK cells are expanded and activated by recognition of activating ligands on the infected cells and

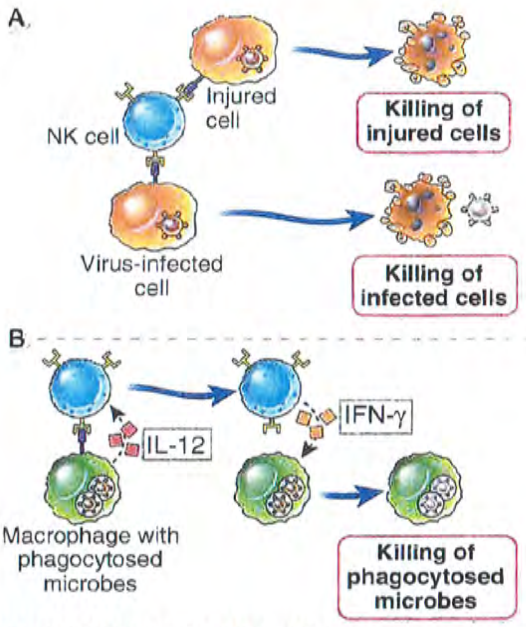


FIGURE 4.9 Functions of NK cells. A, NK cells recognize ligands on infected cells or cells undergoing other types of stress and kill the host cells. In this way, NK cells eliminate reservoirs of infection as well as dysfunctional cells. **B,** NK cells respond to IL-12 produced by macrophages and secrete IFN-γ, which activates the macrophages to kill phagocytosed microbes.

by the cytokines IL-12 and IL-15, and they kill infected cells before antigen-specific CTLs can become fully active, which will usually take 5 to 7 days. NK cells may also be important later in the course of viral infection by killing infected cells that have escaped CTL-mediated immune attack by reducing expression of class I major histocompatibility complex (MHC) molecules. Some tumors, especially those of hematopoietic origin, are targets of NK cells, perhaps because the tumor cells do not express normal levels or types of class I MHC molecules, which inhibit NK cell activation, discussed later.

NK cell–derived IFN-γ increases the capacity of macrophages to kill phagocytosed bacteria, similar to IFN-γ produced by T cells (see Chapter 10). This IFN-γ–dependent NK cell–macrophage interaction can control an infection with intracellular bacteria such as *Listeria monocytogenes* for several days or weeks and thus allow time for T cell–mediated immunity to develop and eradicate the infection. IFN-γ produced by NK cells in lymph nodes can also direct the differentiation of naive T cells into Th1 cells (see Chapter 10). Some human NK cells do not express CD16 nor are they cytotoxic, but they do produce abundant IFN-γ. Predictably, deficiency of NK cells, seen in rare individuals, leads to increased susceptibility to infection by some viruses and intracellular bacteria.

Activating and Inhibitory Receptors of Natural Killer Cells

NK cells distinguish infected and stressed cells from healthy cells, and NK cell function is regulated by a balance between signals that are generated from activating receptors and inhibitory receptors. These receptors recognize molecules on the surface of other cells and generate activating or inhibitory signals that promote or inhibit NK responses. The activating receptors stimulate protein kinases that phosphorylate downstream signaling substrates, while inhibitory receptors stimulate phosphatases that counteract the kinases. In general, the activating receptors recognize ligands on infected and injured cells, and the inhibitory receptors recognize ligands on healthy normal cells (Fig. 4.10). When an NK cell interacts with another cell, the outcome is determined by the integration of signals generated from the array of inhibitory and activating receptors that are expressed by the NK cell and that interact with ligands on the other cell. Engagement of activating receptors stimulates the killing

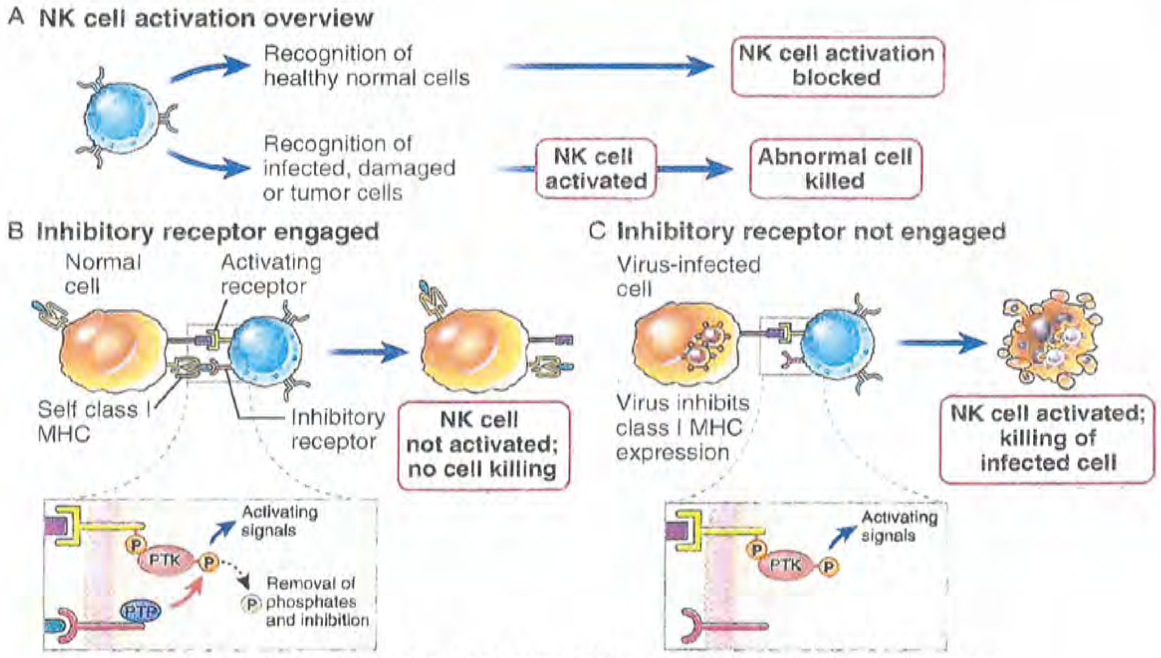


FIGURE 4.10 Functions of activating and inhibitory receptors of NK cells. A, Overview of NK cell activation. **B,** Activating receptors of NK cells recognize ligands on target cells and activate protein tyrosine kinases (PTKs), whose activities are inhibited by inhibitory receptors that recognize class I MHC molecules and activate protein tyrosine phosphatases (PTP). NK cells do not efficiently kill class I MHC–expressing healthy cells. **C,** If a virus infection or other stress inhibits class I MHC expression on infected cells and induces expression of additional activating ligands, the NK cell inhibitory receptor is not engaged and the activating receptor is unopposed to trigger responses of NK cells, including killing of target cells and cytokine secretion. In addition, cells stressed by infection or neoplastic transformation may express increased amounts of activating ligands, which bind NK cell–activating receptors and induce more tyrosine phosphorylation than can be removed by inhibitory receptor–associated phosphatases, resulting in killing of the stressed cells (not shown). Structural details and ligands of inhibitory and activating NK cell receptors are shown in Fig. 4.9.

activity of the NK cells, resulting in destruction of stressed or infected cells. In contrast, engagement of inhibitory receptors shuts off NK cell activity and prevents destruction of healthy cells. Because of the stochastic nature of their expression, there is significant diversity in the array of activating and inhibitory receptors that different NK cells express in any one individual. The result of this is that individual NK cells, even in the same person, may respond to different types of microbes or infected cells.

Activating receptors on NK cells recognize a heterogeneous group of ligands, some of which may be expressed on normal cells and others mainly on cells that have undergone stress, are infected with microbes, or are neoplastic (Fig. 4.11). Many of the NK cell–activating receptors are called **killer cell immunoglobulin (Ig)-like receptors (KIRs)** because they contain a structural domain named the immunoglobulin (Ig) fold, first identified in antibody (also known as Ig) molecules, discussed in Chapter 5. All proteins with Ig folds are members of the Ig superfamily. A second important group of activating NK receptors belongs to the family of C-type lectins, which are proteins with carbohydrate-binding properties similar to the CLRs discussed earlier in the chapter. One well-studied NK cell–activating receptor in the C-type lectin family is NKG2D, which binds class I MHC–like proteins, including MIC-A and MIC-B, found on virally infected cells and tumor cells but not normal cells. The NKG2D receptor associates with a signaling subunit named DAP10, which has signaling functions that stimulate NK cell cytotoxicity against target cells.

Another important activating receptor on NK cells is CD16 (FcγRIIIA), which is a low-affinity receptor for IgG

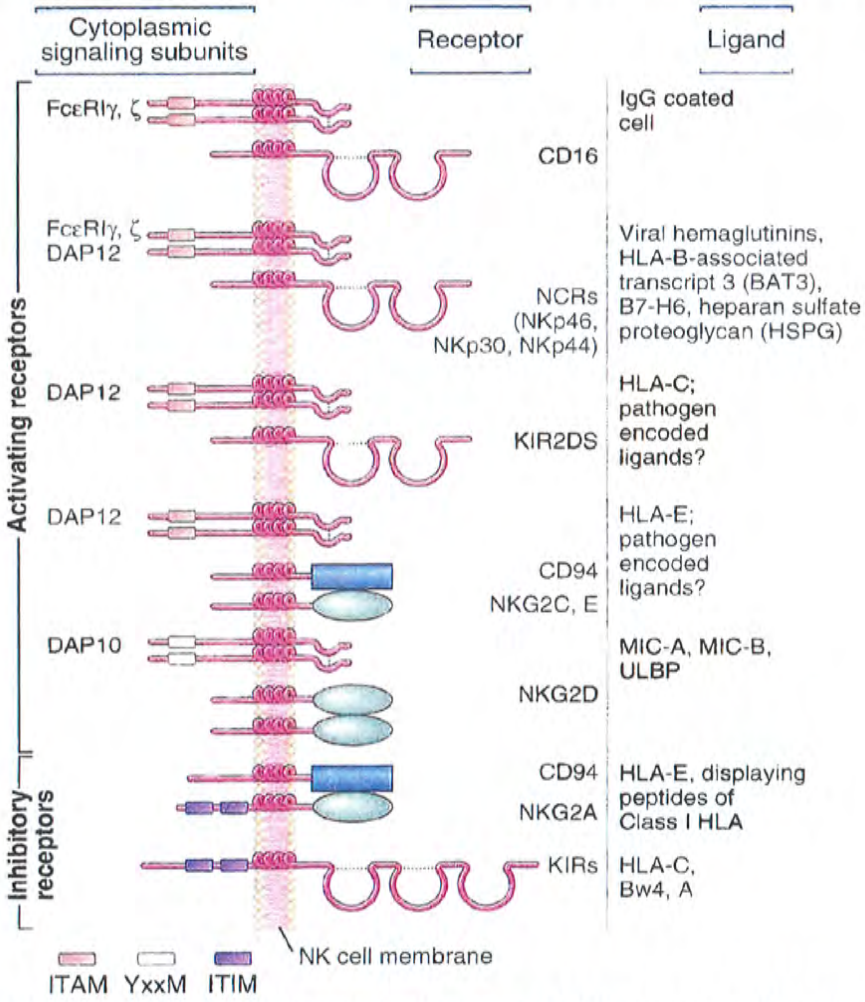


FIGURE 4.11 Structure and ligands of activating and inhibitory receptors of NK cells. The activating and inhibitory receptors are indicated in bold. CD16 and the NCRs associate with ζ chain homodimers, FcεRIγ homodimers, or ζ-FcεRIγ heterodimers. There are multiple different KIRs, with different ligand specificities. *KIR*, killer cell immunoglobulin (Ig) like receptors; *MIC*, MHC class I polypeptide-related sequence; *NCR*, natural cytotoxicity receptor. *ULBP*, UL-16 binding protein.

antibodies. Antibody molecules have highly variable antigen-binding ends, and on the opposite end, they have an invariant portion, called the Fc region, that interacts with various other molecules in the immune system. We will describe the structure of antibodies in detail in Chapter 5, but for now, it is sufficient to know that CD16 binds to the Fc regions of certain types of antibodies called IgG1 and IgG3. CD16 associates with one of three different signaling proteins (e.g., FcεRIγ, ζ, and DAP12 proteins). During an infection, the adaptive immune system produces IgG1 and IgG3 antibodies that bind to microbial antigens expressed on the surface of infected cells, and CD16 on NK cells can bind to the Fc regions of these antibodies. As a result, CD16 generates activating signals, through its associated signaling partners, and the NK cells kill the infected cells that have been coated with antibody molecules. This process is called **antibody-dependent cell-mediated cytotoxicity**; it is an effector function of adaptive immunity, which we will discuss in Chapter 13 when we consider humoral immunity.

Inhibitory receptors of NK cells recognize class I MHC molecules, which are cell surface proteins normally expressed on all healthy nucleated cells in the body (see Fig. 4.11). A major function of class I MHC molecules, distinct from their role in regulating NK cell activation, is to display peptides derived from cytosolic proteins, including microbial proteins, on the cell surface for recognition by $CD8^+$ T lymphocytes. We will describe the structure and function of MHC molecules in relation to T cell antigen recognition in Chapter 6. For now, it is important to understand that NK cells use fundamentally different types of receptors than do T cells to recognize class I MHC molecules. These NK receptors respond to recognition of class I MHC molecules by inhibiting NK activation. This is useful because normal cells express class I MHC molecules, and many viruses and other causes of cell stress lead to a loss of cell surface expression of class I MHC. Thus, NK cells interpret the presence of class I MHC molecules as markers of normal, healthy self, and their absence is an indication of infection or damage. As a result, NK cells will be inhibited by healthy cells but will not receive inhibitory signals from infected or stressed cells. At the same time, the NK cells are likely to receive activating signals from infected or damaged cells through activating receptors. The net result will be activation of the NK cells to secrete cytokines and to kill the infected or stressed cell. This ability of NK cells to become activated by host cells that lack class I MHC has been called "recognition of missing self."

The largest group of NK inhibitory receptors belong to the same KIR family that includes activating receptors, discussed earlier. These inhibitory KIRs bind a variety of different class I MHC molecules. Other inhibitory receptors are lectins, such as the CD94/NKG2A heterodimer, which recognizes a class I MHC molecule called HLA-E. Interestingly, HLA-E displays peptides derived from other class I MHC molecules, so CD94/NKG2A is a surveillance receptor for several different class I MHC molecules.

Activating and inhibitory NK receptors contain structural motifs in their cytoplasmic tails that engage the signaling pathways that respectively promote or inhibit target cell killing and cytokine secretion (see Figs. 4.10 and 4.11). Activating receptors have **immunoreceptor tyrosine-based activation motifs (ITAMs)**, which contain tyrosine residues that become phosphorylated by cytoplasmic kinases after binding of ligands to the receptors. Other protein kinases are recruited to the modified ITAMs and become activated, and these kinases contribute to further signaling by phosphorylating additional proteins, eventually leading to cytotoxic activity and cytokine secretion. ITAMs also are found in the cytoplasmic tails of other signaling receptors in the immune system, including the antigen receptor complexes of T and B cells, and we will discuss their structure and signaling functions in more detail in Chapter 7. In some activating receptors, a single polypeptide chain contains the cytoplasmic ITAM as well as the extracellular ligand-binding portion. In other receptors, the ITAMs are in separate polypeptide chains, such as FcεRIγ, ζ, and DAP12, which do not bind ligands but are noncovalently associated with the ligand-binding chains (see Fig. 4.10).

Inhibitory receptors of NK cells have **immunoreceptor tyrosine-based inhibition motifs (ITIMs)**, which engage molecules that block the signaling pathways of activating receptors (see Figs. 4.10 and 4.11). ITIMs contain tyrosine residues that are phosphorylated on ligand binding to the inhibitory receptor and serve as docking sites for the recruitment and activation of phosphatases, which remove phosphates from several signaling proteins or lipids generated by the signaling downstream of NK activating receptors. The end result is blocking of the signaling functions of activating receptors. ITIMs also are found in cytoplasmic tails of other receptors besides NK inhibitory receptors, and we will discuss their structure and signaling functions in more detail in Chapter 7.

KIR genes are polymorphic, meaning that there are several allelic variants in the human population. As a result, one person may express different receptors than another person. Groups of KIR alleles are often inherited together from a single parent. These groups of linked genes are called *KIR haplotypes*. There are two major *KIR* haplotypes and some rarer ones. Haplotypes differ in the number of receptors encoded, and some have more or fewer activating receptors than others. Some haplotypes are associated with increased susceptibility to some diseases, including spontaneous abortion and a type of eye inflammation called uveitis.

Cytokines can enhance the functional responses of NK cells. The major cytokines of the innate immune system that stimulate NK function are IL-12, IL-15, IL-18, and type I interferons (discussed later). Each of these cytokines enhances the cytotoxic activity of NK cells, and they can stimulate IFN-γ secretion by the NK cells independent of activating receptors. In addition, IL-15 is an important growth factor for NK cells.

T and B Lymphocytes With Limited Antigen Receptor Diversity

As we will discuss in greater detail in later chapters, most T and B lymphocytes are components of the adaptive

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immune system and are characterized by a highly diverse repertoire of specificities for different antigens. However, certain small populations of lymphocytes express antigen receptors that are structurally the same as those of T and B cells, but these receptors have very little diversity. These T and B cell subsets may recognize structures expressed by many different or commonly encountered microbial species. T cells with limited antigen receptor diversity include invariant natural killer T (iNKT) cells, γδ T cells, mucosa-associated invariant T (MAIT) cells, and intraepithelial T cells with αβ TCRs (mentioned earlier). B cell subsets that produce antibodies with a limited set of specificities include B-1 cells and marginal-zone B cells. Although these T and B cells perform similar functions as their more clonally diverse counterparts, the nature of their specificities places them in a special category of lymphocytes that is more akin to cells of innate immunity than to cells of adaptive immunity. These special T and B cell subsets are described in Chapters 10 and 12, respectively.

Mast Cells

Mast cells are sentinel cells present in the skin, mucosal epithelium, and connective tissues that rapidly secrete proinflammatory cytokines and lipid mediators in response to infections and other stimuli. We introduced mast cells in Chapter 2. Recall that these cells contain abundant cytoplasmic granules filled with various inflammatory mediators that are released when the cells are activated, either by microbial products or by a special antibody-dependent mechanism. The granule contents include vasoactive amines (such as histamine) that cause vasodilation and increased capillary permeability, and proteolytic enzymes that can kill bacteria or inactivate microbial toxins. Mast cells also synthesize and secrete lipid mediators (such as prostaglandins) and cytokines (such as TNF). Because mast cells are usually located adjacent to blood vessels (see Fig. 2.1B), their released granule contents rapidly induce changes in the blood vessels that promote acute inflammation. Mast cells express TLRs, and TLR ligands can induce mast cell degranulation. Mast cell–deficient mice are impaired in controlling bacterial infections, probably because of defective innate immune responses. Mast cell products also provide defense against helminths and are responsible for symptoms of allergic diseases. We will return to a detailed discussion of mast cells in the context of allergic diseases in Chapter 20.

SOLUBLE EFFECTOR MOLECULES OF INNATE IMMUNITY

Several different kinds of molecules that recognize microbes and promote innate responses exist in soluble form in the blood and extracellular fluids. These molecules provide early defense against pathogens that enter the circulation or are present outside host cells at some stage of their life cycle. The soluble effector molecules function in two major ways:

- By binding to microbes, they act as **opsonins** and enhance the ability of macrophages and neutrophils to phagocytose the microbes. This is because the phagocytic cells express membrane receptors specific for the opsonins, and these receptors can efficiently mediate the internalization of the complex of opsonin and bound microbe and subsequent destruction of the ingested microbe.
- After binding to microbes, soluble mediators of innate immunity promote inflammatory responses that bring more phagocytes to sites of infections, and they may also directly kill microbes.

The soluble effector molecules are sometimes called the humoral branch of innate immunity, analogous to the humoral branch of adaptive immunity mediated by antibodies. The major components of the humoral innate immune system are the complement system, collectins, pentraxins, and ficolins, which are described next.

The Complement System

The complement system consists of several plasma proteins that work together to opsonize microbes, to promote the recruitment of phagocytes to the site of infection, and in some cases, to directly kill the microbes (Fig. 4.12). Complement activation involves proteolytic cascades in which an inactive precursor enzyme, called a zymogen, is altered to become an active protease that cleaves and thereby induces the proteolytic activity of the next complement protein in the cascade. Enzymatic cascades result in tremendous amplification of the amount of proteolytic products that are generated at each step. These products perform the effector functions of the complement system. Besides the complement system, other medically important proteolytic cascades include the blood coagulation pathways and the kinin-kallikrein system that regulates vascular permeability.

The first step in activation of the complement system is recognition of molecules on microbial surfaces but not host cells, and this occurs in three ways, each referred to as a distinct pathway of complement activation.

- The **classical pathway**, so called because it was discovered first, uses a plasma protein called C1q to detect antibodies bound to the surface of a microbe or other structure (see Fig. 4.11). Once C1q binds to the Fc portion of the antibodies, two associated serine proteases, called C1r and C1s, become active and initiate a proteolytic cascade involving other complement proteins. The classical pathway is one of the major effector mechanisms of the humoral arm of adaptive immune responses (see Chapter 13). Innate immune system soluble proteins called pentraxins, which are discussed later, can also bind C1q and initiate the classical pathway.
- The **alternative pathway**, which was discovered later but is phylogenetically older than the classical pathway, is triggered when a complement protein called C3 directly recognizes certain microbial surface structures, such as bacterial LPS. C3 is also constitutively activated in solution at a low level

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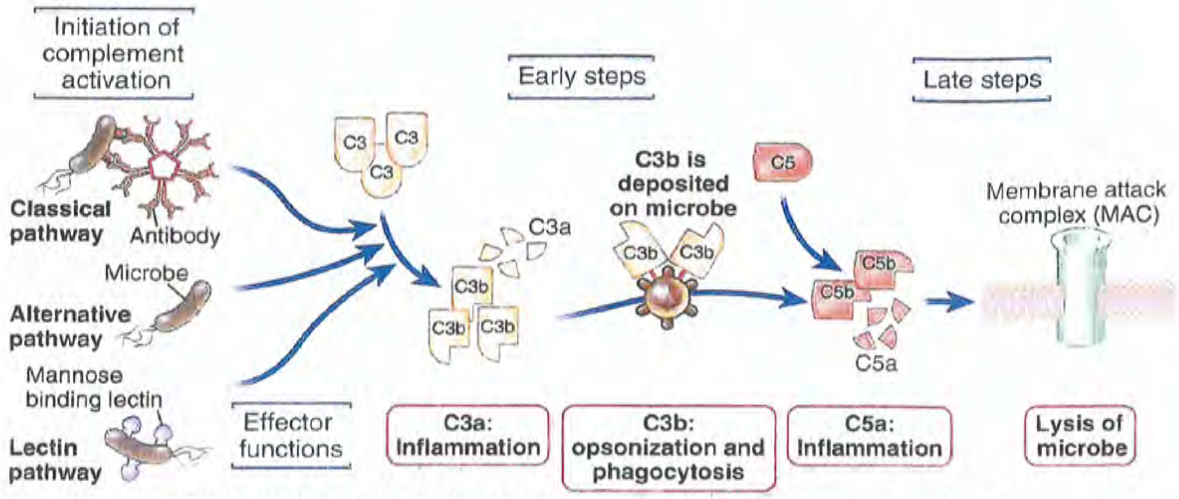


FIGURE 4.12 Pathways of complement activation. The activation of the complement system may be initiated by three distinct pathways, all of which lead to the production of C3a, which stimulates inflammation, and C3b (early steps). C3b initiates the late steps of complement activation, culminating in the production of peptides that also stimulate inflammation (C5a) and polymerized C9, which forms the membrane attack complex, so called because it creates holes in plasma membranes (late steps). The principal functions of major proteins produced at different steps are shown. The activation, functions, and regulation of the complement system are discussed in much more detail in Chapter 13.

and binds to cell surfaces, but it is then inhibited by regulatory molecules present on mammalian cells. Because microbes lack these regulatory proteins, the spontaneous activation can be amplified on microbial surfaces. Thus, this pathway can distinguish normal self from foreign microbes on the basis of the presence or absence of the regulatory proteins.

- The **lectin pathway** is triggered by a plasma protein called mannose-binding lectin (MBL), which recognizes terminal mannose residues on microbial glycoproteins and glycolipids, similar to the mannose receptor on phagocytes described earlier (see Fig. 4.12). MBL is a member of the collectin family (discussed later) with a hexameric structure similar to the C1q component of the complement system. After MBL binds to microbes, two zymogens called MASP1 (mannose-associated serine protease 1, or mannan-binding lectin-associated serine protease) and MASP2, with similar functions to C1r and C1s, associate with MBL and initiate downstream proteolytic steps identical to the classical pathway.

Recognition of microbes by any of the three complement pathways results in sequential recruitment and assembly of additional complement proteins into protease complexes (see Fig. 4.12). One of these complexes, called **C3 convertase**, cleaves the central protein of the complement system, **C3**, producing C3a and C3b. The larger C3b fragment becomes covalently attached to the microbial surface where the complement pathway was activated. The sequential enzymatic activity of complement proteins provides such tremendous amplification that millions of C3b molecules can deposit on the surface of a microbe within 2 or 3 minutes! C3b serves as an opsonin to promote phagocytosis of the microbes. The smaller fragment, C3a, is released and stimulates inflammation by acting as a chemoattractant for neutrophils, by inducing mast cell degranulation, and by directly increasing vascular permeability so that plasma proteins and fluid leak into sites of infections. C3b binds other complement proteins to form a protease called **C5 convertase** that cleaves C5, generating a released peptide (C5a) and a larger fragment (C5b) that remains attached to the microbial cell membranes. C5a exerts the same proinflammatory effects as C3a and is more potent. C5b initiates the formation of a complex of the complement proteins C6, C7, C8, and C9, which are assembled into a membrane pore called the **membrane attack complex** (MAC) that causes lysis of the cells where the complement is activated.

The complement system is an essential component of innate immunity, and patients with deficiencies in C3 are highly susceptible to recurrent, often lethal, bacterial infections. Genetic deficiencies in MAC formation (the terminal product of the classical pathway) increase susceptibility to only a limited number of microbes, notably *Neisseria* bacteria, which have thin cell walls that make them especially susceptible to the lytic action of the MAC. We will discuss the complement system in more detail in Chapter 13.

Pentraxins

Several plasma proteins that recognize microbial structures and participate in innate immunity belong to the pentraxin family, which is a phylogenetically old

group of structurally homologous pentameric proteins. Prominent members of this family include the short pentraxins, C-reactive protein (CRP) and serum amyloid P (SAP), and the long pentraxin PTX3. Both CRP and SAP bind to several different species of bacteria and fungi. The molecular ligands recognized by CRP and SAP include phosphorylcholine and phosphatidylethanolamine, respectively, which are found on bacterial membranes and become exposed on apoptotic cells. CRP, SAP, and PTX3 all activate complement by binding C1q and initiating the classical pathway.

Plasma concentrations of CRP are very low in healthy individuals but can increase up to 1000-fold during infections and in response to other inflammatory stimuli. The increased levels of CRP are a result of increased synthesis by the liver induced by the cytokines IL-6 and IL-1, which are produced by phagocytes and DCs as part of the innate immune response. Liver synthesis and plasma levels of several other proteins, including SAP and some unrelated to the pentraxins, also increase in response to IL-1 and IL-6. All these plasma proteins are called **acute-phase reactants** because they are elevated in the blood during acute inflammatory reactions, and their increased production is part of the **acute phase response** to infection and other insults.

PTX3 is produced by several cell types, including DCs, macrophages, and endothelial cells, in response to TLR ligands and inflammatory cytokines, such as TNF, and may be considered an acute-phase reactant. PTX3 is also stored in neutrophil granules and released as neutrophils die. PTX3 recognizes various molecules on fungi, certain bacteria, and viruses, as well as apoptotic cells, and activates the classical complement pathway. Studies with knockout mice reveal that PTX3 provides protection against these microbes, including the fungus *Aspergillus fumigatus* and the influenza virus.

Collectins and Ficolins

The **collectins** are a family of trimeric or hexameric proteins, each subunit of which contains a collagen-like tail connected by a neck region to a calcium-dependent (C-type) lectin head. Three members of this family serve as soluble effector molecules in the innate immune system; these are mannose-binding lectin (MBL) and pulmonary surfactant proteins SP-A and SP-D.

MBL, which is a soluble pattern recognition receptor that binds carbohydrates with terminal mannose and fucose, was discussed earlier in relation to the lectin pathway of complement activation (Fig. 4.13). MBL also functions as an opsonin by binding to and enhancing phagocytosis of microbes. Recall that opsonins simultaneously bind microbes and a surface receptor on phagocyte membranes, and in the case of MBL, the surface receptor is called the C1q receptor because it also binds C1q. This receptor mediates the internalization of microbes that are opsonized by MBL. The gene encoding MBL is polymorphic, and certain alleles are associated with impaired hexamer formation and reduced blood levels. Low MBL levels result in increased susceptibility to a variety of infections, especially in individuals who have other immune defects.

Surfactant protein A (SP-A) and surfactant protein D (SP-D) are collectins with lipophilic properties shared

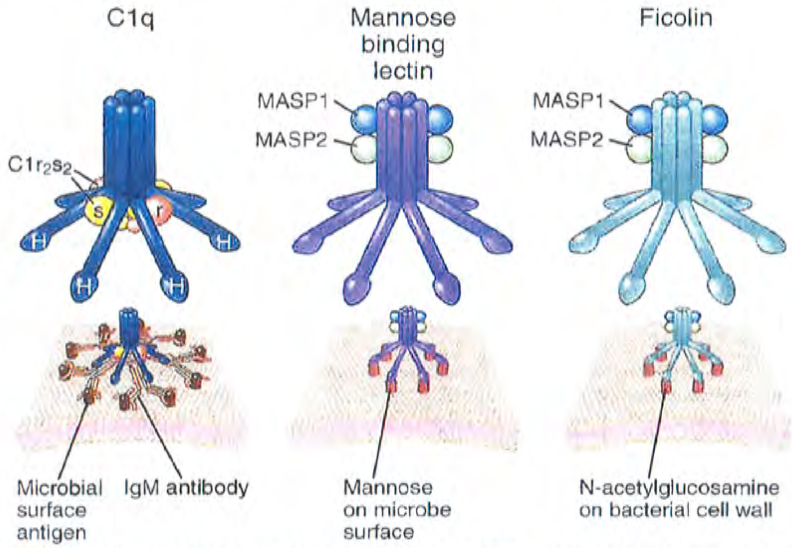


FIGURE 4.13 C1, mannose-binding lectin, and ficolin. These three homologous hexameric proteins can all initiate complement activation on binding to their ligands on cell surfaces. C-type lectin–like globular heads *(H)* at the end of collagenous-like stalks in the C1q and mannose-binding lectin proteins bind the Fc regions of IgM or mannose on the surface of microbes, respectively. Fibrinogen-like globular heads on ficolin bind *N*-acetylglucosamine on the surface of microbes. Binding results in conformational changes that activate the serine protease activity of C1r and C1s, associated with C1q, or MASP1 and MASP2, associated with mannose-binding lectin and ficolin.

by other surfactants. They are found in the alveoli of the lungs, and their major functions are to maintain the ability of alveoli to expand upon inhalation by reducing surface tension of alveolar fluid, and as mediators of innate immune responses in the lung. They bind to various microorganisms and act as opsonins, facilitating ingestion by alveolar macrophages. SP-A and SP-D can also directly inhibit bacterial growth, and they may activate macrophages. SP-A– and SP-D–deficient mice have impaired abilities to resist a variety of pulmonary infections.

Ficolins are plasma proteins that are structurally similar to collectins. They possess a collagen-like domain, but instead of a C-type lectin domain, they have a fibrinogen-type carbohydrate recognition domain (see Fig. 4.13). Ficolins have been shown to bind several species of bacteria, opsonizing them and activating complement in a manner similar to that of MBL. The molecular ligands of the ficolins include *N*-acetylglucosamine and the lipoteichoic acid component of the cell walls of gram-positive bacteria.

Now that we have discussed the general properties and various components of the innate immune system, including the cells, cellular pathogen recognition receptors, and soluble effector molecules, we can consider how these various components work to protect against pathogens. The three major mechanisms by which the innate immune system protects against infections are by inducing inflammation, inducing antiviral defense, and stimulating adaptive immunity.

THE INFLAMMATORY RESPONSE

The principal way by which the innate immune system deals with infections and tissue injury is to stimulate acute inflammation, which is the accumulation of leukocytes, plasma proteins, and fluid derived from the blood at an extravascular tissue site of infection or injury. Leukocytes and plasma proteins, which are critical for innate defense against microbes, normally circulate in the blood and are recruited to sites of infection and injury, where they perform the effector functions that kill microbes and begin to repair damaged tissue. Typically, the leukocyte that is recruited first from the blood into sites of inflammation is the neutrophil because it is the most abundant leukocyte in the blood and the most rapid responder to chemotactic signals. Blood monocytes, which become macrophages in the tissue, become increasingly prominent over time and may be the dominant population in some reactions. Among the important plasma proteins that enter inflammatory sites are complement proteins, antibodies, and acute-phase reactants.

The delivery of cells and proteins to the inflammatory site is dependent on reversible changes in blood vessels in the infected or damaged tissue. These changes include increased blood flow into the tissue due to vascular dilation, increased adhesiveness of circulating leukocytes to the endothelial lining of venules (see Chapter 3), and increased permeability of the capillaries and venules to plasma proteins and fluid. All of these changes are induced by cytokines and small-molecule mediators initially derived from resident sentinel cells in the tissue, such as mast cells, macrophages, DCs, and endothelial cells, in response to PAMP or DAMP stimulation. As the inflammatory process develops, the mediators may be derived from newly arrived and activated leukocytes and complement proteins.

Acute inflammation can develop in minutes to hours and last for days. Chronic inflammation is a process that takes over from acute inflammation if the infection is not eliminated or the tissue injury is prolonged. It usually involves recruitment and activation of monocytes and lymphocytes. Chronic inflammatory sites also often undergo tissue remodeling, with angiogenesis and fibrosis. Although innate immune stimuli may contribute to chronic inflammation, the adaptive immune system may also be involved because cytokines produced by T cells are powerful inducers of inflammation (see Chapter 10). Detailed descriptions of the various mediators and pathologic manifestations of acute and chronic inflammation can be found in pathology textbooks. We will focus our discussion on particular aspects of the acute inflammatory process that have broad relevance to both innate and adaptive immunity and immune-mediated inflammatory diseases.

The Major Proinflammatory Cytokines of Innate Immunity

One of the earliest responses of the innate immune system to infection and tissue damage is the secretion of cytokines by tissue cells, which is critical for the acute inflammatory response. The cytokines of innate immunity have some important general properties and functions (Table 4.5):

- They are produced mainly by tissue macrophages and DCs, although other cell types, including mast cells, endothelial cells, and some epithelial cells, can also produce them.
- Most of these cytokines act on cells close to their cell of origin (paracrine action). In some severe infections, enough of the cytokines may be produced so that significant amounts enter the circulation and act at a distance (endocrine action).
- Different cytokines have similar or overlapping actions, or are functionally unique. One cytokine may stimulate the production of others, thus setting up cascades that amplify the reaction or induce new reactions.
- The cytokines of innate immunity serve several roles: inducing inflammation, inhibiting viral replication, promoting T cell responses, and limiting innate immune responses. These functions are described next and later in the chapter.
- Many cytokines that are produced by innate immune cells, such as TNF, IL-17, IL-5, and IFN-γ, are also produced by T lymphocytes in adaptive immune responses.

Three of the most important proinflammatory cytokines of the innate immune system are TNF, IL-1 (both

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TABLE 4.5 Cytokines of Innate Immunity

Cytokine	Size	Principal Cell Source	Principal Cellular Targets and Biologic Effects
TNF	17 kD; 51 kD homotrimer	Macrophages, T cells	Endothelial cells: activation (inflammation, coagulation) Neutrophils: activation Hypothalamus: fever Muscle, fat: catabolism (cachexia) Many cell types: apoptosis
IL-1	17 kD mature form; 33 kD precursors	Macrophages, endothelial cells, some epithelial cells	Endothelial cells: activation (inflammation, coagulation) Hypothalamus: fever Liver: synthesis of acute-phase proteins T cells: T_H17 differentiation
Chemokines (see Table 3.2)	8–12 kD	Macrophages, endothelial cells, T cells, fibroblasts, platelets	Leukocytes: chemotaxis, activation; migration into tissues
IL-12	Heterodimer of 35 kD and 40 kD subunits	Macrophages, DCs	T cells: T_H1 differentiation NK cells and T cells: IFN-γ synthesis, increased cytotoxic activity
Type I interferons (IFN-α, IFN-β)	IFN-α: 15–21 kD IFN-β: 20–25 kD	IFN-α: macrophages, plasmacytoid DCs IFN-β: fibroblasts	All cells: antiviral state, increased class I MHC expression NK cells: activation
IL-10	Homodimer of 34–40 kD and 18 kD subunits	Macrophages, T cells (mainly regulatory T cells)	Macrophages, DCs: inhibition of expression of IL-12, costimulators and class II MHC molecules
IL-6	19–26 kD	Macrophages, endothelial cells, T cells	Liver: synthesis of acute-phase proteins B cells: proliferation of antibody-producing cells T cells: T_H17 differentiation
IL-15	13 kD	Macrophages, others	NK cells: proliferation T cells: proliferation (memory $CD8^+$ cells)
IL-18	17 kD	Macrophages	NK cells and T cells: IFN-γ synthesis
IL-23	Heterodimer of unique 19 kD subunit and 40 kD subunit of IL-12	Macrophages and DCs	T cells: maintenance of IL-17–producing T cells
IL-27	Heterodimer of 28 kD and 13 kD subunits	Macrophages and DCs	T cells: T_H1 differentiation; inhibition of T_H17 cells NK cells: IFN-γ synthesis

DC, Dendritic cells; *MHC*, major histocompatibility complex; *IFN*, interferon; *IL*, interleukin; *TNF*, tumor necrosis factor. (Also see Appendix I.)

of which we have mentioned several times), and IL-6. We will discuss the major features of these cytokines, focusing mainly on TNF and IL-1, before describing their role in acute inflammation.

Tumor Necrosis Factor

TNF is a mediator of the acute inflammatory response to bacteria and other infectious microbes. The name of this cytokine derives from its original identification as a serum substance (factor) that caused necrosis of tumors, now known to be the result of inflammation and thrombosis of tumor blood vessels. TNF is also called TNF-α to distinguish it from the closely related TNF-β, which is also called lymphotoxin. TNF is produced mainly by macrophages and DCs, and also by other cell types. In macrophages, it is synthesized as a nonglycosylated type II membrane homotrimeric protein that is able to bind to one form of TNF receptor. The membrane form of TNF is cleaved by a membrane-associated metalloproteinase, releasing a polypeptide fragment, and three of these polypeptide chains polymerize to form a triangular pyramid-shaped circulating TNF protein

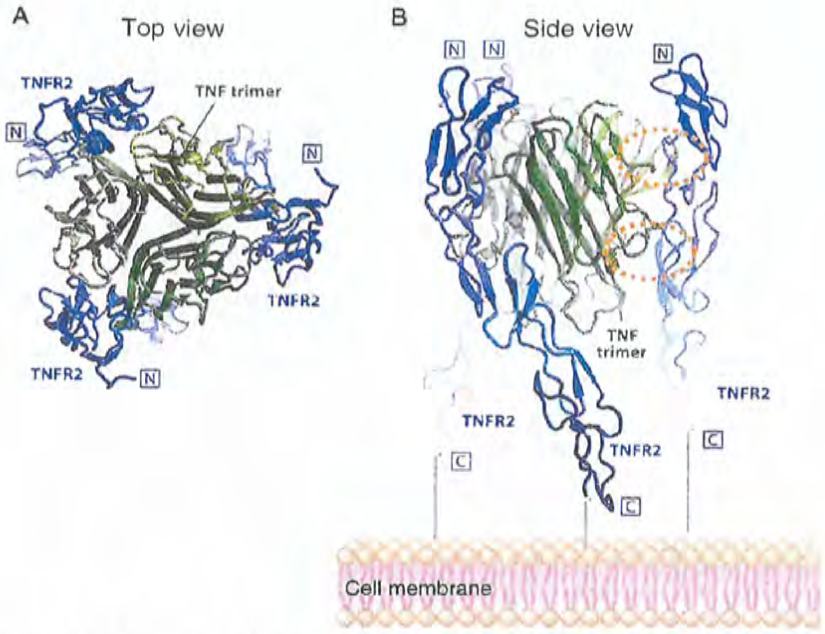


FIGURE 4.14 Structure of the TNF receptor with bound TNF. The ribbon structure depicts a top view (**A**) and a side view (**B**) of a complex of three type 2 TNF receptors (TNF-R2) and one molecule of bound trimeric TNF, revealed by x-ray crystallography. The three TNF-R2 molecules, colored blue, together bind one homotrimer of TNF, colored green, with each receptor molecule interacting with two different TNF monomers in the homotrimer complex. The binding regions of only one of the three TNFR2 molecules to two TNF monomers are highlighted in the side view by orange ovals. *(Modified from Mukai Y, Nakamura T, Yoshikawa M, et al: Solution of the structure of the TNF-TNFR2 complex,* Science Signal *3:ra83, 2010.)*

(Fig. 4.14). The receptor-binding sites are at the base of the pyramid, allowing simultaneous binding of the cytokine to three receptor molecules. TNF-α is a member of a large family of homologous proteins called the TNF superfamily, all of which share the feature of forming homotrimers (see Appendix I).

There are two distinct TNF receptors called type I (TNF-RI) and type II (TNF-RII). The affinities of TNF for its receptors are unusually low for a cytokine, the K_d being only ~1×10^{-9} M for binding to TNF-RI and approximately 5×10^{-10} M for binding to TNF-RII. Both TNF receptors are present on most cell types. The TNF receptors are members of a large family of proteins called the TNF receptor superfamily, many of which are involved in immune and inflammatory responses. These receptors exist as trimers in the plasma membrane. Ligand binding to some TNF receptor family members, such as TNF-RI, TNF-RII, and CD40, leads to the recruitment of proteins called TNF receptor–associated factors (TRAFs) to the cytoplasmic domains of the receptors. The TRAFs activate transcription factors, notably NF-κB and AP-1 (see Chapter 7). Cytokine binding to some family members, such as TNF-RI, may lead to recruitment of an adaptor protein that activates caspases and triggers apoptosis. Thus, different members of the TNF receptor family can induce gene expression or cell death, and some can do both.

TNF production by macrophages is stimulated by PAMPs and DAMPs. TLRs, NLRs, RLRs, and CDSs can all induce TNF gene expression, in part by activation of the NF-κB transcription factor. Many different microbial products can therefore induce TNF production. Large amounts of this cytokine may be produced during infections with gram-negative and gram-positive bacteria, which express and release the cell wall TLR ligands LPS and lipoteichoic acid, respectively. Septic shock, a life-threatening condition resulting from severe infections, is mediated in large part by TNF. We will discuss septic shock later in this chapter. TNF is also a major contributor to inflammation in several human inflammatory diseases, and anti-TNF agents have become the mainstay of treatment of many of these diseases.

Interleukin-1

IL-1 is also a mediator of the acute inflammatory response and has many similar actions as TNF. The major cellular source of IL-1, like that of TNF, is activated mononuclear phagocytes. Unlike TNF, IL-1 is also produced by many cell types other than macrophages, such as neutrophils, epithelial cells (e.g., keratinocytes), and endothelial cells. There are two forms of IL-1, called IL-1α and IL-1β, that are less than 30% homologous to each other, but they bind to the same cell surface receptors and have the same biologic activities. The main biologically active secreted

form in the setting of infections and most other immune responses is IL-1β.

IL-1 production usually requires two distinct signals, one that activates new gene transcription and production of a 33-kD precursor pro–IL-1β polypeptide and a second that activates the inflammasome to proteolytically cleave the precursor to generate the 17-kD mature IL-1β protein (see Fig. 4.6). As discussed earlier in this chapter, IL-1β gene transcription is induced by TLR and NLR signaling pathways that activate NF-κB, whereas pro–IL-1β cleavage is mediated by caspase-1, which is activated in the inflammasome. TNF can also stimulate phagocytes and other cell types to produce IL-1. This is an example of a cascade of cytokines that have similar biologic activities. IL-1 is secreted by a nonclassical pathway because, unlike most secreted proteins, neither IL-1α nor IL-1β has hydrophobic signal sequences to target the nascent polypeptide to the endoplasmic reticulum membrane. One way IL-1β may be secreted is through membrane pores formed by oligomerized fragments of a protein called gasdermin D, which are proteolytically generated by caspase-11, discussed earlier as a mediator of pyroptosis.

IL-1 mediates its biologic effects through a membrane receptor called the type I IL-1 receptor, which is expressed on many cell types, including endothelial cells, epithelial cells, and leukocytes. This receptor is an integral membrane protein that contains an extracellular ligand-binding Ig domain and a TIR signaling domain in the cytosolic region, which we described earlier in reference to TLRs. The signaling events that occur when IL-1 binds to the type I IL-1 receptor are similar to those triggered by TLRs and result in the activation of NF-κB and AP-1 transcription factors (see Chapter 7). A second IL-1 receptor, called the type II IL-1 receptor, appears incapable of activating downstream signals, and serves as a decoy receptor that limits responses to IL-1.

Interleukin-6

IL-6 is another important cytokine in acute inflammatory responses that has both local and systemic effects. It induces the synthesis of acute phase reactants by the liver, stimulates neutrophil production in the bone marrow, and promotes the differentiation of IL-17–producing helper T cells. IL-6 is synthesized by mononuclear phagocytes, DCs, vascular endothelial cells, fibroblasts, and other cells in response to PAMPs and in response to IL-1 and TNF. IL-6 is a homodimer that belongs to the type I cytokine family (see Chapter 7). The receptor for IL-6 consists of a cytokine-binding polypeptide chain and a signal-transducing subunit (called gp130) that is also the signaling component of receptors for other cytokines. The IL-6 receptor engages a signaling pathway that activates the transcription factor STAT3 (see Chapter 7). IL-6 is a major contributor to inflammation in several human inflammatory diseases, including rheumatoid arthritis, and antibodies specific for the IL-6 receptor are used to treat some forms of arthritis. Some lymphoproliferative disorders such as Castleman's disease are caused by human herpesvirus-8 (HHV-8), a virus that encodes a homolog of IL-6, and IL-6 blockade has been used to treat these diseases.

Other Cytokines Produced During Innate Immune Responses

In addition to TNF, IL-1, and IL-6, DCs and macrophages activated by PAMPs and DAMPs produce other cytokines that have important roles in innate immune responses (see Table 4.5). We will discuss the main features of some of these cytokines and their roles in innate immunity in this section; interferons and inhibitory cytokines are discussed later in the chapter.

IL-12 is secreted by DCs and macrophages and stimulates IFN-γ production by ILC1s, NK cells, and T cells; enhances NK cell and CTL-mediated cytotoxicity; and promotes differentiation of Th1 cells. IL-12 exists as a disulfide-linked heterodimer of 35-kD (p35) and 40-kD (p40) subunits. The p35 subunit is a member of the type I cytokine family, and the p40 subunit is also a component of the cytokine IL-23, which is involved in the differentiation of Th17 cells. Therefore, antibodies specific for p40 block both IL-12 and IL-23 and thus inhibit the IL-12–dependent development of Th1 cells and the IL-23–dependent development of Th17 cells. These antibodies are approved for the treatment of inflammatory diseases such as inflammatory bowel disease and psoriasis, which are caused by Th1 and/or Th17 cytokines.

The principal sources of IL-12 are activated DCs and macrophages. Many cells appear to synthesize the p35 subunit, but macrophages and DCs are the main cell types that produce the p40 component and therefore the biologically active cytokine. During innate immune reactions to microbes, IL-12 is produced in response to TLR and other pattern recognition receptor signaling induced by many microbial stimuli, including bacterial LPS or lipoteichoic acid and virus infections. IFN-γ produced by NK cells or T cells also stimulates IL-12 production, contributing to a positive feedback loop.

The receptor for IL-12 is a heterodimer composed of β1 and β2 subunits, both of which are members of the type I cytokine receptor family. Both chains are required for high-affinity binding of IL-12 and for signaling, which activates the transcription factor STAT4. Expression of the β2 chain of the IL-12 receptor is itself enhanced by IFN-γ, whose production is stimulated by IL-12. This is an example of a positive amplification loop in immune responses. Studies with gene knockout mice and the phenotype of rare patients with mutations in the IL-12 receptor support the conclusion that IL-12 is important for IFN-γ production by NK cells and T cells and for host resistance to intracellular bacteria and some viruses. For example, patients with mutations in the IL-12 receptor β1 subunit have been described, and they are highly susceptible to infections with intracellular bacteria, notably *Salmonella* and atypical mycobacteria. IL-12 secreted by DCs during antigen presentation to naive $CD4^+$ T cells promotes the differentiation of these cells into the Th1 subset of helper T cells, which are important for defense against intracellular infections (see Chapter 10). This is a key way in which innate immunity shapes adaptive immune responses.

IL-18 enhances the functions of NK cells, similar to IL-12. Recall that the production of IL-18, like that of IL-1, is dependent on the inflammasome. Also like

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IL-1, IL-18 binds to a receptor that signals through a TIR domain.

IL-15 stimulates growth and functions of ILC1s, NK cells, and T cells. IL-15 is structurally homologous to the T cell growth factor IL-2, and the heterotrimeric IL-15 receptor shares two subunits with the IL-2 receptor. An interesting feature of IL-15 is that it can be expressed on the cell surface bound to the α chain of its receptor and in this form can be presented to and stimulate nearby cells that express a receptor composed of the other two chains (β and γ). IL-15 presented this way by DCs to NK cells in lymph nodes activates signaling pathways that promote NK cell IFN-γ production. IL-15 also serves as a survival factor for NK and memory $CD8^+$ T cells.

IL-25, thymic stromal lymphopoietin (TSLP), and IL-33 are structurally unrelated cytokines produced by epithelial barrier cells, as well as other cell types, which stimulate ILC2s, Th2 cells, and mast cells to produce IL-4, IL-5, and IL-13. The latter cytokines are important for defense against helminths, but also contribute to allergic disease (see Chapter 20). IL-33 is constitutively expressed by barrier epithelial cells, and stored in their nuclei. It is often called an alarmin because it is rapidly released from damaged epithelial cells and then stimulates innate and adaptive responses.

In addition to the cytokines discussed here, other cytokines play important roles in both innate and adaptive immune responses, including IL-5, IL-17, and IFN-γ. These cytokines will be discussed in detail in Chapter 10, when we consider helper T cell subsets that produce them.

Recruitment of Leukocytes to Sites of Infection

Recruitment of large numbers of neutrophils, followed by monocytes, from blood into tissues typically occurs as part of the acute inflammatory response to infections and tissue injury. The cytokines TNF, IL-1 and IL-6, and chemokines, all of which are secreted at the sites of infection or tissue injury, have multiple effects on vascular endothelial cells, leukocytes, and bone marrow, which together increase the local delivery of cells that can fight infections and repair tissues (Fig. 4.15; see also Fig. 3.3). Leukocyte recruitment was described in Chapter 3 and will be only briefly considered here.

TNF and IL-1 induce postcapillary venule endothelial cells to express E-selectin and to increase their expression of ligands for leukocyte integrins. These changes in endothelial adhesion molecule expression are the result of

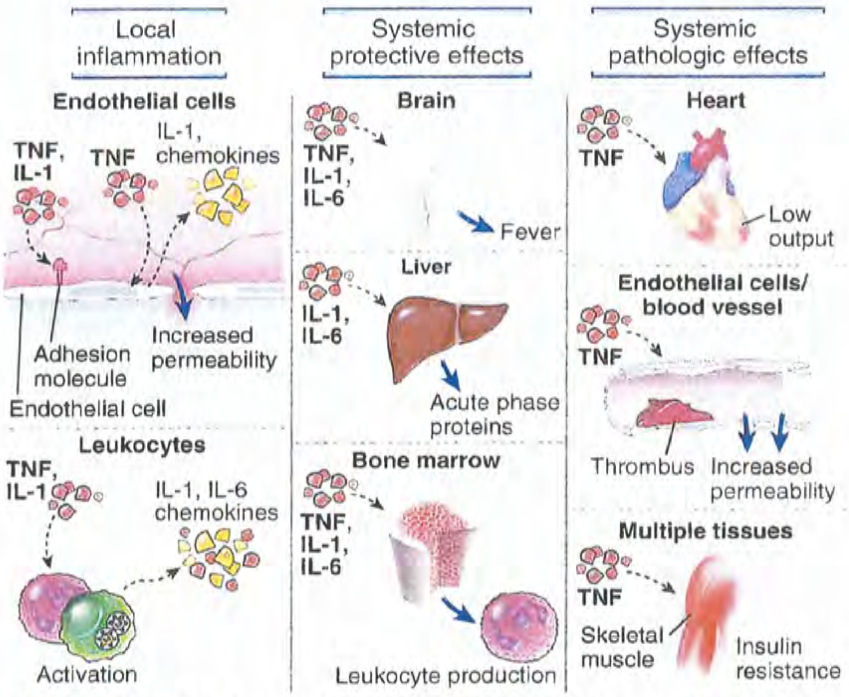


FIGURE 4.15 Local and systemic actions of cytokines in inflammation. TNF, IL-1, and IL-6 have multiple local and systemic inflammatory effects. TNF and IL-1 act on leukocytes and endothelium to induce acute inflammation, and both cytokines induce the expression of IL-6 from leukocytes and other cell types. TNF, IL-1, and IL-6 mediate protective systemic effects of inflammation, including induction of fever, acute-phase protein synthesis by the liver, and increased production of leukocytes by the bone marrow. Systemic TNF can cause the pathologic abnormalities that lead to septic shock, including decreased cardiac function, thrombosis, capillary leak, and metabolic abnormalities due to insulin resistance.

TNF and IL-1 activation of transcription factors, including NF-κB.

TNF and IL-1 also stimulate various cells to secrete chemokines, such as CXCL8 and CCL2, which bind to receptors on neutrophils and monocytes, respectively. As discussed in Chapter 3, these chemokines increase the affinity of leukocyte integrins for their ligands and stimulate directional movement of leukocytes. The result of increased selectin, integrin, and chemokine expression is an increase in neutrophil and monocyte adhesion to endothelial cells and transmigration through the vessel wall. The leukocytes accumulate in the tissues, forming an inflammatory infiltrate. The actions of TNF on endothelium and leukocytes are critical for local inflammatory responses to microbes. If inadequate quantities of TNF are present (e.g., in patients treated with drugs that block TNF or in TNF gene knockout mice), a consequence may be failure to contain infections.

In addition, TNF, IL-1, and IL-6 produced at inflammatory sites may enter the blood and be delivered to the bone marrow, where they enhance the production of neutrophils from bone marrow progenitors, usually acting in concert with colony-stimulating factors. In this way, these cytokines increase the supply of cells that can be recruited to the sites of infection and replace leukocytes that are consumed during inflammatory reactions.

Ingestion and Killing of Microbes by Activated Phagocytes

Neutrophils and macrophages that are recruited into sites of infections ingest microbes into vesicles by the process of phagocytosis and destroy these microbes (Fig. 4.16). Phagocytosis is an active, energy-dependent process of engulfment of large particles (>0.5 μm in diameter) into vesicles. Phagocytic vesicles fuse with lysosomes, where the ingested particles are destroyed. In this way, the mechanisms of killing, which could potentially injure the phagocyte, are isolated from the rest of the cell.

Neutrophils and macrophages express receptors that specifically recognize microbes, and binding of microbes to these receptors is the first step in phagocytosis. Some of these receptors are pattern recognition receptors, including C-type lectins and scavenger receptors, which we discussed earlier. Pattern recognition receptors can contribute to phagocytosis only of organisms that express particular molecular patterns, such as mannose for the mannose receptor. Phagocytes also have high-affinity receptors for certain opsonins, including antibody molecules, complement proteins, and plasma lectins; these receptors are critical for phagocytosis of many different microbes that are coated with the opsonins. Coating microbes with antibodies is one of the most efficient systems for opsonization. Phagocytes express a high-affinity Fc receptor called FcγRI, specific for one type of antibody called IgG (see Chapters 5 and 13). Thus, if an individual responds to an infection by making IgG antibodies against microbial antigens, the IgG molecules bind to these antigens, the Fc ends of the bound antibodies can interact with FcγRI on phagocytes, and the end result is efficient phagocytosis of the microbes. Antibody-dependent phagocytosis illustrates a link between innate and adaptive immunity—antibodies are a product of the adaptive immune system (B lymphocytes) that engages innate immune system effector cells (phagocytes) to perform their protective functions.

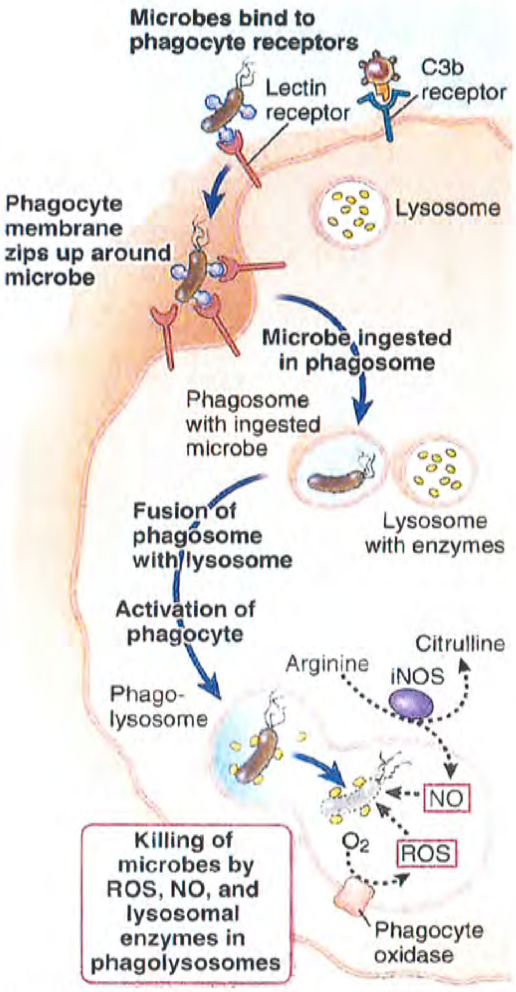


FIGURE 4.16 Phagocytosis and intracellular destruction of microbes. Microbes may be ingested by different membrane receptors of phagocytes; some directly bind microbes, and others bind opsonized microbes. (Note that the Mac-1 integrin binds microbes opsonized with complement proteins, not shown.) The microbes are internalized into phagosomes, which fuse with lysosomes to form phagolysosomes, where the microbes are killed by reactive oxygen and nitrogen species and proteolytic enzymes. *iNOS*, inducible nitric oxide synthase; *NO*, nitric oxide; *ROS*, reactive oxygen species.

Once a microbe or particle binds to receptors on a phagocyte, the plasma membrane in the region of the receptors begins to invaginate and extends a cup-shaped projection around the microbe. When the protruding membrane cup extends beyond the diameter of the particle, the top of the cup closes over and pinches off the interior of the cup to form an inside-out intracellular

vesicle (see Fig. 4.16). This vesicle, called a phagosome, contains the ingested foreign particle, and it breaks away from the plasma membrane. The cell surface receptors also deliver activating signals that stimulate the microbicidal activities of phagocytes. Phagocytosed microbes are destroyed, as described next. At the same time, peptides are generated from microbial proteins and presented to T lymphocytes to initiate adaptive immune responses (see Chapter 6).

Activated neutrophils and macrophages kill phagocytosed microbes by the action of microbicidal molecules in phagolysosomes (see Fig. 4.16). Signals from various receptors, including pattern recognition receptors (such as TLRs), opsonin receptors (such as Fc and C3 receptors), receptors for cytokines (mainly IFN-γ), and CD40, function cooperatively to activate phagocytes to kill ingested microbes. Fusion of phagocytic vacuoles (phagosomes) with lysosomes results in the formation of phagolysosomes, where most of the microbicidal mechanisms are concentrated. Three classes of microbicidal molecules are known to be the most important.

- ***Reactive oxygen species.*** Activated neutrophils and, to a lesser extent, macrophages convert molecular oxygen into ROS, which are highly reactive oxidizing agents with free radicals that destroy microbes (and other cells). The primary free radical–generating system is the phagocyte oxidase system. Phagocyte oxidase is a multi-subunit enzyme that is assembled in activated phagocytes, mainly in the phagolysosomal membrane. Phagocyte oxidase is activated by many stimuli, including IFN-γ and signals from TLRs. The function of this enzyme is to reduce molecular oxygen into ROS such as superoxide radicals, with the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) acting as a cofactor. Superoxide is enzymatically dismutated into hydrogen peroxide, which is used by the enzyme myeloperoxidase to convert normally unreactive halide ions into reactive hypohalous acids that are toxic for bacteria. The process by which ROS are produced is called the **respiratory burst**, because it requires oxygen consumption (cellular respiration). A disease called **chronic granulomatous disease** is caused by an inherited deficiency of one of the components of phagocyte oxidase; this deficiency compromises the capacity of phagocytes to kill certain species of bacteria (see Chapter 21).
- ***Nitric oxide.*** Macrophages produce reactive nitrogen species, mainly nitric oxide (NO), by the action of an enzyme called inducible nitric oxide synthase (iNOS). iNOS is a cytosolic enzyme that is absent in resting macrophages but can be induced in response to microbial products that activate TLRs, especially in combination with IFN-γ. iNOS catalyzes the conversion of arginine to citrulline, and freely diffusible nitric oxide gas is released. Within phagolysosomes, nitric oxide may combine with hydrogen peroxide or superoxide, generated by phagocyte oxidase, to produce highly reactive peroxynitrite radicals that can kill microbes. The cooperative and redundant function of ROS and nitric oxide is demonstrated by the finding that knockout mice lacking both iNOS and phagocyte oxidase are more susceptible to bacterial infections than single phagocyte oxidase or iNOS knockout animals.
- ***Proteolytic enzymes.*** Activated neutrophils and macrophages produce several proteolytic enzymes in the phagolysosomes that function to destroy microbes. One of the important enzymes in neutrophils is elastase, a broad-spectrum serine protease known to be required for killing many types of bacteria. Another important enzyme is cathepsin G. Mouse gene knockout studies have confirmed the essential requirement for these enzymes in phagocyte killing of bacteria.

Neutrophils also kill microbes by extruding their DNA and granule contents, which form extracellular threads on which bacteria and fungi are trapped and killed. The extruded chromatin contents, which are called **neutrophil extracellular traps** (NETs), are composed of strands of DNA and histones to which high concentrations of antimicrobial granule contents are bound, including lysozyme, elastase, and defensins. NET formation requires citrullination of histones by an enzyme called peptidylarginine deiminase (PAD4), as well as neutrophil serine protease elastase, myeloperoxidase, and phagocyte oxidase. The extrusion of nuclear contents during NET formation leads to neutrophil cell death, referred to as NETosis. The importance of NETs in innate protection against infections remains unclear, but growing evidence indicates that excessive NET formation contributes to autoimmune and other inflammatory diseases.

Other Functions of Activated Macrophages

In addition to killing phagocytosed microbes, macrophages serve many other functions in defense against infections (Fig. 4.17). Several of these functions are mediated by the cytokines the macrophages produce. We have already described how TNF, IL-1, and chemokines made by phagocytes enhance the inflammatory reactions to microbes and bring in more leukocytes and plasma proteins. Some activated macrophages also produce growth factors for fibroblasts and endothelial cells that participate in the repair of tissues after infections and injury. The role of macrophages in cell-mediated immunity is described in Chapter 10.

Macrophages may be activated in different ways, which favor microbicidal and proinflammatory functions or, in contrast, reparative and antiinflammatory functions. These different types of macrophage activation, called classical and alternative, respectively, are discussed in more detail in Chapter 10.

Systemic and Pathologic Consequences of Inflammation

TNF, IL-1, and IL-6 produced during the innate immune response to infection or tissue damage have systemic effects that contribute to host defense and are responsible for many of the clinical manifestations of infection and inflammatory disease (see Fig. 4.15).

- ***TNF and IL-1 act on the hypothalamus to induce an increase in body temperature (fever).*** These cytokines

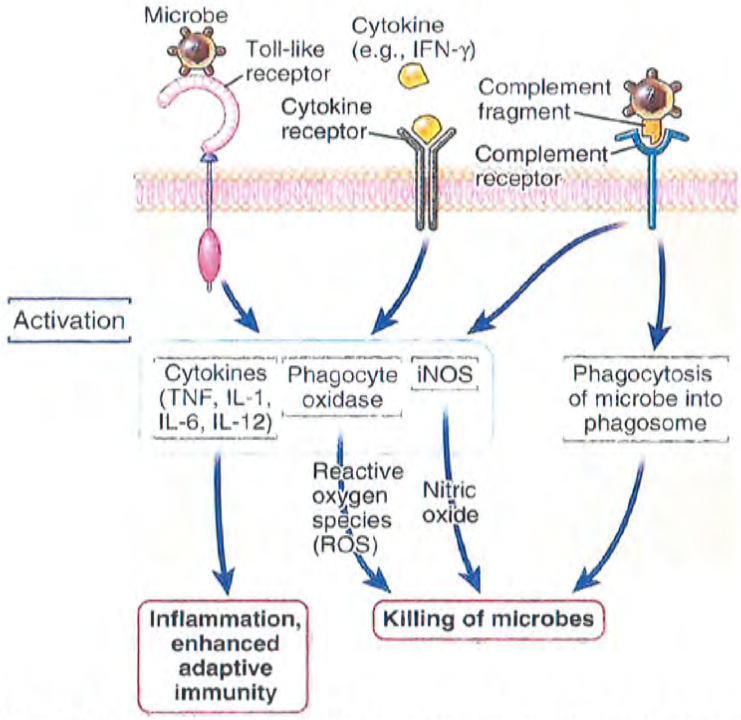


FIGURE 4.17 Functions of macrophages. Macrophages are activated by microbial products such as LPS and by NK cell–derived IFN-γ. The process of macrophage activation leads to the activation of transcription factors, the transcription of various genes, and the synthesis of proteins that mediate the functions of these cells. In adaptive cell-mediated immunity, macrophages are activated by stimuli from T lymphocytes (CD40 ligand and IFN-γ) and respond in essentially the same way (see Fig. 10.7). Macrophages also may be activated by other signals to promote tissue repair and fibrosis (not shown).

are therefore called endogenous pyrogens (i.e., host-derived fever-causing agents, to distinguish them from LPS, which was considered an exogenous [microbe-derived] pyrogen). This distinction is mainly of historical significance because we now know that even LPS induces fever by stimulating production of the cytokines TNF and IL-1, which in turn increase synthesis of prostaglandins in hypothalamic cells. Prostaglandin synthesis inhibitors, such as aspirin, reduce fever by blocking this action of the cytokines. The role of fever in host defense is not well understood but may relate to enhanced metabolic functions of immune cells, impaired metabolic functions of microbes, and changes in the behavior of the febrile host that reduces risk of worsening infections and injury.

- IL-1 and IL-6 induce hepatocytes to produce acute-phase reactants, including CRP, SAP, and fibrinogen, which are secreted into the blood. Elevated plasma levels of these proteins are commonly used clinically as signs of infection or other inflammatory processes. The pentraxins CRP and SAP play protective roles in infections, as we discussed earlier in the chapter, and fibrinogen, the precursor of fibrin, contributes to hemostasis and tissue repair.

In severe infections, TNF may be produced in large amounts and causes systemic clinical and pathologic abnormalities. If the stimulus for cytokine production is sufficiently strong, the quantity of TNF may be so large that it enters the blood stream and acts at distant sites (see Fig. 4.15). The principal systemic actions of TNF are as follows:

- TNF inhibits myocardial contractility and vascular smooth muscle tone, resulting in a marked decrease in blood pressure, or shock.
- TNF causes intravascular thrombosis, mainly as a result of impairment of the normal anticoagulant properties of the endothelium. TNF stimulates endothelial cell expression of tissue factor, a potent activator of coagulation, and inhibits expression of thrombomodulin, an inhibitor of coagulation. The endothelial alterations are exacerbated by activation of neutrophils, leading to vascular plugging by these cells.
- Prolonged production of TNF causes wasting of muscle and fat cells, called cachexia. This wasting results from TNF-induced appetite suppression and reduced synthesis of lipoprotein lipase, an enzyme needed to release fatty acids from circulating lipoproteins so that they can be used by the tissues.

A systemic complication of severe infection, usually bacterial, is a syndrome called **sepsis**, clinically characterized by fever, fast heart and respiratory rates, metabolic abnormalities, and mental disturbances. The infection may involve microbes in the blood, but this is not documented in most cases. Bacterial sepsis is most often initiated by LPS (also called endotoxin) released from gram-negative bacteria or lipoteichoic acid released from gram-positive bacteria, which may enter the blood stream. TLR signaling is then induced in cells in many organs by LPS or lipoteichoic acid, leading to the production of TNF and other cytokines, including IL-12, IFN-γ, and IL-1. In the most severe form of sepsis, called **septic shock**, there is vascular collapse and disseminated intravascular coagulation, caused by the effects of high doses of TNF discussed earlier. The concentration of serum TNF may be predictive of the outcome of severe sepsis. Septic shock can be reproduced in experimental animals by administration of LPS, lipoteichoic acid, or TNF. Antagonists of TNF can prevent mortality in the experimental models, but clinical trials with anti-TNF antibodies or with soluble TNF receptors have not shown benefit in patients with sepsis. The cause of this therapeutic failure is not known, but it may be because other cytokines elicit the same responses as TNF.

A syndrome similar to septic shock may occur as a complication of noninfectious disorders, such as severe burns, trauma, pancreatitis, and other serious conditions. This has been called the systemic inflammatory response syndrome (SIRS).

Acute inflammation may cause tissue injury because the effector mechanisms that phagocytes use to kill microbes are also toxic to host tissues. The proteolytic enzymes and reactive oxygen species produced by phagocytes that accumulate at a site of infection can injure host cells and degrade extracellular matrix if they are generated in large quantities, especially if the microbes resist being killed and continue to stimulate the innate immune responses. In fact, at least part of the pathology associated with infections is due to the inflammatory responses and not the direct toxic effects of the microbes. Acute inflammation also causes tissue damage in the setting of autoimmune diseases, in which case neutrophils and macrophages accumulate and become activated as a result of stimulation of the adaptive immune system by self antigens (see Chapter 15). As in inflammation induced by infections, TNF, IL-1, IL-6, and IL-12 are the key inducers of inflammation in autoimmune diseases. Antagonists against all of these cytokines or their receptors are in clinical use to reduce inflammation in patients with inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease, and psoriasis.

THE ANTIVIRAL RESPONSE

The major way by which the innate immune system blocks viral infections is to induce the expression of type I interferons, whose most important action is to inhibit viral replication. Earlier in the chapter, we discussed how several pattern recognition receptors, including some TLRs, NLRs, RLRs, and CDSs, generate signals that stimulate IFN-α and IFN-β gene expression in many different cell types. These type I interferons are secreted from the cells and act on other cells to prevent the spread of viral infection. In this section, we will describe the major properties of type I interferons and the antiviral actions of these cytokines.

Type I interferons are a large family of structurally related cytokines that mediate the early innate immune response to viral infections. The term interferon derives from the ability of these cytokines to interfere with viral infection. There are many type I interferons, which are structurally homologous and are encoded by genes in a single cluster on chromosome 9. The most important type I interferons in viral defense are IFN-α (which actually includes 13 different closely related proteins) and IFN-β, which is a single protein. Plasmacytoid DCs are the major sources of IFN-α, but it also may be produced by mononuclear phagocytes. IFN-β is produced by many cell types in response to viral infection. The most potent stimuli for type I interferon synthesis are viral nucleic acids. Recall that RIG-like receptors and DNA sensors in the cytosol, and TLRs 3, 7, 8, and 9 in endosomal vesicles, recognize viral nucleic acids and initiate signaling pathways that activate the IRF family of transcription factors, which induce type I interferon gene expression (see Fig. 4.3).

The receptor for type I interferons, which binds both IFN-α and IFN-β, is a heterodimer of two structurally related polypeptides, IFNAR1 and IFNAR2, which are expressed on all nucleated cells. This receptor signals to activate STAT1, STAT2, and IRF9 transcription factors, which induce expression of several different genes whose protein products contribute to antiviral defense in various ways:

- ***Type I interferons, signaling through the type I interferon receptor, activate transcription of several genes that confer on the cells a resistance to viral infection called an antiviral state*** (Fig. 4.18). Type I interferon–induced genes include double-stranded RNA–activated serine/threonine protein kinase (PKR), which blocks viral transcriptional and translational events, and 2′,5′-oligoadenylate synthetase and RNase L, which promote viral RNA degradation. The antiviral action of type I interferon is primarily a paracrine action in that a virally infected cell secretes interferon to act on and protect neighboring cells that are not yet infected. The effects of type I interferons are not specific to viral gene expression, and part of the ability of these cytokines to block the spread of infection is due to their toxicity to host cells that are near infected cells. Interferon secreted by an infected cell may also act in an autocrine fashion to inhibit viral replication in that cell.
- Type I interferons cause sequestration of lymphocytes in lymph nodes, thus maximizing the opportunity for encounter with microbial antigens. The mechanism for this effect of type I interferons is the induction of a molecule on the lymphocytes called CD69, which forms a complex with and reduces surface expression of the sphingosine 1-phosphate (S1P) receptor S1PR1. Recall from Chapter 3 that lymphocyte egress from lymphoid tissues depends on S1P binding to S1PR1.

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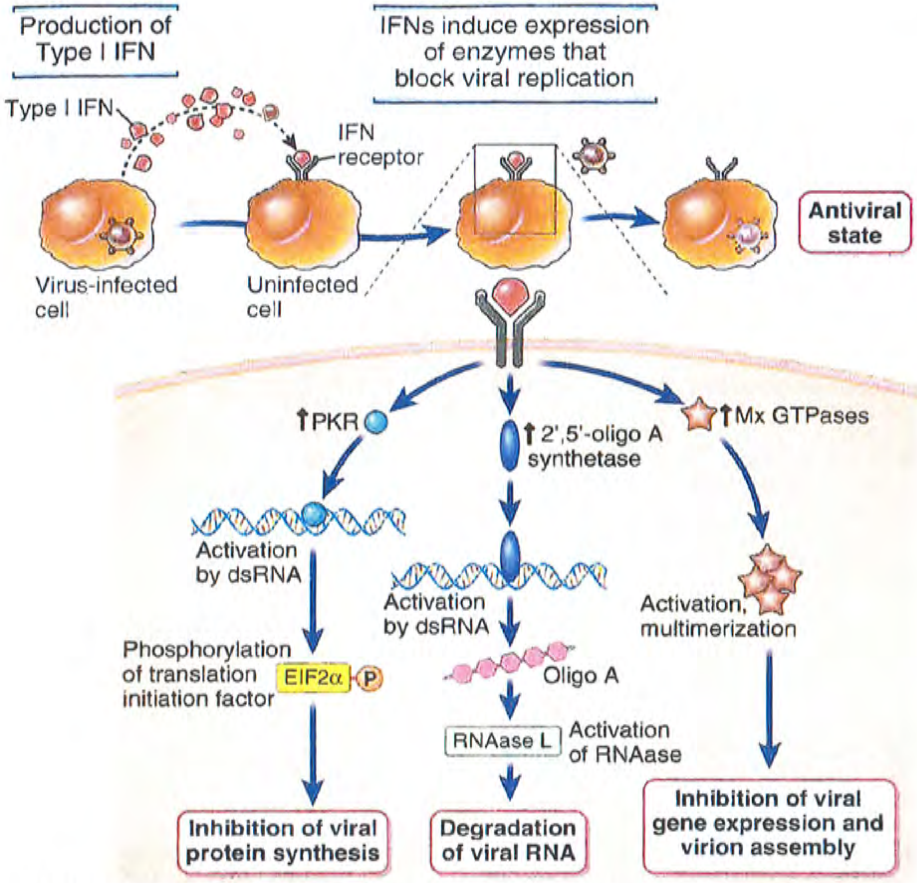


FIGURE 4.18 Biologic actions of type I interferons. Type I interferons (IFN-α, IFN-β) are produced by virus-infected cells in response to intracellular TLR signaling and other sensors of viral RNA. Type I interferons bind to receptors on neighboring uninfected cells and activate JAK-STAT signaling pathways, which induce expression of genes whose products interfere with viral replication. Type I interferons also bind to receptors on infected cells and induce expression of genes whose products enhance the cell's susceptibility to CTL-mediated killing. *PKR*, Double-stranded RNA-activated protein kinase.

Therefore, reduced S1PR1 inhibits this egress and keeps lymphocytes in lymphoid organs.

- Type I interferons increase the cytotoxicity of NK cells and $CD8^+$ CTLs and promote the differentiation of naive T cells to the Th1 subset of helper T cells. These effects of type I interferons enhance both innate and adaptive immunity against intracellular infections, including viruses and some bacteria.
- Type I interferons upregulate expression of class I MHC molecules and thereby increase the probability that virally infected cells will be recognized and killed by $CD8^+$ CTLs. Virus-specific $CD8^+$ CTLs recognize peptides derived from viral proteins bound to class I MHC molecules on the surface of infected cells. (We will discuss the details of T cell recognition of peptide-MHC and CTL killing of cells in Chapters 6 and 11.) Therefore, by increasing the amount of class I MHC synthesized by a virally infected cell, type I interferons will increase the number of viral peptide–class I MHC complexes on the cell surface that the CTLs can see and respond to. The end result is increased killing of virus-infected cells and eradication of viral infections.

Thus, the principal activities of type I interferon work in concert to combat viral infections. Knockout mice lacking the receptor for type I interferons are susceptible to viral infections. IFN-α is in clinical use as an antiviral agent in certain forms of viral hepatitis. IFN-α is also used for the treatment of some tumors, perhaps because it boosts CTL activity or inhibits cell proliferation. IFN-β is used as a therapy for multiple sclerosis, but the mechanism of its beneficial effect in this disease is not known.

Protection against viruses is due, in part, to the activation of intrinsic apoptotic death pathways in infected cells and enhanced sensitivity to extrinsic inducers of apoptosis. Viral proteins synthesized in infected cells may be misfolded, and their accumulation triggers an unfolded protein response that may culminate in apoptosis of the infected cells if the misfolded protein accumulation cannot be corrected. In addition, virally infected cells are hypersensitive to TNF-induced apoptosis. Abundant TNF is made by plasmacytoid DCs and macrophages in response to viral infections, in addition to type I interferons. The type I TNF receptor engages both proinflammatory and proapoptosis death pathways. The dominant pathway that is activated upon TNF binding depends on the state of protein synthesis in the responding cells, and viral infection can shift this balance toward apoptosis.

STIMULATION OF ADAPTIVE IMMUNITY

The innate immune response provides signals that function in concert with antigen to stimulate the proliferation and differentiation of antigen-specific T and B lymphocytes. As the innate immune response is providing the initial defense against microbes, it also sets in motion the adaptive immune response. The activation of lymphocytes requires two distinct signals, the first being antigen and the second being molecules that are produced during innate immune responses to microbes or injured cells (Fig. 4.19). This idea is called the **two-signal hypothesis** for lymphocyte activation. The requirement for antigen (so-called signal 1) ensures that the ensuing immune response is specific. The requirement for additional stimuli triggered by innate immune reactions to microbes (signal 2) ensures that adaptive immune responses are induced when there is a dangerous infection and not when lymphocytes recognize harmless antigens, including self antigens. The molecules produced during innate immune reactions that function as second signals for lymphocyte activation include costimulators (for T cells), cytokines (for both T and B cells), and complement breakdown products (for B cells). We will return to the nature of second signals for lymphocyte activation in Chapters 9 and 12.

The second signals generated during innate immune responses to different microbes not only enhance the magnitude of the subsequent adaptive immune response but also influence the nature of the adaptive response. A major function of T cell–mediated immunity is to activate macrophages to kill intracellular microbes and to induce robust inflammatory responses so that a sufficiently large army of phagocytes is called into a site of infection. When dendritic cells or phagocytes encounter microbes, TLRs and other pattern recognition receptors stimulate cytokine secretion and T cell–mediated immune responses, which in turn activate and recruit phagocytes to kill microbes. These processes are mediated by cytokines. Thus, the innate immune response to microbes in macrophages stimulates the adaptive T cell response that is effective against such microbes.

By contrast, many extracellular microbes that enter the blood activate the alternative complement pathway,

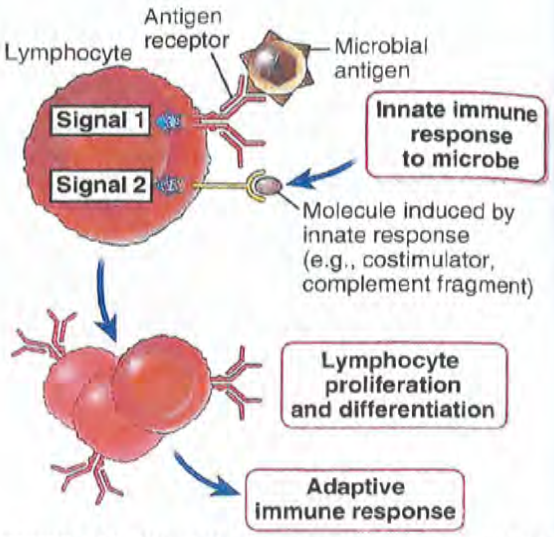


FIGURE 4.19 Stimulation of adaptive immunity by innate immune responses. Antigen recognition by lymphocytes provides signal 1 for the activation of the lymphocytes, and molecules induced on host cells during innate immune responses to microbes provide signal 2. In this illustration, the lymphocytes are B cells, but the same principles apply to T lymphocytes. The nature of second signals differs for B and T cells and is described in later chapters.

and some of the proteolytic products of complement activation enhance the production of antibodies by B lymphocytes (see Chapter 12). These antibodies opsonize the microbes and thereby promote their phagocytosis by neutrophils and macrophages, or kill the microbes by complement-dependent mechanisms. Thus, blood-borne microbes induce an innate response (complement activation) that triggers the adaptive response that is designed to eliminate these extracellular pathogens.

Cytokines produced by cells during innate immune responses to microbes stimulate the proliferation and differentiation of lymphocytes in adaptive immune responses. Examples of cytokines secreted by PAMP-stimulated cells acting on B cells, $CD4^+$ T cells, and $CD8^+$ T cells are given here. We have mentioned these cytokines previously and will discuss the details of their roles in lymphocyte responses in later chapters.

- IL-12 stimulates the differentiation of naive $CD4^+$ T cells to the Th1 subset of effector cells (see Chapter 10).
- IL-1, IL-6, and IL-23 stimulate the differentiation of naive $CD4^+$ T cells to the Th17 subset of effector cells (see Chapter 10).
- IL-25, IL-33, and TSLP stimulate the differentiation of naive $CD4^+$ T cells to the Th2 subset of effector cells.
- IL-15 promotes the survival of memory $CD8^+$ T cells.
- IL-6 promotes the production of antibodies by activated B cells (see Chapter 12).

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Adjuvants are substances that need to be administered together with purified protein antigens to elicit maximal T cell–dependent immune responses (see Chapter 6). They work by stimulating innate immune responses at the site of antigen exposure. Adjuvants are useful in experimental immunology and in clinical vaccines. Many adjuvants in experimental use are microbial products that engage TLRs, such as killed mycobacteria and LPS. The only routinely used adjuvant in human vaccines is alum, which is composed of either aluminum hydroxide or aluminum phosphate, and is a stimulus for inflammasome activation. Among their important effects, adjuvants activate DCs to express more major histocompatibility molecules that are part of the antigen (signal 1) that T cells recognize, increase the expression of costimulators (signal 2) and cytokines needed for T cell activation, and stimulate migration of the DCs to lymph nodes where T cells are located.

MECHANISMS THAT LIMIT INNATE IMMUNE RESPONSES

The magnitude and duration of innate immune responses are regulated by a variety of inhibitory mechanisms that limit potential damage to tissues. Whereas the inflammatory response is critically important for protection against microbes, it has the potential to cause tissue injury and disease. Several mechanisms have evolved to provide a brake on inflammation, and these mechanisms come into play at the same time as or shortly after the initiation of inflammation. Furthermore, the stimuli for the initiation of many of these control mechanisms include the same PAMPs and DAMPs that induce inflammation. We will describe a selected group of these regulatory mechanisms.

IL-10 is a cytokine that is produced by and inhibits activation of macrophages and DCs. IL-10 inhibits the production of various inflammatory cytokines by activated macrophages and DCs, including IL-1, TNF, and IL-12. Because it is both produced by macrophages and DCs and inhibits the functions of these cells, IL-10 is an excellent example of a negative feedback regulator. Alternatively activated macrophages make more IL-10 than classically activated macrophages. IL-10 is produced by some nonlymphoid cell types (e.g., keratinocytes). IL-10 is also produced by regulatory T cells, and we will discuss the details of IL-10 in this context in Chapter 15. Loss-of-function mutations in the IL-10 receptor result in severe colitis developing in infancy.

Mononuclear phagocytes produce a natural antagonist of IL-1 that is structurally homologous to the cytokine and binds to the same receptors but is biologically inactive, so that it functions as a competitive inhibitor of IL-1. It is therefore called **IL-1 receptor antagonist (IL-1RA)**. Synthesis of IL-1RA is induced by many of the same stimuli that induce IL-1 production, and some studies in IL-1RA–deficient mice suggest that this inhibitory cytokine is required to prevent inflammatory diseases of joints and other tissues. Recombinant IL-1RA has been developed as a drug for the treatment of rheumatoid arthritis and familial fever syndromes in which IL-1 production is dysregulated. Regulation of IL-1–mediated inflammation may also occur by expression of the type II receptor, which binds IL-1 but does not transduce an activating signal. The major function of this receptor may be to act as a "decoy" that competitively inhibits IL-1 binding to the type I signaling receptor.

Secretion of inflammatory cytokines from a variety of cell types appears to be regulated by the products of autophagy genes. Targeted mutations in different autophagy genes result in enhanced secretion of IL-1 and IL-18 by various cell types and the development of inflammatory bowel disease. The mechanisms by which autophagy proteins impair cytokine synthesis are not well understood; they may regulate inflammasome activation or production of reactive oxygen species. The linkage of polymorphisms in a human autophagy gene with inflammatory bowel disease may be because these proteins affect inflammation or epithelial integrity.

There are numerous negative regulatory signaling pathways that block the activating signals generated by pattern recognition receptors and inflammatory cytokines. Suppressors of cytokine signaling (SOCS) proteins are inhibitors of JAK-STAT signaling pathways linked to cytokine receptors. TLR signaling in macrophages and DCs induces the expression of SOCS proteins, which limit responses of these cells to exogenous cytokines such as type I interferons. Proinflammatory responses of cells to TLR signaling are negatively regulated by SHP-1, an intracellular protein phosphatase that negatively regulates numerous tyrosine kinase–dependent signaling pathways in lymphocytes. There are many other examples of kinases and phosphatases that inhibit TLR, NLR, and RLR signaling, and small inhibitory RNAs that inhibit production of many of the mediators of innate immunity.

SUMMARY

- The innate immune system provides the first line of host defense against microbes, before adaptive immune responses have had sufficient time to develop. The mechanisms of innate immunity exist before exposure to microbes. The cellular components of the innate immune system include epithelial barriers and leukocytes (neutrophils, macrophages, NK cells, lymphocytes with invariant antigen receptors, and mast cells).
- The innate immune system uses cell-associated pattern recognition receptors, present on plasma and endosomal membranes and in the cytosol, to recognize structures called PAMPs, which are shared by microbes, are not present on mammalian cells, and are often essential for survival of the microbes, thus limiting the capacity of microbes to evade detection by mutating or losing expression of these molecules. In addition, these receptors recognize molecules made by the host but whose expression or location indicates cellular damage; these are called DAMPs.

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- TLRs, present on the cell surface and in endosomes, are the most important family of pattern recognition receptors, recognizing a wide variety of ligands, including bacterial cell wall components and microbial nucleic acids. Cytosolic pattern recognition receptors exist that recognize microbial molecules. These receptors include the RLRs, which recognize viral RNA, CDSs which recognize microbial DNA, and NLRs, which recognize bacterial cell wall constituents and also serve as recognition components of many inflammasomes.
- Pattern recognition receptors, including TLRs, NLRs, and RLRs, signal to activate the transcription factors NF-κB and AP-1, which stimulate expression of cytokines, costimulators, and other molecules involved in inflammation, and the IRF transcription factors, which stimulate expression of the antiviral type I interferon genes.
- The inflammasome, a specialized caspase-1 containing enzyme complex that forms in response to a wide variety of PAMPs and DAMPs, includes recognition structures, which are often NLR family proteins, an adaptor, and the enzyme caspase-1, the main function of which is to produce active forms of the inflammatory cytokines IL-1 and IL-18.
- Soluble pattern recognition and effector molecules are found in the plasma, including pentraxins (e.g., CRP), collectins (e.g., MBL), and ficolins. These molecules bind microbial ligands and enhance clearance by complement-dependent and complement-independent mechanisms.
- Innate lymphoid cells are cells with lymphocyte morphology and functions similar to T lymphocytes, but do not express clonally distributed T cell antigen receptors. Three helper subsets of ILCs secrete the same cytokines as Th1, Th2, and Th17 helper T cells.
- NK cells are one type of innate lymphoid cells that have cytotoxic functions and secrete FIN-γ, similar to CTLs. NK cells defend against intracellular microbes by killing infected cells and providing a source of the macrophage-activating cytokine IFN-γ. NK cell recognition of infected cells is regulated by a combination of activating and inhibitory receptors. Inhibitory receptors recognize class I MHC molecules, because of which NK cells do not kill normal host cells but do kill cells in which class I MHC expression is reduced, such as virus-infected cells.
- The complement system includes several plasma proteins that become activated in sequence by proteolytic cleavage to generate fragments of the C3 and C5 proteins, which promote inflammation, or opsonize and promote phagocytosis of microbes. Complement activation also generates membrane pores that kill some types of bacteria. The complement system is activated on microbial surfaces and not on normal host cells, because microbes lack regulatory proteins that inhibit complement. In innate immune responses, complement is activated mainly spontaneously on microbial cell surfaces and by mannose-binding lectin to initiate the alternative and lectin pathways, respectively.
- The two major effector functions of innate immunity are to induce inflammation, which involves the delivery of microbe-killing leukocytes and soluble effector molecules from blood into tissues, and to block viral infection of cells by the antiviral actions of type I interferons. Both types of effector mechanism are induced by the PAMPs and DAMPs.
- Several cytokines produced mainly by macrophages, DCs, and other innate immune cells mediate inflammation. TNF and IL-1 activate endothelial cells, stimulate chemokine production, and increase neutrophil production in the bone marrow. IL-1 and TNF both induce IL-6 production, and all three cytokines mediate systemic effects, including fever and acute-phase protein synthesis by the liver. IL-12 and IL-18 stimulate production of the macrophage-activating cytokine IFN-γ by NK cells and T cells. These cytokines function in innate immune responses to different classes of microbes, and some (IL-1, IL-6, IL-12, IL-18) modify adaptive immune responses that follow the innate immune response.
- Neutrophils and monocytes (the precursors of tissue macrophages) migrate from blood into inflammatory sites during innate immune responses because of the effects of cytokines and chemokines produced by PAMP- and DAMP-stimulated tissue cells.
- Neutrophils and macrophages phagocytose microbes and kill them by producing ROS, nitric oxide, and enzymes in phagolysosomes. Macrophages also produce cytokines that stimulate inflammation and promote tissue repair at sites of infection. Phagocytes recognize and respond to microbial products by several different types of receptors, including TLRs, C-type lectins, scavenger receptors, and *N*-formyl met-leu-phe receptors.
- Molecules produced during innate immune responses stimulate adaptive immunity and influence the nature of adaptive immune responses. DCs activated by microbes produce cytokines and costimulators that enhance T cell activation and differentiation into effector T cells. Complement fragments generated by the alternative pathway provide second signals for B cell activation and antibody production.
- Innate immune responses are regulated by negative feedback mechanisms that limit potential damage to tissues. IL-10 is a cytokine that is produced by and inhibits activation of macrophages and DCs. Inflammatory cytokine secretion is regulated by autophagy gene products. Negative signaling pathways block the activating signals generated by pattern recognition receptors and inflammatory cytokines.

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CHAPTER

5

Antibodies and Antigens

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SUMMARY, 115

Antibodies are circulating proteins that are produced in vertebrates in response to exposure to foreign structures known as antigens, and are the mediators of humoral immunity against all classes of microbes. Antibodies are extremely diverse and specific in their ability to recognize foreign molecular structures. Because these proteins were discovered as serum molecules that provided protection against diphtheria toxin, they were initially called antitoxins. When it was appreciated that similar proteins could be generated against many substances, not just microbial toxins, they were given the general name **antibodies**. The substances that stimulated production of or were recognized by antibodies were then called **antigens.** Antibodies and T cell antigen receptors (see Chapter 7) are the two classes of molecules used by the adaptive immune system to specifically recognize and respond to antigens (Table 5.1). Major histocompatibility complex (MHC) molecules also bind peptide antigens, but their specificity is very different and their function is to display the peptides to T cells, not respond to the antigens (see Chapter 6). Antibodies were the first type of antigen binding molecule to be discovered, recognize the widest range of antigenic structures, have the greatest ability to discriminate between different antigens, and bind antigens with the greatest strength. In this chapter we describe the structure and antigen-binding properties of antibodies.

Antibodies are synthesized only by cells of the B lymphocyte lineage and exist in two forms: membrane-bound antibodies on the surface of B lymphocytes function as antigen receptors, and secreted antibodies function to protect against microbes. The recognition of antigens by membrane-bound antibodies on naive B cells activates these lymphocytes and initiates a humoral immune response. The activated B cells differentiate into plasma cells that secrete antibodies of the same specificity as the antigen receptor. Secreted forms of antibodies are present in the plasma (the fluid portion of the blood), in mucosal secretions, and in the interstitial fluid of tissues. In the effector phase of humoral immunity, these secreted antibodies neutralize microbial toxins, prevent the entry and spread of pathogens, and trigger several effector mechanisms that eliminate the microbes.

The elimination of antigens often requires interaction of antibodies with other components of the immune system, including molecules such as complement proteins and cells such as phagocytes and mast cells. Antibody-mediated effector functions include neutralization of microbes or toxic microbial products; activation of the complement system; opsonization of pathogens for enhanced phagocytosis; antibody-dependent cell-mediated cytotoxicity, by which antibodies target infected cells for lysis by cells of the innate immune system; and antibody-mediated mast cell activation to expel parasitic worms. We will describe these functions of antibodies in detail in Chapter 13.

When blood or plasma removed from an individual forms a clot, antibodies remain in the residual fluid, which is called **serum**. Serum lacks coagulation factors (which are consumed during clot formation) but contains all the other proteins found in plasma. Any serum sample that contains detectable antibody molecules that bind to a particular antigen is commonly called an **antiserum**. The study of antibodies and their reactions with antigens is therefore called **serology**. The concentration of antibody molecules in serum specific for a particular antigen

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TABLE 5.1 Features of Antigen Binding by Antigen Receptors

Feature	Immunoglobulin (Ig)	T Cell Receptor (TCR)
		
Antigen-binding site	Made up of three CDRs in V_H and three CDRs in V_L domains	Made up of three CDRs in Vα and three CDRs in Vβ domains (in the most common form of TCR)
Nature of antigen that may be bound	Macromolecules (proteins, lipids, polysaccharides) and small chemicals	Peptide-MHC complexes
Nature of antigenic determinants recognized	Linear and conformational determinants of various macromolecules and chemicals	Linear determinants of peptides; only few amino acid residues of a peptide bound to an MHC molecule
Affinity of antigen binding	K_d 10^{-7}–10^{-11} M; average affinity of Igs increases during immune response	K_d 10^{-5}–10^{-7} M
On-rate and off-rate	Rapid on-rate, variable off-rate	Slow on-rate, slow off-rate

The structure and function of TCR molecules are discussed in Chapter 7.
CDR, Complementarity-determining region; K_d, dissociation constant; *MHC*, major histocompatibility complex; V_H, variable domain of heavy chain Ig; V_L, variable domain of light chain Ig.

is often estimated by determining how many serial dilutions of the serum can be made before binding to the antigen can no longer be detected. When determined by this method, the antibody concentration is called the titer (after titration). The more dilutions that are required, the higher the titer of the antibody molecules specific for a particular antigen.

A healthy 70-kg adult human produces about 2 to 3 g of antibodies every day. Almost two-thirds of this is a type of antibody called IgA, most of which is produced by activated B cells and plasma cells in the gastrointestinal tract.

ANTIBODY STRUCTURE

An understanding of the structure of antibodies has provided important insights into their function. The analysis of antibody structure also laid the foundation for elucidating the mechanisms of antigen receptor diversity that we will consider in depth in Chapter 8.

Early studies of antibody structure relied on antibodies purified from the blood of individuals immunized with various antigens. It was not possible, using this approach, to define antibody structure precisely because serum contains a mixture of different antibodies produced by many clones of B lymphocytes that may each bind to different portions (epitopes) of an antigen. These antibody mixtures are called polyclonal antibodies. A major breakthrough in obtaining antibodies whose structures could be elucidated was the discovery that patients with multiple myeloma, a monoclonal tumor of antibody-producing plasma cells, often have large amounts of biochemically identical antibody molecules (produced by the neoplastic clone) in their blood and urine. Immunologists found that these antibodies could be purified to homogeneity and analyzed. The recognition that myeloma cells make monoclonal immunoglobulins led to the development of the technology to produce monoclonal antibodies, described later in the chapter. The availability of homogeneous populations of antibodies and monoclonal antibody–producing plasma cells facilitated the detailed structural analysis of antibody molecules and molecular cloning of the genes for individual antibodies. These were important advances in our understanding of the adaptive immune system.

General Features of Antibody Structure

Plasma or serum proteins can be physically separated based on solubility characteristics into albumins and globulins, and may be more precisely separated, based on differences in charge, using a technique called electrophoresis. In electrophoretic separations of serum or plasma most antibodies are found in the third fastest migrating group of globulins, named **gamma globulins** for the third letter of the Greek alphabet. (Note that gamma globulins include all classes of antibodies, described later, not just the IgG class.) Another common name for antibody is **immunoglobulin (Ig)**, referring to the immunity-conferring portion of the globulin fraction of serum or plasma. The terms *immunoglobulin* and *antibody* are used interchangeably throughout this book.

All antibody molecules share the same basic structural characteristics but display remarkable variability in the regions that bind antigens. This variability of the antigen-binding regions accounts for the capacity of different antibodies to bind a tremendous number of

structurally diverse antigens. In every individual, there are millions of different clones of B cells, each producing antibody molecules with identical antigen-binding sites but which differ from the antigen-binding sites of antibodies produced by other clones. The effector functions and common physicochemical properties of antibodies are associated with the non-antigen-binding portions, which exhibit relatively few variations among different antibodies.

An antibody molecule has a symmetric core structure composed of two identical light chains and two identical heavy chains (Fig. 5.1). Both the light chains and heavy chains contain a series of repeating homologous structural units, each about 110 amino acid residues in

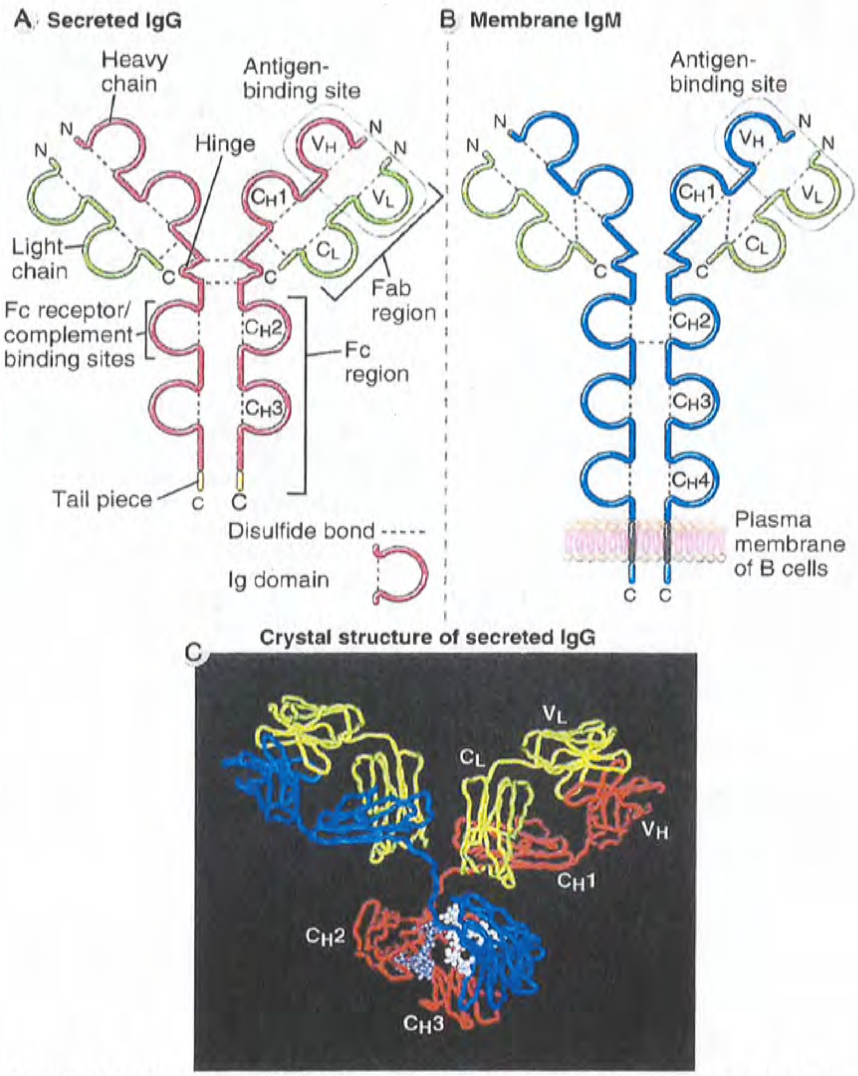


FIGURE 5.1 Structure of an antibody molecule. A, Schematic diagram of a secreted IgG molecule. The antigen-binding sites are formed by the juxtaposition of V_L and V_H domains. The heavy chain C regions end in tail pieces. The locations of complement- and Fc receptor–binding sites within the heavy chain constant regions are approximations. **B,** Schematic diagram of a membrane-bound IgM molecule on the surface of a B lymphocyte. The IgM molecule has one more C_H domain than IgG has, and the membrane form of the antibody has C-terminal transmembrane and cytoplasmic portions that anchor the molecule in the plasma membrane. **C,** Structure of a human IgG molecule as revealed by x-ray crystallography. In this ribbon diagram of a secreted IgG molecule, the identical heavy chains are colored blue and red so that they can be easily visualized, although they are identical, and the light chains are colored green; carbohydrates are shown in gray. *(Courtesy of Dr. Alex McPherson, University of California, Irvine.)*

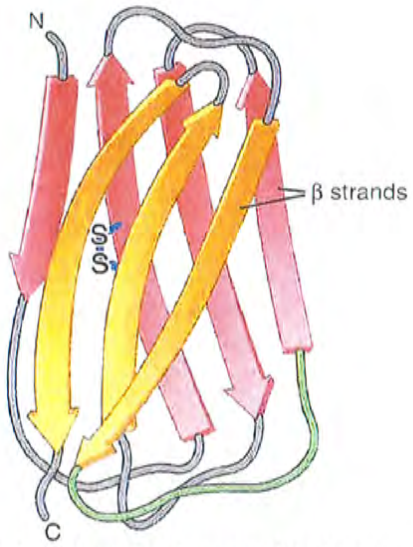


FIGURE 5.2 Structure of an Ig domain. Each domain is composed of two antiparallel arrays of β strands, colored yellow and red, to form two β-pleated sheets held together by a disulfide bond. The diagram shows an Ig constant (C) domain containing three and four β strands in the two adjacent sheets. Note that the loops connect β strands that are sometimes adjacent in the same β-pleated sheet, but the loops sometimes represent connections between the two different sheets that make up an Ig domain.

length, that fold independently in a globular motif that is called an **Ig domain**, which we introduced in Chapters 3 and 4. An Ig domain contains two layers of β-pleated sheet, each layer composed of three to five strands of antiparallel polypeptide chain (Fig. 5.2). The two layers are held together by a disulfide bridge, and adjacent strands of each β sheet are connected by short loops. It is the amino acids in some of these loops that are the most variable and critical for antigen recognition, as discussed later in the chapter.

Antibody heavy chains and light chains both consist of amino-terminal variable (V) regions that participate in antigen recognition and carboxy-terminal constant (C) regions; the C regions of the heavy chains help mediate some of the protective or effector functions of antibodies. In the heavy chains, the V region is composed of one Ig domain, and the C region is composed of three or four Ig domains. Each light chain is composed of one V region Ig domain and one C region Ig domain. Variable regions are so named because their amino acid sequences vary among antibodies made by different B cell clones. The V region of one heavy chain (V_H) and the adjoining V region of one light chain (V_L) form an antigen-binding site (see Fig. 5.1). Because the core structural unit of each antibody molecule contains two heavy chains and two light chains, every antibody molecule has at least two antigen-binding sites.

The C region Ig domains are spatially separated from the antigen-binding sites and do not participate in antigen recognition. The heavy chain C regions interact with other molecules and cells of the immune system and therefore help mediate most of the biologic functions of antibodies, sometimes called "effector" functions. In addition, heavy chains exist in two forms that differ at their carboxy-terminal ends: one form of the heavy chain anchors membrane-bound antibodies in the plasma membranes of B lymphocytes, and the other form is found only in secreted antibodies. The C regions of light chains do not participate in effector functions and are not directly attached to cell membranes.

Heavy and light chains are covalently linked by disulfide bonds formed between cysteine residues in the carboxy terminus of the light chain and the C_H1 domain of the heavy chain. Noncovalent interactions between the V_L and V_H domains and between the C_L and C_H1 domains may also contribute to the association of heavy and light chains. The two heavy chains of each antibody molecule are also covalently linked by disulfide bonds. There are different kinds of antibodies, called classes or isotypes, which have different heavy chain structures, discussed in detail later in the chapter. In the IgG isotype, these disulfide bonds are formed between cysteine residues in the C_H2 domains, close to the region known as the hinge, described later in the chapter. In other isotypes, the disulfide bonds may be in different locations. Noncovalent interactions (e.g., between the third C_H domains [C_H3]) also contribute to heavy chain pairing.

The antigen-binding portion of an antibody molecule is the Fab region, and the C-terminal end that is involved in effector functions is the Fc region. These regions were identified by proteolysis of rabbit IgG molecules. In these molecules, the unfolded hinge region between the C_H1 and C_H2 domains of the heavy chain is the segment most susceptible to proteolytic cleavage. If rabbit IgG is treated with the enzyme papain under conditions of limited proteolysis, the enzyme acts on the hinge region and cleaves the IgG into three separate pieces (Fig. 5.3A). Two of the pieces are identical to each other and consist of the complete light chain (V_L and C_L) associated with a V_H-C_H1 fragment of the heavy chain. These fragments retain the ability to bind antigen because each contains paired V_L and V_H domains, and they are called **Fab** (fragment, antigen binding). The third piece is composed of two identical disulfide-linked peptides, each containing the heavy chain C_H2 and C_H3 domains. This piece of IgG has a propensity to self-associate and to crystallize into a lattice, and is therefore called **Fc** (fragment, crystallizable). When pepsin (instead of papain) is used to cleave rabbit IgG under limiting conditions, proteolysis occurs distal to the hinge region, generating a $F(ab')_2$ fragment of IgG with the hinge and the interchain disulfide bonds intact and two identical antigen-binding sites (see Fig. 5.3B).

The basic organization of the antibody molecule deduced from the rabbit IgG proteolysis experiments is common to all Ig molecules of all classes and all species, and the terms Fab, $F(ab')_2$, and Fc are widely used to describe these different portions of human and mouse antibodies. In fact, these experiments provided the first evidence that the antigen recognition functions and the effector functions of Ig molecules are spatially separated.

Many other proteins in the immune system, as well as numerous proteins with no known immunologic

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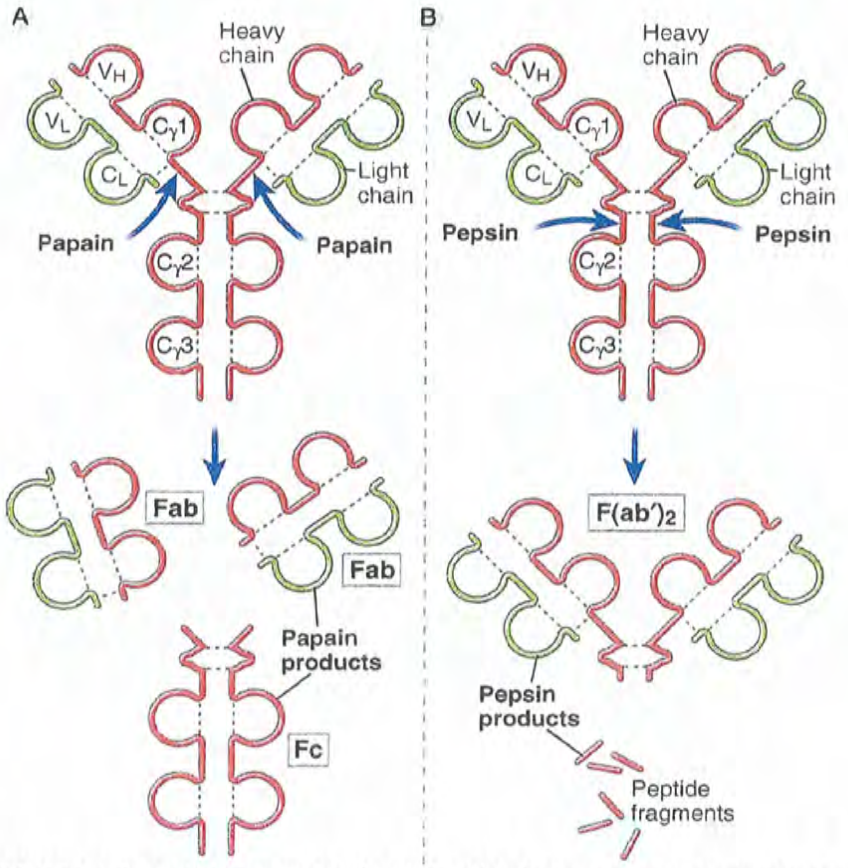


FIGURE 5.3 Proteolytic fragments of an IgG molecule. Rabbit IgG molecules are cleaved by the enzymes papain **(A)** and pepsin **(B)** at the sites indicated by arrows. Papain digestion allows separation of two antigen-binding regions (the Fab fragments) from the portion of the IgG molecule that binds to complement and Fc receptors (the Fc fragment). Pepsin generates a single bivalent antigen-binding fragment, $F(ab')_2$.

function, contain domains with an Ig fold structure—that is, two adjacent β-pleated sheets held together by a disulfide bridge. All molecules that contain this type of domain are said to belong to the **Ig superfamily**, and all gene segments encoding the Ig domains of these molecules are believed to have evolved from one ancestral gene. Ig domains are classified as V-like or C-like on the basis of closest homology to either Ig V or Ig C domains. V domains are formed from a longer polypeptide than C domains and contain two extra β strands within the β sheet sandwich. Some members of the Ig superfamily were described in Chapter 3 (endothelial adhesion molecules ICAM-1 and VCAM-1) and Chapter 4 (NK cell KIR receptors). Examples of Ig superfamily members of relevance in the immune system are depicted in Fig. 5.4.

Structural Features of Antibody Variable Regions

Most of the sequence differences and variability among different antibodies are confined to three short stretches in the V region of the heavy chain and to three stretches in the V region of the light chain. These segments of the greatest diversity are known as **hypervariable regions**. They correspond to three protruding loops connecting adjacent strands of the β sheets that make up the V domains of Ig heavy and light chain proteins (Fig. 5.5). The hypervariable regions are each about 10 amino acid residues long, and they are held in place by the more conserved framework sequences that make up the Ig domain of the V region. In an antibody molecule, the three hypervariable regions of a V_L domain and the three hypervariable regions of a V_H domain are brought together to create an antigen-binding surface. The hypervariable loops can be thought to resemble fingers protruding from each variable domain, with three fingers from the heavy chain and three fingers from the light chain coming together to form the antigen-binding site (Fig. 5.6). Because these sequences form a surface that is complementary to the three-dimensional shape of the bound antigen, the hypervariable regions are also called **complementarity-determining regions**

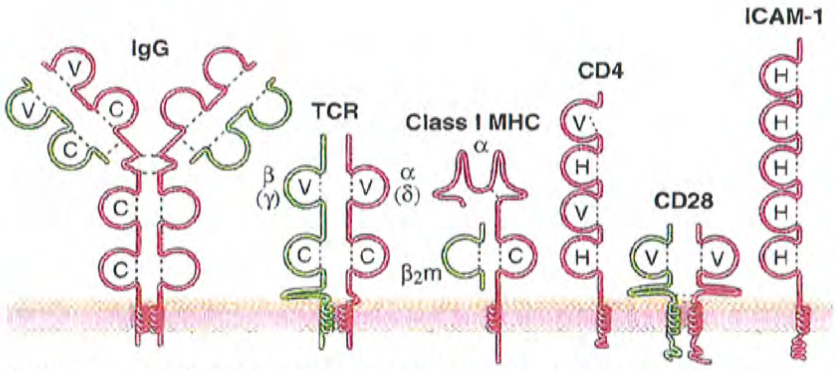


FIGURE 5.4 Examples of Ig superfamily proteins in the immune system. Shown here are a membrane-bound IgG molecule; the T cell receptor; an MHC class I molecule; the CD4 coreceptor of T cells; CD28, a costimulatory receptor on T cells; and the adhesion molecule ICAM-1.

(CDRs). Proceeding from either the V_L or the V_H amino terminus, these regions are called CDR1, CDR2, and CDR3. The CDR3s of both the V_H segment and the V_L segment are the most variable of the CDRs. As we will discuss in Chapter 8, there are special mechanisms for generating more sequence diversity in CDR3 than in CDR1 and CDR2. Sequence differences among the CDRs of different antibody molecules contribute to distinct interaction surfaces and therefore to specificities of individual antibodies. The ability of a V region to fold into an Ig domain is mostly determined by the conserved sequences of the framework regions adjacent to the CDRs. Confinement of the sequence variability to three short stretches allows the basic structure of all antibodies to be maintained despite the variability of specificities among different antibodies.

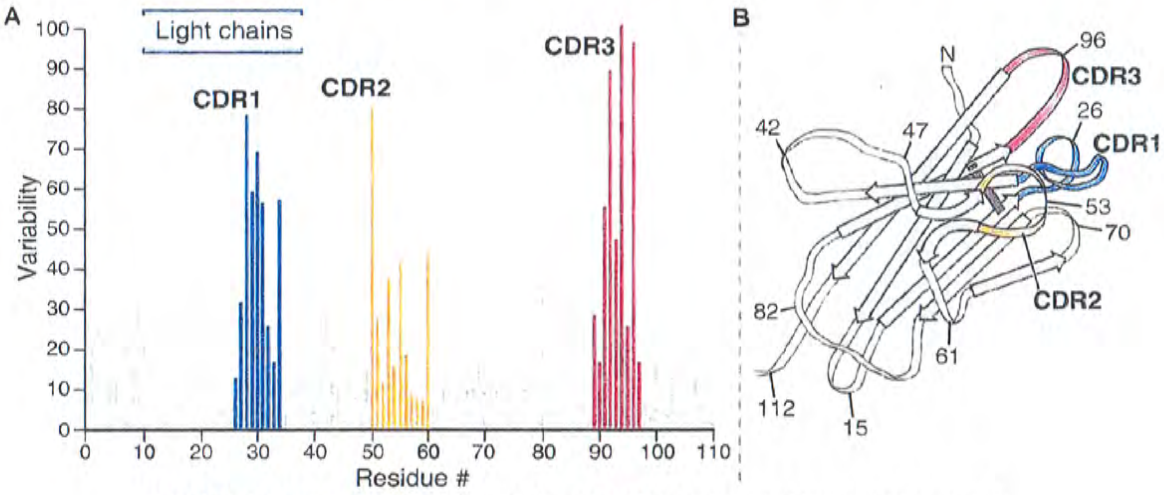


FIGURE 5.5 Hypervariable regions in Ig molecules. A, The vertical lines depict the extent of variability, defined as the number of differences in each amino acid residue among various independently sequenced Ig light chains, plotted against amino acid residue number, measured from the amino terminus. This analysis indicates that the most variable residues are clustered in three "hypervariable" regions, colored in blue, yellow, and red, corresponding to CDR1, CDR2, and CDR3, respectively. Three hypervariable regions are also present in heavy chains (not shown). This way of displaying amino acid variability in Ig molecules is called a Kabat-Wu plot after the two scientists who devised the assay. **B,** Three-dimensional view of the hypervariable CDR loops in a light chain V domain. The V region of a light chain is shown with CDR1, CDR2, and CDR3 loops, colored in blue, yellow, and red, respectively. These loops correspond to the hypervariable regions in the variability plot in **A**. Heavy chain hypervariable regions (not shown) are also located in three loops, and all six loops are juxtaposed in the antibody molecule to form the antigen-binding surface (see Fig. 5.6). Note that in Fig. 5.2 an Ig constant domain, which does not have CDRs, is depicted. ***(A,** Courtesy of Dr. EA. Kabat, Department of Microbiology, Columbia University College of Physicians and Surgeons, New York.)*

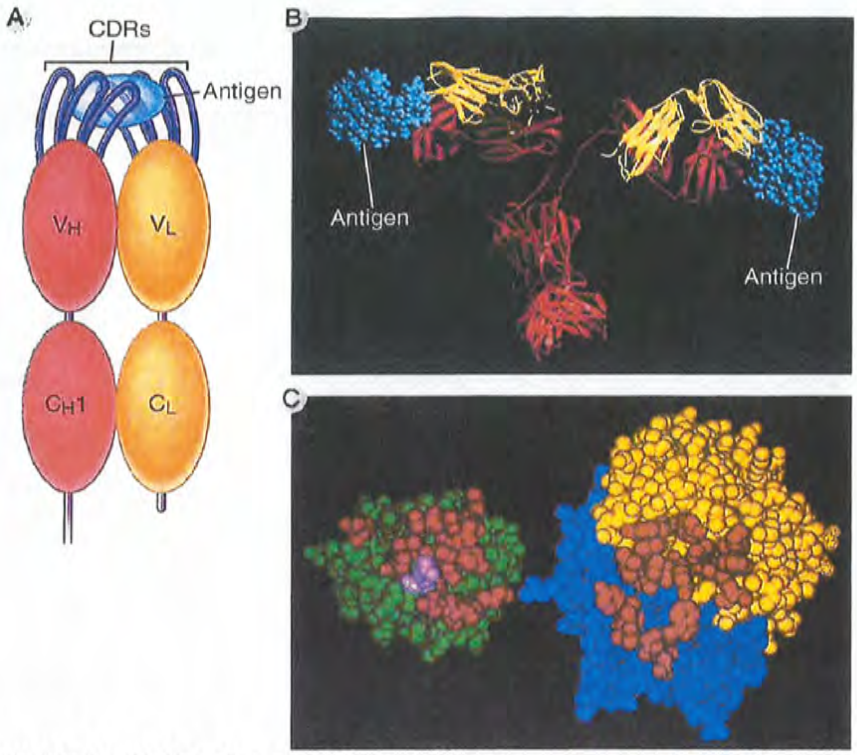


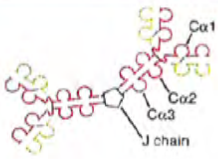
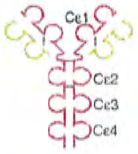
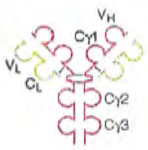
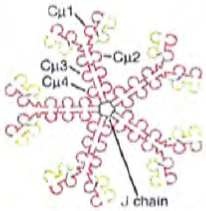
FIGURE 5.6 Binding of an antigen by an antibody. A, A schematic view of complementarity-determining regions (CDRs) generating an antigen-binding site. CDRs from the heavy chain and the light chain are loops that protrude from the surface of the two Ig V domains and in combination create an antigen-binding surface. **B,** This model of a globular protein antigen (hen egg lysozyme) bound to an antibody molecule shows how the antigen-binding site can accommodate soluble macromolecules in their native (folded) conformation. The heavy chains of the antibody are red, the light chains are yellow, and the antigen is blue. **C,** A view of the interacting surfaces of hen egg lysozyme (in green) and a Fab fragment of a monoclonal anti-hen egg lysozyme antibody (V_H in blue and V_L in yellow) is provided. The residues of hen egg lysozyme and of the Fab fragment that interact with each other are shown in red. A critical glutamine residue on lysozyme (in magenta) fits into a "cleft" in the antibody. *(**B,** Courtesy of Dr. Dan Vaughn, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. **C,** Reprinted with permission from Amit AG, Mariuzza RA, Phillips SE, Poljak RJ: Three dimensional structure of an antigen antibody complex at 2.8A resolution. Science 233:747–753, 1986. Copyright 1986 by AAAS.)*

Crystallographic analyses of antigen-antibody complexes show that the amino acid residues of the hypervariable regions form multiple contacts with bound antigens (see Fig. 5.6). The most extensive contact is with the third hypervariable region (CDR3). However, antigen binding is not solely a function of the CDRs, and framework residues may also contact the antigen. Moreover, in the binding of some antigens, one or more of the CDRs may be outside the region of contact with the antigen, and thus not participating in antigen binding.

Structural Features of Antibody Constant Regions

Antibody molecules can be divided into distinct classes and subclasses on the basis of differences in the structure of their heavy chain C regions. The classes of antibody molecules are also called **isotypes** and are named IgA, IgD, IgE, IgG, and IgM (Table 5.2). In humans, IgA and IgG isotypes can be further subdivided into closely related subclasses, or subtypes, called IgA1 and IgA2 and IgG1, IgG2, IgG3, and IgG4. (Mice, which are often used in the study of immune responses, differ from humans in that the IgG isotype is divided into the IgG1, IgG2a, IgG2b, and IgG3 subclasses; certain strains of mice, including C57BL/6, lack the gene for IgG2a but produce a related isotype called IgG2c.) The heavy chain C regions of all antibody molecules of one isotype or subtype have essentially the same amino acid sequence. This sequence is different in antibodies of other isotypes or subtypes. Heavy chains are designated by the letter of the Greek alphabet corresponding to the isotype of the antibody: IgA1 contains α1 heavy chains; IgA2, α2; IgD, δ; IgE, ε; IgG1, γ1; IgG2, γ2; IgG3, γ3; IgG4, γ4; and IgM, μ. In human IgM and IgE antibodies, the C regions contain four tandem Ig domains (see Fig. 5.1). The C regions of IgG, IgA, and IgD contain only three Ig domains. These

TABLE 5.2 Human Antibody Isotypes

Isotype of Antibody	Subtypes (H chain)	Plasma Concentration (mg/mL)	Half-Life (days)	Secreted Form		Functions
IgA	IgA1,2 (α1 or α2)	3.5	6	Mainly dimer; also monomer, trimer		Mucosal immunity
IgD	None (δ)	Trace	3	Monomer		B cell antigen receptor
IgE	None (ε)	0.05	2	Monomer		Defense against helminthic parasites, immediate hypersensitivity
IgG	IgG1-4 (γ1, γ2, γ3, or γ4)	13.5	23	Monomer		Opsonization, complement activation, antibody-dependent cell-mediated cytotoxicity, neonatal immunity, feedback inhibition of B cells
IgM	None (μ)	1.5	5	Pentamer		Naive B cell antigen receptor (monomeric form), complement activation

The effector functions of antibodies are discussed in detail in Chapter 13.

domains are generically designated C_H domains and are numbered sequentially from amino terminus to carboxy terminus (e.g., C_H1, C_H2, and so on). In each isotype, these regions may be designated more specifically (e.g., Cγ1, Cγ2 in IgG).

Different isotypes and subtypes of antibodies perform different effector functions. The reason for this is that most of the effector functions of antibodies are mediated by the binding of heavy chain C regions to Fc receptors (FcRs) on different cells, such as phagocytes, NK cells, and mast cells, and to plasma proteins, such as complement proteins. Antibody isotypes and subtypes differ in their C regions and therefore in what they bind to and what effector functions they perform. The effector functions mediated by each antibody isotype are listed in Table 5.2 and are discussed in more detail later in this chapter and in Chapter 13.

Antibody molecules are flexible, permitting them to bind to different arrays of antigens. Every antibody contains at least two antigen-binding sites, each formed by a pair of V_H and V_L domains. Many Ig molecules can orient these binding sites so that two antigen molecules on a planar (e.g., cell) surface may be engaged at once (Fig. 5.7). This flexibility is conferred, in large part, by a **hinge region** located between C_H1 and C_H2 in certain isotypes. The hinge region varies in length from 10 to more than 60 amino acid residues in different isotypes. Portions of this sequence assume an unfolded and flexible conformation, permitting molecular motion between the C_H1 and C_H2 domains. Some of the greatest differences between the constant regions of the IgG subclasses are concentrated in the hinge. This leads to different overall shapes of the IgG subtypes. In addition, some flexibility of antibody molecules is due to the ability of each V_H domain to rotate with respect to the adjacent C_H1 domain.

There are two classes, or isotypes, of light chains, called κ and λ, that have distinct carboxy-terminal constant (C) regions. Each antibody molecule has either two identical κ light chains or two identical λ light chains. In humans, about 60% of antibody molecules have κ light chains, and about 40% have λ light chains. Marked changes in

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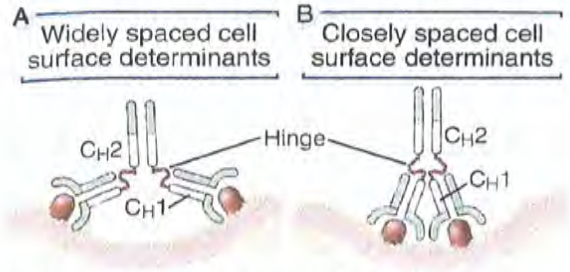


FIGURE 5.7 Flexibility of antibody molecules. The two antigen-binding sites of an Ig monomer can simultaneously bind to two determinants separated by varying distances. In **(A)** an Ig molecule is depicted binding to two widely spaced determinants on a cell surface, and in **(B)** the same antibody is binding to two determinants that are close together. This flexibility is mainly due to the hinge regions located between the C_H1 and C_H2 domains, which permit independent movement of antigen-binding sites relative to the rest of the molecule.

this ratio can occur in patients with B cell tumors because the many neoplastic cells, being derived from one B cell clone, produce a single species of antibody molecules, all with the same light chain. In fact, an abnormal predominance of either κ-bearing cells or λ-bearing cells is often used clinically for the diagnosis of B cell lymphomas. In mice, κ-containing antibodies are about 10 times more abundant than λ-containing antibodies. Unlike in heavy chain isotypes, there are no known differences in function between κ-containing antibodies and λ-containing antibodies.

Secreted and membrane-associated antibodies differ in the amino acid sequence of the carboxy-terminal end of the heavy chain C region. The secreted form, found in blood, mucosal secretions, and other extracellular fluids, contains a carboxy-terminal hydrophilic region called the tail piece. The membrane-bound form of antibody contains a carboxy-terminal stretch that includes two segments: a hydrophobic α-helical transmembrane region, followed by an intracellular juxtamembrane positively charged stretch (Fig. 5.8). The positively charged amino acids bind to negatively charged phospholipid head groups on the inner leaflet of the plasma membrane and help anchor the protein in the membrane. In membrane IgM and IgD molecules, the cytoplasmic portion of the heavy chain is short (only three amino acid residues in length); in membrane IgG and IgE molecules, it is longer (up to 30 amino acid residues in length).

Secreted IgG and IgE and all membrane Ig molecules, regardless of isotype, are monomeric with respect to the basic antibody structural unit (i.e., they contain two heavy chains and two light chains). In contrast, the secreted forms of IgM and IgA form multimeric complexes in which two or more of the four-chain core antibody structural units are covalently joined. IgM is secreted mainly as pentamers but also some hexamers of the core four-chain structure, whereas IgA is usually secreted as a dimer. These complexes are formed by interactions between regions called tail pieces that are located at the carboxy-terminal ends of the secreted forms of μ and α heavy chains (see Table 5.2). Multimeric IgM and IgA molecules contain an additional non-Ig 15-kD polypeptide called the joining (J) chain, which is disulfide bonded to the tail pieces of the Ig C regions and serves to stabilize the multimeric complexes and to transport multimers across epithelial cells from the basolateral to the luminal end. As we will see later, multimeric forms of antibodies bind to antigens more avidly than monomeric forms.

Antibodies of different species differ from each other in the C regions and in framework parts of the V regions. Therefore, when Ig molecules from one species are introduced into another (e.g., horse serum antibodies or mouse monoclonal antibodies injected into humans), the recipient sees them as foreign, mounts an immune response, and makes antibodies largely against the C regions of the introduced Ig. The response sometimes results in an illness called serum sickness (see Chapter 19), which greatly limits the ability to treat individuals with antibodies produced in other species. Much effort has been devoted to overcoming this problem, especially for treating patients with therapeutic monoclonal antibodies, and we will discuss this issue in more detail later in the chapter.

Smaller sequence differences are present in antibodies from different individuals even of the same species, reflecting inherited polymorphisms in the genes encoding the C regions of Ig heavy and light chains. When a polymorphic variant found in some individuals of a species can be recognized by an antibody, the variants are referred to as **allotypes**, and the antibody that recognizes an allotypic determinant is called an antiallotypic antibody. The differences between antibody V regions are

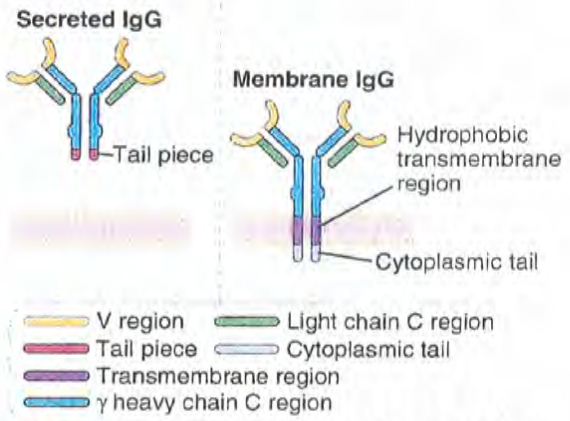


FIGURE 5.8 Membrane and secreted forms of Ig heavy chains. The membrane forms of the Ig heavy chains, but not the secreted forms, contain transmembrane regions made up of hydrophobic amino acid residues and cytoplasmic domains that differ significantly among the different isotypes. The cytoplasmic portion of the membrane form of the μ chain contains only three residues, whereas the cytoplasmic region of IgG heavy chains (γ heavy chains) contains 20 to 30 residues. The secreted forms of the antibodies end in C-terminal tail pieces, which also differ among isotypes: μ has a long tail piece (21 residues) that is involved in pentamer formation, whereas IgGs have a short tail piece (3 residues).

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concentrated in the CDRs and constitute the **idiotypes** of antibodies. An antibody that recognizes some aspect of the CDRs of another antibody is therefore called an anti-idiotypic antibody. There have been interesting theories that individuals produce anti-idiotypic antibodies against their own antibodies that control immune responses, but there is little evidence to support the importance of this potential mechanism of immune regulation.

Monoclonal Antibodies

A **monoclonal antibody** is a pure collection of identical antibodies molecules with the same specificity. A tumor of plasma cells (myeloma or plasmacytoma), like most tumors of any cellular origin, is monoclonal and therefore produces antibodies of a single specificity. In most cases, the specificity of the tumor-derived antibody is not known, so the myeloma antibody cannot be used to detect or bind to molecules of interest. However, the discovery of monoclonal antibodies produced by these tumors led to the idea that it may be possible to produce similar monoclonal antibodies of any desired specificity by immortalizing individual antibody-secreting cells from an animal immunized with a known antigen. A technique to accomplish this was described by Georges Kohler and Cesar Milstein in 1975, and this has proved to be one of the most valuable advances in all of scientific research and clinical medicine. The method relies on fusing B cells from an immunized animal (typically a mouse) with an immortal myeloma cell line and growing the cells under conditions in which the unfused normal and tumor cells cannot survive (Fig. 5.9). The resultant fused cells that grow out are called **hybridomas** because they are hybrids of normal B cells and a myeloma tumor. Each hybridoma makes only one Ig, derived from one B cell from the immunized animal. The antibodies secreted by many hybridoma clones are screened for binding to the antigen of interest, and the clone with the desired specificity is selected and expanded. The products of these individual clones are monoclonal antibodies, and each antibody is specific for a single epitope on the antigen used to immunize the animal.

Monoclonal antibodies have many applications in research and in medical diagnosis and therapy. Some of their common applications include the following:

- ***Identification of phenotypic markers unique to particular cell types.*** The basis for the modern classification of lymphocytes and other leukocytes is the recognition of individual cell populations by specific monoclonal antibodies. These antibodies have been used to define clusters of differentiation (CD) markers for various cell types (see Chapter 2 and Appendix II).
- ***Immunodiagnosis.*** The diagnosis of many infectious and systemic diseases relies on the detection of particular antigens or antibodies in the blood, urine, or tissues by use of monoclonal antibodies in immunoassays (see Appendix III).
- ***Tumor identification.*** Labeled monoclonal antibodies specific for various cell proteins are used to determine the tissue source of tumors by staining histological tumor sections.
- ***Therapy.*** Advances in medical research have led to the identification of cells and molecules that are involved in the pathogenesis of many diseases. Monoclonal antibodies, because of their exquisite specificity, provide a means of targeting these cells and molecules. Many monoclonal antibodies are used therapeutically today (Table 5.3). Some examples include antibodies against the cytokine tumor necrosis factor (TNF) used to treat rheumatoid arthritis and other inflammatory diseases, antibodies against CD20 for the treatment of B cell–derived tumors and for depleting B cells in certain autoimmune disorders, antibodies against epidermal growth factor receptors to target cancer cells, antibodies against vascular endothelial growth factor (a cytokine that promotes angiogenesis) in patients with macular degeneration, and so on.
- ***Functional analysis of cell surface and secreted molecules.*** In biologic research, monoclonal antibodies that bind to cell surface molecules and either stimulate or inhibit particular cellular functions are invaluable tools for defining the functions of these molecules, including receptors for antigens. Monoclonal antibodies are also widely used to purify selected cell populations from complex mixtures to facilitate the analysis of the properties and functions of these cells, and to block or deplete secreted molecules and particular cells for studying their functions.

One of the limitations of monoclonal antibodies for therapy is that these antibodies are most easily produced by immunizing mice, but patients treated with mouse antibodies will make antibodies against the mouse Ig, called human antimouse antibody (HAMA). These anti-Ig antibodies block the function or enhance clearance of the injected monoclonal antibody and also can cause serum sickness (see Chapter 19). Genetic engineering techniques have been used to minimize the generation of HAMAs, and therefore expand the usefulness of monoclonal antibodies. The complementary DNAs (cDNAs) that encode the polypeptide chains of a monoclonal antibody can be isolated from a hybridoma, and these genes can be manipulated in vitro. As discussed earlier, only small portions of the antibody molecule are responsible for binding to antigen; the remainder of the antibody molecule can be thought of as a framework. This structural organization allows the DNA segments encoding the antigen-binding sites from a mouse monoclonal antibody to be inserted into a cDNA encoding a human myeloma protein, creating a hybrid gene. When it is expressed, the resultant protein, which retains the antigen specificity of the original mouse monoclonal but has the core structure of a human Ig, is referred to as a humanized antibody. Fully human monoclonal antibodies are also in clinical use. These are derived using phage display methods or in mice with B cells expressing human Ig transgenes. Humanized antibodies are far less likely than mouse monoclonals to appear foreign in humans and to induce anti-antibody responses. However, a proportion of subjects receiving fully humanized monoclonal antibodies for therapy develop blocking anti-antibodies, for unknown reasons.

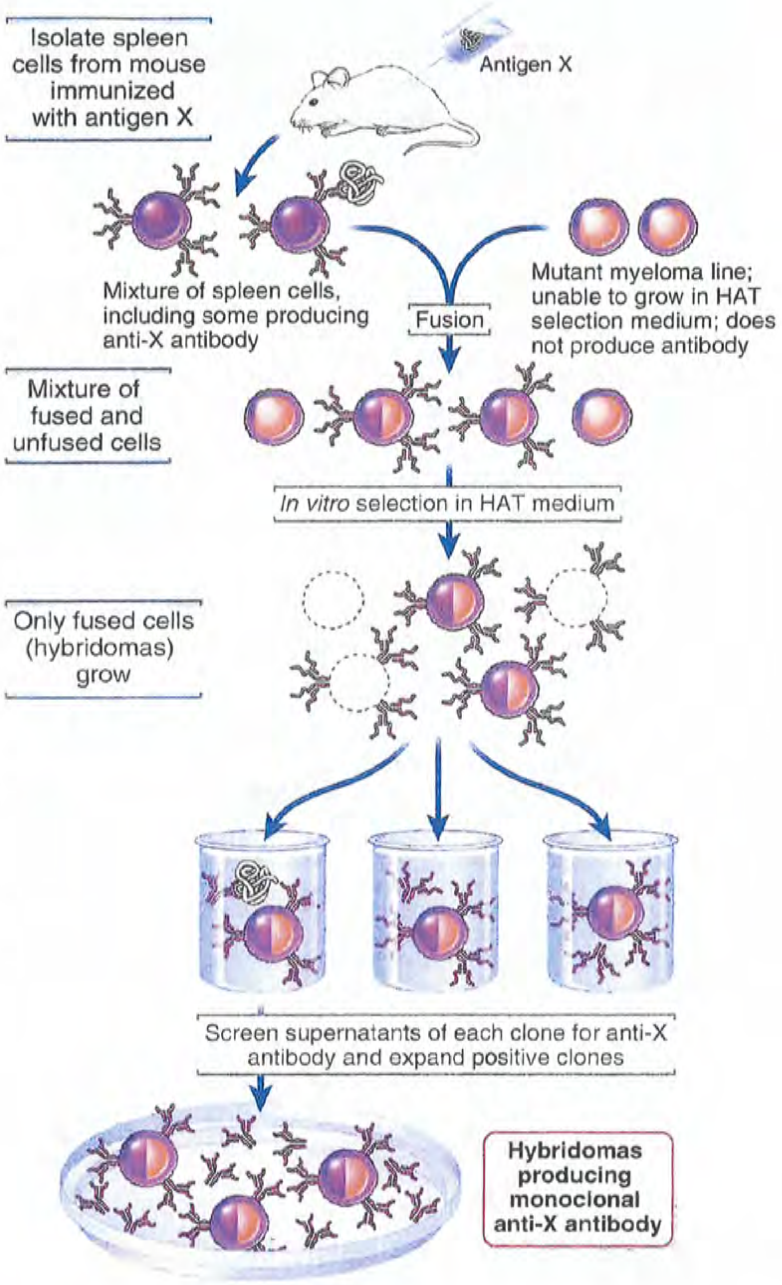


FIGURE 5.9 The generation of monoclonal antibodies. In this procedure, spleen cells from a mouse that has been immunized with a known antigen or mixture of antigens are fused with an enzyme-deficient partner myeloma cell line, with use of chemicals such as polyethylene glycol that can facilitate the fusion of plasma membranes and the formation of hybrid cells that retain many chromosomes from both fusion partners. The myeloma partner used is one that does not secrete its own Ig. These hybrid cells are then placed in a selection medium that permits the survival of only immortalized hybrids; these hybrid cells are then grown as single cell clones and tested for the secretion of the antibody of interest. The selection medium includes hypoxanthine, aminopterin, and thymidine and is therefore called HAT medium. There are two pathways of purine synthesis in most cells, a de novo pathway that needs tetrahydrofolate and a salvage pathway that uses the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT). Myeloma cells that lack HGPRT are used as fusion partners, and they normally survive using de novo purine synthesis. In the presence of aminopterin, tetrahydrofolate is not made, resulting in a defect in de novo purine synthesis and also a specific defect in pyrimidine biosynthesis, namely, in generating TMP from dUMP. Hybrid cells receive HGPRT from the splenocytes and have the capacity for uncontrolled proliferation from the myeloma partner; if they are given hypoxanthine and thymidine, these cells can make DNA in the absence of tetrahydrofolate. As a result, only hybrid cells survive in HAT medium.

SYNTHESIS, ASSEMBLY, AND EXPRESSION OF IMMUNOGLOBULIN MOLECULES

Ig heavy and light chains, like most secreted and membrane proteins, are synthesized on membrane-bound ribosomes in the rough endoplasmic reticulum. The protein is translocated into the endoplasmic reticulum, and Ig heavy chains are *N*-glycosylated during the translocation process. The proper folding of Ig heavy chains and their assembly with light chains are regulated by proteins resident in the endoplasmic reticulum called chaperones. These proteins, which include calnexin and a molecule called binding protein (BiP), bind to newly

TABLE 5.3 Examples of Monoclonal Antibodies in Clinical Use

Target	Effect	Diseases
Inflammatory (Immunological) Diseases		
α4 integrins	Blocking of immune cell egress to intestine and CNS	Crohn's disease, multiple sclerosis
CD20	Depletion of B cells	B cell lymphomas, rheumatoid arthritis, multiple sclerosis, other autoimmune diseases
IgE	Blocking of IgE function	Allergy related asthma
TNF	Blocking of inflammation	Rheumatoid arthritis, Crohn's disease, psoriasis
Other Diseases		
C5	Blocking of complement-mediated lysis	Paroxysmal nocturnal hemoglobinuria, atypical hemolytic uremic syndrome
Glycoprotein IIb/IIIa	Inhibition of platelet aggregation	Cardiovascular disease
RANK ligand	Blocking of RANK signaling	Postmenopausal osteoporosis, bone metastases of solid tumors
RSV F protein	Blocking of viral entry	Respiratory syncytial virus infection
Cancer (see Table 18.1, Chapter 18)		

RANK, receptor activator of nuclear factor-κB; *RSV*, respiratory syncytial virus; *TNF*, tumor necrosis factor. Additional anti-cytokine antibodies in clinical use are listed in Table 19.5, Chapter 19.

synthesized Ig polypeptides and ensure that they are retained or targeted for degradation unless they fold properly and assemble into complete Ig molecules. The covalent association of heavy and light chains is stabilized by the formation of disulfide bonds, and also occurs in the endoplasmic reticulum during the assembly process. After assembly, the Ig molecules are released from chaperones, transported into the cisternae of the Golgi complex where carbohydrates are modified, and then routed to the plasma membrane in vesicles. Antibodies of the membrane form are anchored in the plasma membrane, and the secreted form is transported out of the cell.

The maturation of B cells from bone marrow progenitors is accompanied by specific changes in Ig gene expression, resulting in the production of Ig molecules in different forms (Fig. 5.10). The earliest cell in the B lymphocyte lineage that produces Ig polypeptides, called the pre-B cell, synthesizes the membrane form of the μ heavy chain. These μ chains associate with proteins called surrogate light chains to form the pre-B cell receptor, and a small proportion of the synthesized pre-B cell receptor

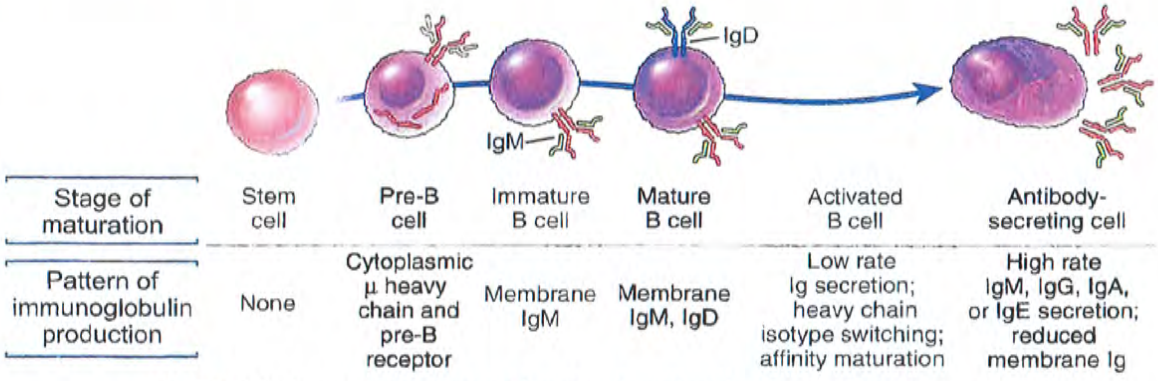


FIGURE 5.10 Ig expression during B lymphocyte maturation. Stages in B lymphocyte maturation are shown with associated changes in the production of Ig heavy and light chains. IgM heavy chains are shown in red, IgD heavy chains in blue, and light chains in green. The molecular events accompanying these changes are discussed in Chapters 8 and 12.

is expressed on the cell surface. Immature and mature B cells produce κ or λ light chains, which associate with μ proteins to form IgM molecules. Mature B cells express membrane forms of IgM and IgD (the μ and δ heavy chains associated with κ or λ light chains). These membrane Ig receptors serve as cell surface receptors that recognize antigens and initiate the process of B cell activation. The pre-B cell receptor and the B cell antigen receptor are noncovalently associated with two other integral membrane proteins, Igα and Igβ, which serve signaling functions and are essential for surface expression of IgM and IgD. We will discuss the molecular and cellular events in B cell maturation underlying these changes in antibody expression in Chapter 8.

When mature B lymphocytes are activated by antigens and other stimuli, the cells differentiate into antibody-secreting plasma cells. This process is also accompanied by changes in the pattern of Ig production. One such change is the increased production of the secreted form of Ig relative to the membrane form. This alteration occurs at the level of posttranscriptional processing. The second change is the expression of Ig heavy chain isotypes other than IgM and IgD, by a process called heavy chain isotype (or class) switching. A third change involves the introduction of new amino acid substitutions into the variable domains of the antibody heavy and light chains in order to create high affinity antibodies, resulting in a change in antibodies that is called affinity maturation. Changes in antibody expression that occur after B cell activation will be discussed later in this chapter and in more detail in Chapter 12.

Half-Life of Antibodies

The half-life of circulating antibodies is a measure of how long those antibodies remain in blood after secretion from B cells (or after injection in the case of an administered antibody). The half-life is the mean time before the number of antibody molecules is reduced by half. Different antibody isotypes have very different half-lives in circulation. IgE has a very short half-life of about 2 days in the circulation (although cell-bound IgE associated with the high-affinity IgE receptor on mast cells has a very long half-life; see Chapter 20). Circulating IgA has a half-life of about 3 days (although most IgA is produced at mucosal sites and is secreted directly into the lumen of the gut or airway), and circulating IgM has a half-life of about 4 days. In contrast, circulating IgG molecules have a half-life of about 21 to 28 days.

The long half-life of IgG is attributed to its ability to bind to a specific Fc receptor called the **neonatal Fc receptor** (FcRn), which is also involved in the transport of IgG from the maternal circulation across the placental barrier. FcRn structurally resembles MHC class I molecules (described in Chapter 6), and in the placenta, it transports IgG molecules from the maternal blood, across cells, and into the fetal circulation. In adult vertebrates, FcRn is found on the surface of endothelial cells, macrophages, and other cell types, and binds to micropinocytosed IgG in acidic endosomes. FcRn does not target bound IgG to lysosomes (the usual fate of many ingested molecules) but recycles it to the cell surface and releases it at neutral pH, returning the IgG to the circulation (Fig. 5.11). This

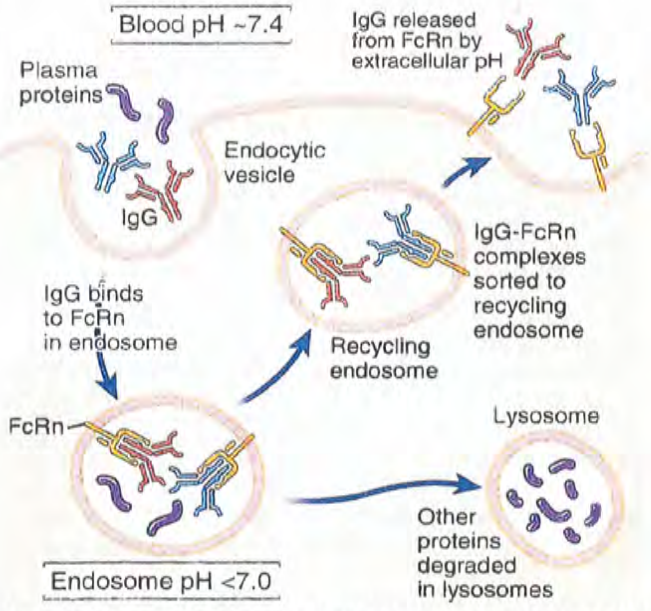


FIGURE 5.11 FcRn (neonatal Fc receptor) contributes to the long half-life of IgG molecules. Micropinocytosed IgG molecules in endothelial cells bind the FcRn, an IgG-binding receptor in the acidic environment of endosomes. In endothelial cells, FcRn directs the IgG molecules away from lysosomal degradation and releases them when vesicles fuse with the cell surface, exposing FcRn-IgG complexes to neutral pH.

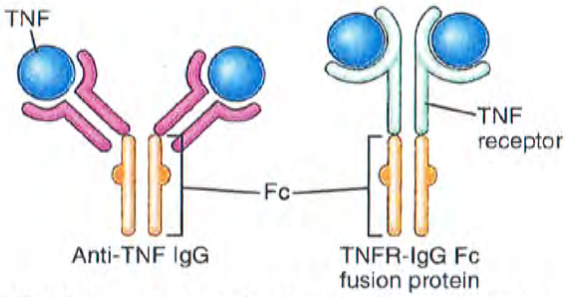


FIGURE 5.12 A monoclonal antibody and a cytokine receptor–IgG Fc fusion protein, both used therapeutically. An antibody specific for the cytokine tumor necrosis factor (TNF) (left) can bind to and block the activity of the cytokine. The extracellular domain of the TNF receptor (right) is also an antagonist of the cytokine, and linking this soluble receptor domain to an IgG Fc domain (by recombinant DNA technology) increases the half-life of the receptor in the circulation.

intracellular sequestration of IgG away from lysosomes prevents the IgG from being degraded as rapidly as most other plasma proteins, including other antibody isotypes, and as a result, this antibody isotype has a relatively long half-life. There are some differences in the half-lives of the four human IgG isotypes. IgG3 is relatively short-lived because it binds poorly to FcRn. IgG1 and IgG2 are the most long-lived and most efficient in terms of effector functions, as will be discussed in Chapter 13.

The long half-life of IgG has been used to provide a therapeutic advantage for certain injected proteins by producing fusion proteins containing the biologically active part of the protein and the Fc portion of IgG. The Fc portion enables the proteins to bind to the FcRn and thus extends the half-lives of the injected proteins. One therapeutically useful fusion protein is TNFR-Ig, which consists of the extracellular domain of the type II TNF receptor (TNFR) fused to an IgG Fc domain. This fusion protein blocks the inflammatory actions of TNF, similar to an anti-TNF antibody, and is used to treat certain immune disorders such as rheumatoid arthritis, inflammatory bowel disease, and psoriasis (Fig. 5.12). Another fusion protein used therapeutically is CTLA4-Ig, containing the extracellular domain of the CTLA-4 receptor, which binds to and blocks B7 costimulators, fused to the Fc portion of human IgG; it has also been used in the treatment of rheumatoid arthritis and kidney transplant rejection.

ANTIBODY BINDING OF ANTIGENS

All of the functions of antibodies are dependent on their ability to specifically bind antigens. We will now consider the nature of antigens and how they are recognized by antibodies.

Features of Biologic Antigens

An antigen is any substance that may be specifically bound by an antibody molecule or T cell receptor. Antibodies can recognize as antigens almost every kind of biologic molecule, including simple intermediary metabolites, sugars, lipids, autacoids, and hormones, as well as macromolecules such as complex carbohydrates, phospholipids, nucleic acids, and proteins. This is in contrast to T cells, which mainly recognize peptides (see Chapter 6).

Not all antigens recognized by specific lymphocytes or by secreted antibodies are capable of activating lymphocytes. Molecules that stimulate immune responses are called **immunogens**. Macromolecules are effective at stimulating B lymphocytes to initiate humoral immune responses because B cell activation requires the bringing together (cross-linking) of multiple antigen receptors. Small chemicals, such as dinitrophenol, may bind to antibodies and are therefore antigens, but they cannot activate B cells on their own (i.e., they are not immunogenic). To generate antibodies specific for such small chemicals, immunologists commonly attach multiple copies of the small molecules to a protein or polysaccharide before immunization. In these cases, the small chemical is called a **hapten**, and the large molecule to which it is conjugated is called a **carrier**. The hapten-carrier complex, unlike free hapten, can act as an immunogen (see Chapter 12).

Macromolecules, such as proteins, polysaccharides, and nucleic acids, are usually much bigger than the antigen-binding region of an antibody molecule (see Fig. 5.6). Therefore, any antibody binds to only a portion of the macromolecule, which is called a **determinant** or an **epitope**. These two words are synonymous and are used interchangeably throughout this book. Macromolecules typically contain multiple determinants, some of which may be repeated and each of which, by definition, can be bound by an antibody. The presence of multiple identical determinants in an antigen is referred to as **polyvalency** or **multivalency**. Most globular proteins do not contain multiple identical determinants and are not individually polyvalent, but many identical proteins may be displayed in a polyvalent array on cell surfaces, including the surface of microbes. In the case of polysaccharides and nucleic acids, many identical epitopes may be regularly spaced, and the molecules are said to be polyvalent. Polyvalent arrays of carbohydrate antigens can also be displayed on cell surfaces. Polyvalent antigens can induce clustering of the B cell receptor and thus initiate the process of B cell activation (see Chapter 12).

The spatial arrangement of different epitopes on a single protein molecule may influence the binding of antibodies in several ways. When determinants are well separated, two or more antibody molecules can be bound to the same protein antigen without influencing each other; such determinants are said to be nonoverlapping. When two determinants are close to each other, the binding of antibody to the first determinant may cause steric interference with the binding of antibody to the second; such determinants are said to be overlapping. In rare cases, the binding of one antibody may cause a conformational change in the structure of the antigen, positively or negatively influencing the binding of a second antibody at another site on the protein by means other than steric hindrance. Such interactions are called allosteric effects.

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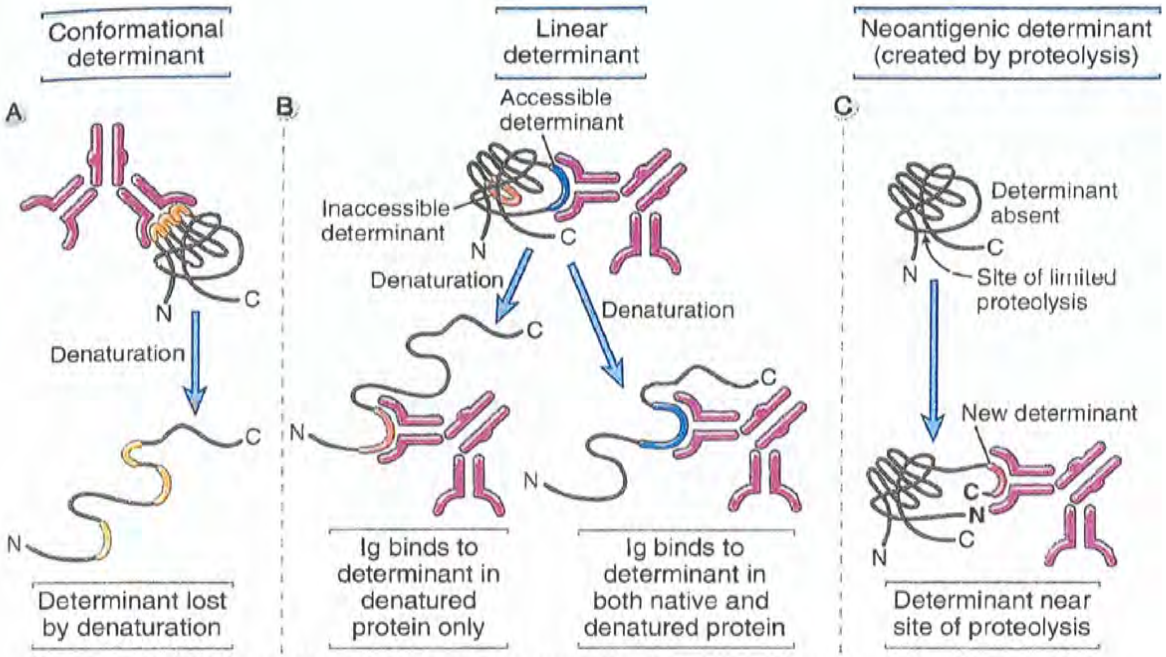


FIGURE 5.13 The nature of antigenic determinants. Antigenic determinants (shown in orange, red, and blue) may depend on protein folding (conformation) as well as on primary structure. Some determinants are accessible in native proteins and are lost on denaturation **(A)**, whereas others are exposed only on protein unfolding **(B)**. Neodeterminants arise from postsynthetic modifications such as peptide bond cleavage **(C)**.

Any available shape or surface on a molecule that may be recognized by an antibody constitutes an antigenic determinant or epitope. Antigenic determinants may be delineated on any type of compound, including but not restricted to carbohydrates, proteins, lipids, and nucleic acids. In the case of proteins, the formation of some epitopes depends only on the primary structure, and the formation of other determinants reflects tertiary structure, or conformation (shape) (Fig. 5.13). Epitopes formed by several adjacent amino acid residues are called linear epitopes. The antigen-binding site of an antibody can usually accommodate a linear epitope made up of about six amino acids. If linear epitopes appear on the external surface or in a region of extended conformation in the native folded protein, they may be accessible to antibodies. In other cases, linear epitopes may be inaccessible in the native conformation and appear only when the protein is denatured. In contrast, conformational epitopes are formed by amino acid residues that are not in a sequence but become spatially juxtaposed in the folded protein. Antibodies specific for certain linear epitopes and antibodies specific for conformational epitopes can be used to ascertain whether a protein is denatured or in its native conformation, respectively. Proteins may be subjected to modifications such as glycosylation, phosphorylation, ubiquitination, acetylation, and proteolysis. These modifications, by altering the structure of the protein, can produce new epitopes. Such epitopes are called neoantigenic epitopes, and they too may be recognized by specific antibodies.

Structural and Chemical Basis of Antigen Binding

The antigen-binding sites of many antibodies are planar surfaces that can accommodate conformational epitopes of macromolecules, allowing the antibodies to bind large macromolecules (see Fig. 5.6). The six CDRs, three from the heavy chain and three from the light chain, can spread out to form a broad surface. In a number of antibodies specific for small molecules, such as monosaccharides and drugs, the antigen is bound in a cleft generated by the close apposition of CDRs from the V_L and V_H domains.

The recognition of antigens by antibodies involves noncovalent, reversible binding. Various types of noncovalent interactions may contribute to antibody binding of antigens, including electrostatic forces, hydrogen bonds, van der Waals forces, and hydrophobic interactions. The relative importance of each of these depends on the structures of the binding site of the individual antibody and of the antigenic determinant. The strength of the binding between a single combining site of an antibody and an epitope of an antigen is called the **affinity** of the antibody. The affinity is commonly represented by a dissociation constant (K_d), which indicates how easy it is to separate (dissociate) an antigen-antibody complex

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into its constituents. A smaller K_d indicates a stronger or higher affinity interaction because a lower concentration of antigen and of antibody is required for complex formation. The K_d of antibodies produced in typical humoral immune responses usually varies from about 10^{-7} to 10^{-11} M. Serum from an immunized individual will contain a mixture of antibodies with different affinities for the antigen, depending primarily on the amino acid sequences of the CDRs.

Because the hinge region of antibodies gives them flexibility, a single antibody may attach to a single multivalent antigen by more than one binding site. For IgG or IgE, this attachment can involve, at most, two binding sites, one on each Fab. For pentameric IgM, however, a single antibody may bind at up to 10 different sites (Fig. 5.14). Polyvalent antigens will have more than one copy of a particular determinant. Although the affinity of any one antigen-binding site will be the same for each epitope of a polyvalent antigen, the strength of attachment of the antibody to the antigen must take into account binding of all the sites to all the available epitopes. This overall strength of attachment is called the **avidity** and is much greater than the affinity of any one antigen-binding site. Thus, a low-affinity IgM molecule can still bind tightly to a polyvalent antigen because many low-affinity interactions (up to 10 per IgM molecule) can produce a high-avidity interaction.

Polyvalent antigens are important from the viewpoint of B cell activation, as discussed earlier. Polyvalent interactions between antigens and antibodies are also of biologic significance because many effector functions of antibodies are triggered optimally when two or more antibody molecules are brought close together by binding to a polyvalent antigen. If a polyvalent antigen is mixed with a specific antibody in a test tube, the two interact to form **immune complexes** (Fig. 5.15). At the correct concentration, called a zone of equivalence, antibody and antigen form an extensively cross-linked network of attached molecules such that most or all of the antigen and antibody molecules are complexed into large masses.

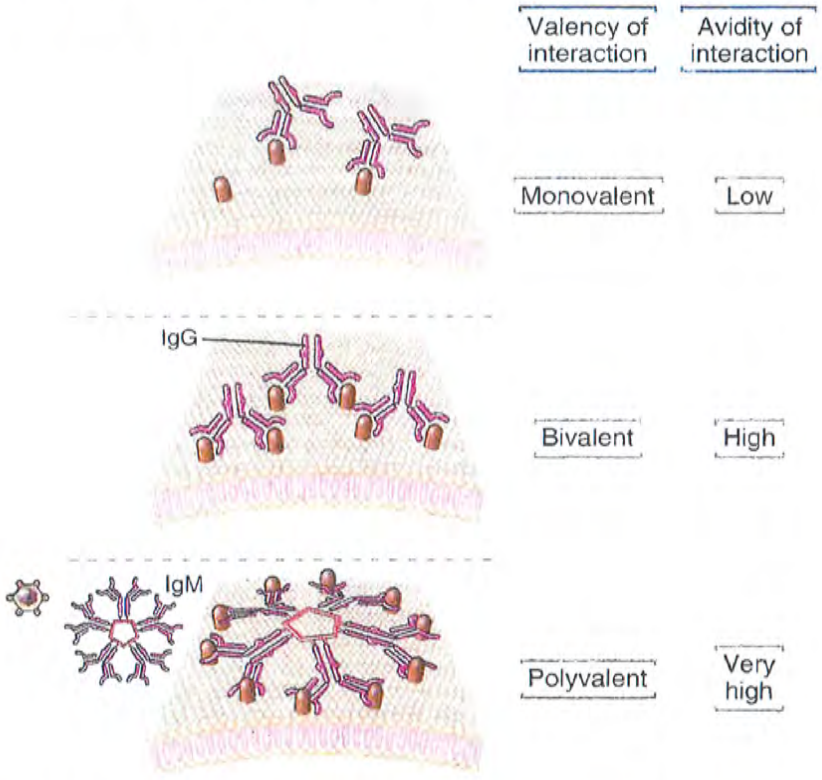


FIGURE 5.14 Valency and avidity of antibody-antigen interactions. Monovalent antigens, or epitopes spaced far apart on cell surfaces, will interact with a single binding site of one antibody molecule. Although the affinity of this interaction may be high, the overall avidity may be relatively low. When repeated determinants on a cell surface are close enough, both the antigen-binding sites of a single IgG molecule can bind, leading to a higher avidity bivalent interaction. The hinge region of the IgG molecule accommodates the shape change needed for simultaneous engagement of both binding sites. IgM molecules have 10 identical antigen-binding sites that can theoretically bind simultaneously with 10 repeating determinants on a cell surface, resulting in a polyvalent, high-avidity interaction.

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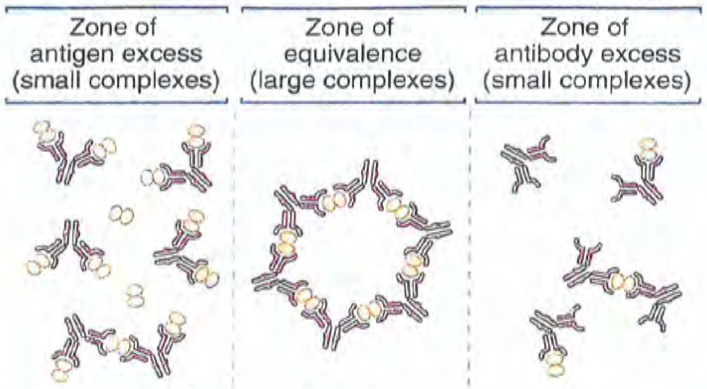


FIGURE 5.15 Antigen-antibody complexes. The sizes of antigen-antibody (immune) complexes are a function of the relative concentrations of antigen and antibody. Large complexes are formed at concentrations of multivalent antigens and antibodies that are termed the zone of equivalence; the complexes are smaller in relative antigen or antibody excess.

Immune complexes may be dissociated into smaller aggregates either by increasing the concentration of antigen so that free antigen molecules will displace antigen bound to the antibody (zone of antigen excess) or by increasing antibody so that free antibody molecules will displace bound antibody from antigen determinants (zone of antibody excess). If a zone of equivalence is reached in vivo, large immune complexes can form in the circulation. Immune complexes that are trapped or formed in the walls of blood vessels can initiate an inflammatory reaction, resulting in immune complex diseases (see Chapter 19).

STRUCTURE-FUNCTION RELATIONSHIPS IN ANTIBODY MOLECULES

Many structural features of antibodies are critical for their ability to recognize antigens and for their effector functions. In the following section, we will summarize how the structure of antibodies contributes to their functions.

Features Related to Antigen Recognition

The ability of antibodies to specifically recognize a wide variety of antigens with varying affinities reflects the properties of the V regions.

Specificity

Antibodies can be remarkably specific for antigens, distinguishing between small differences in chemical structure. The fine specificity of antibodies applies to the recognition of all classes of molecules. For example, antibodies can distinguish between two linear protein determinants differing by only a single conservative amino acid substitution that has little effect on secondary structure. This high degree of specificity is necessary so that antibodies generated in response to the antigens of one microbe usually do not react with structurally similar self molecules or with the antigens of other microbes. However, some antibodies produced against one antigen may bind to a different but structurally related antigen. This is referred to as a **cross-reaction**. Antibodies that are produced in response to a microbial antigen sometimes cross-react with self antigens, and this may be the basis of certain immunologic diseases (see Chapter 19).

Diversity

As we discussed earlier in this chapter, an individual is capable of making a tremendous number of structurally distinct antibodies, perhaps on the order of millions, each with a distinct specificity. The ability of antibodies in any individual to specifically bind a large number of different antigens is a reflection of antibody **diversity**, and the total collection of antibodies with different specificities represents the antibody **repertoire**. The genetic mechanisms that generate such a large antibody repertoire are active only in B lymphocytes (and the same mechanisms for generating TCR diversity are active in T cells). This diversity is generated by random recombination of a limited set of inherited germline DNA sequences to form functional genes that encode the V regions of heavy and light chains as well as by the addition of nucleotide sequences during the recombination process. We will discuss these mechanisms in detail in Chapter 8. The millions of resulting variations in structure are concentrated in the antigen-binding hypervariable regions of both heavy and light chains and thereby determine specificity for antigens.

Affinity Maturation

The ability of antibodies to neutralize toxins and infectious microbes is dependent on tight binding of the antibodies. As we have discussed, tight binding is achieved by high-affinity and high-avidity interactions. A mechanism for the generation of high-affinity antibodies involves subtle changes in the structure of the V regions of antibodies during T cell–dependent humoral immune responses to protein antigens. These changes come about by a process of somatic mutation in antigen-stimulated B lymphocytes that generates new V domain structures, some of which

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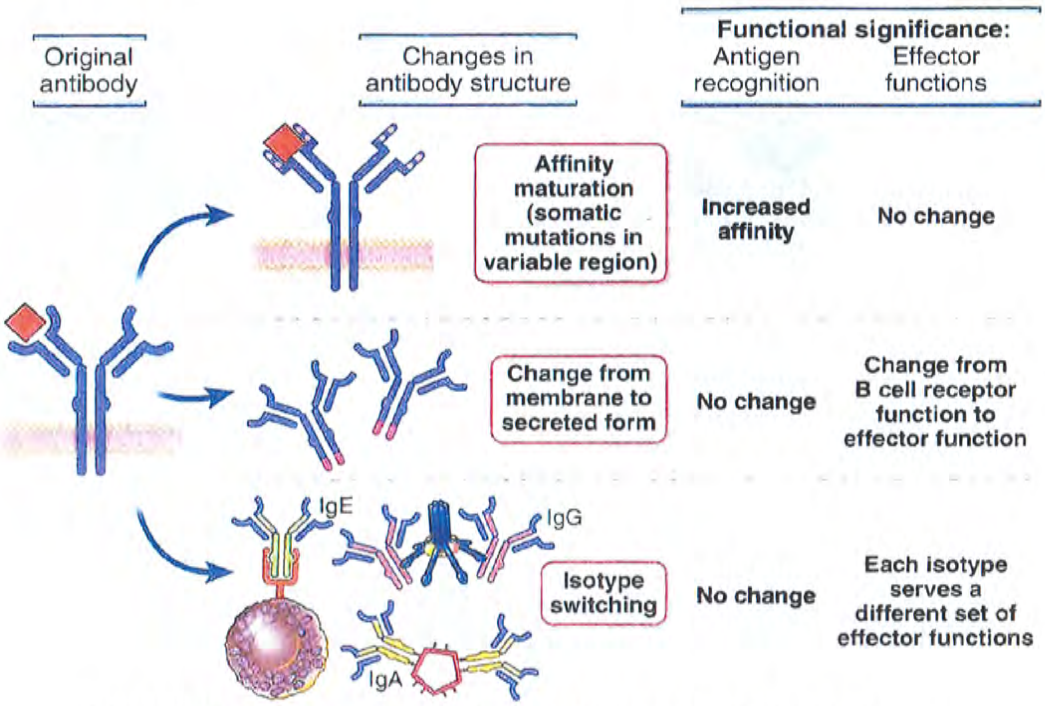


FIGURE 5.16 Changes in antibody structure during humoral immune responses. The illustration depicts the changes in the structure of antibodies that may be produced by the progeny of activated B cells (one clone) and the related changes in function. During affinity maturation, mutations in the V region (indicated by yellow dots) lead to changes in fine specificity without changes in C region–dependent effector functions. Activated B cells may shift production from largely membrane-bound antibodies containing transmembrane and cytoplasmic regions to secreted antibodies. Secreted antibodies may or may not show V gene mutations (i.e., secretion of antibodies occurs before and after affinity maturation). In isotype switching, the C regions change (indicated by color change from purple to green, yellow, or pink) without changes in the antigen-binding V region. Isotype switching is seen in membrane-bound and secreted antibodies. We will discuss the molecular basis for these changes in Chapter 12.

bind the antigen with greater affinity than the original V domains (Fig. 5.16). Those B cells producing higher affinity antibodies preferentially bind to the antigen and, as a result of selection, become the dominant B cells with each subsequent exposure to the antigen. This process, called **affinity maturation**, results in an increase in the average binding affinity of antibodies for an antigen as a humoral immune response evolves. Thus, an antibody produced during a primary immune response to a protein antigen often has a K_d in the range of 10^{-7} to 10^{-9} M; in secondary responses, the affinity increases, with a K_d of 10^{-11} M or even less. We will discuss the mechanism of affinity maturation in Chapter 12.

Features Related to Effector Functions

Many of the effector functions of antibodies are mediated by the Fc portions of the molecules, and Ig isotypes that differ in these Fc regions perform distinct functions. We have mentioned previously that the effector functions of antibodies require the binding of heavy chain C regions, which make up the Fc portions, to other cells and plasma proteins. For example, IgG coats microbes and targets them for phagocytosis by neutrophils and macrophages. This occurs because the IgG molecule is able to simultaneously bind, through its Fab region to the microbe, and through its Fc region to IgG heavy chain–specific Fc receptors that are expressed on neutrophils and macrophages. In contrast, IgE binds to mast cells and triggers their degranulation because mast cells express IgE-specific Fc receptors. Another Fc-dependent effector mechanism of humoral immunity is activation of the classical pathway of the complement system. The system generates inflammatory mediators and promotes microbial phagocytosis and lysis. It is initiated by the binding of a complement protein called C1q to the Fc portions of antigen-complexed IgG or IgM. The Fc receptor– and complement-binding sites of antibodies are found within the heavy chain C domains of the different isotypes (see Fig. 5.1). We will discuss the structure and functions of Fc receptors and complement proteins in Chapter 13.

The effector functions of antibodies are initiated only by Ig molecules that have bound antigens and not by free Ig. The reason that only antibodies with bound antigens

activate effector mechanisms is that two or more adjacent antibody Fc portions are needed to bind to and trigger various effector systems, such as complement proteins and Fc receptors of phagocytes (see Chapter 13). This requirement for adjacent antibody molecules ensures that the effector functions are targeted specifically toward eliminating antigens that are recognized by the antibody and that circulating free antibodies do not, inappropriately and dangerously, trigger effector responses.

Changes in the isotypes of antibodies during humoral immune responses influence how the responses work to eradicate antigens. After stimulation by an antigen, a single clone of B cells may produce antibodies with different isotypes that nevertheless possess identical V domains and therefore identical antigen specificity. Naive B cells simultaneously produce IgM and IgD that function as membrane receptors for antigens. When these B cells are activated by foreign antigens, typically of microbial origin, they may undergo a process called **isotype** (or **class**) **switching** in which the type of C_H region, and therefore the antibody isotype, produced by the B cell changes, but the V regions and the specificity do not (see Fig. 5.16). As a result of isotype switching, different progeny of the original IgM- and IgD-expressing naive B cell may produce isotypes and subtypes that are best able to eliminate the antigen. For example, the antibody response to many bacteria and viruses in the blood is dominated by IgG antibodies, but the same microbes in mucosal tissues (intestines and airways) elicit much more IgA, which is efficiently secreted into the lumens of these organs. Switching to the IgG isotype also prolongs the effectiveness of humoral immune responses because of the long half-life of IgG antibodies. We will discuss the mechanisms and functional significance of isotype switching in Chapter 12.

The heavy chain C regions of antibodies also determine the tissue distribution of antibody molecules. As we mentioned earlier, after B cells are activated, they gradually lose expression of the membrane-bound antibody and express more of it as a secreted protein (see Fig. 5.16). IgA can be secreted efficiently across mucosal epithelia and is the major class of antibody in mucosal secretions and milk (see Chapter 14). Neonates are protected from infections by IgG antibodies they acquire from their mothers through the placenta during gestation. This transfer of maternal IgG is mediated by the FcRn, which we described earlier as the receptor responsible for the long half-life of IgG antibodies.

SUMMARY

- Antibodies, or immunoglobulins, are a family of structurally related glycoproteins produced in membrane-bound or secreted form by B lymphocytes.
- Membrane-bound antibodies serve as receptors that mediate the antigen-triggered activation of B cells.
- Secreted antibodies function as mediators of specific humoral immunity by engaging various effector mechanisms that serve to eliminate the bound antigens.
- The antigen-binding regions of antibody molecules are highly variable, and any one individual has the potential to produce millions of different antibodies, each with distinct antigen specificity.
- All antibodies have a common symmetric core structure of two identical covalently linked heavy chains and two identical light chains, each linked to one of the heavy chains. Each chain consists of two or more independently folded Ig domains of about 110 amino acids containing conserved sequences and intrachain disulfide bonds.
- The *N*-terminal domains of heavy and light chains form the V regions of antibody molecules, which differ among antibodies of different specificities. The V regions of heavy and light chains each contain three separate hypervariable regions of about 10 amino acids that are spatially assembled to form the antigen-combining site of the antibody molecule.
- Antibodies are classified into different isotypes and subtypes on the basis of differences in the heavy chain C regions, which consist of three or four Ig C domains, and these classes and subclasses have different functional properties. The antibody classes are called IgM, IgD, IgG, IgE, and IgA. Both light chains of a single Ig molecule are of the same light chain isotype, either κ or λ, which differ in their single C domains.
- Most of the effector functions of antibodies are mediated by the C regions of the heavy chains, but these functions are triggered by binding of antigens to the combining site in the V region.
- Monoclonal antibodies are produced from a single clone of B cells and recognize a single antigenic determinant. Monoclonal antibodies can be generated in the laboratory and are widely used in research, diagnosis, and therapy.
- Antigens are substances specifically bound by antibodies or T lymphocyte antigen receptors. Antigens that bind to antibodies include a wide variety of biologic molecules, including sugars, lipids, carbohydrates, proteins, and nucleic acids. This is in contrast to most T cell antigen receptors, which recognize only peptide antigens.
- Macromolecular antigens contain multiple epitopes, or determinants, each of which may be recognized by an antibody. Linear epitopes of protein antigens consist of a sequence of adjacent amino acids, and conformational determinants are formed by folding of a polypeptide chain.
- The affinity of the interaction between the combining site of a single antibody molecule and a single epitope is generally represented by the K_a calculated from binding data. Polyvalent antigens contain multiple identical epitopes to which identical antibody molecules can bind. Antibodies can bind to two or, in the case of IgM, up to 10 identical epitopes simultaneously, leading to enhanced avidity of the antibody–antigen interaction.

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- The relative concentrations of polyvalent antigens and antibodies may favor the formation of immune complexes that may deposit in tissues and cause damage.
- Antibody binding to antigen can be highly specific, distinguishing small differences in chemical structures, but cross-reactions may also occur in which two or more antigens may be bound by the same antibody.
- Several changes in the structure of antibodies made by one clone of B cells may occur in the course of an immune response. B cells initially produce only membrane-bound Ig, but in activated B cells and plasma cells, Ig with the same antigen-binding specificity as the original membrane-bound Ig receptor is secreted. Changes in the use of C region gene segments without changes in V regions are the basis of isotype switching, which leads to changes in effector function without a change in specificity. Point mutations in the V regions of an antibody specific for an antigen lead to increased affinity for that antigen (affinity maturation).

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CHAPTER

9

Activation of T Lymphocytes

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The process of T cell activation generates, from a small pool of naive lymphocytes specific for an antigen, a large number of effector cells with the same specificity that function to eliminate that antigen and a population of long-lived memory cells that can rapidly react against the antigen in case it is reintroduced. A fundamental characteristic of the T cell response, like all adaptive immune responses, is that it is highly specific for the antigen that elicits the response. Both the initial activation of naive T cells and the effector phases of T cell-mediated adaptive immune responses are triggered by recognition of antigen by the antigen receptors of T lymphocytes. In Chapter 6, we described the specificity of T cells for peptide fragments, derived from protein antigens, which are bound to and displayed by self major histocompatibility complex (MHC) molecules. In Chapter 7, we described the antigen receptors and other molecules of T cells that are involved in the activation of the cells by antigens, and the biochemical signals initiated by these receptors. In this chapter, we will describe the biology of T cell activation. We begin with a brief overview of T cell activation, discuss the role of costimulators and other signals provided by antigen-presenting cells (APCs) in T cell activation, and describe the sequence of proliferation and differentiation that occurs when $CD4^+$ and $CD8^+$ T cells recognize foreign antigens. The generation and functions of effector $CD4^+$ T cells are described in Chapter 10 and the generation and functions of effector $CD8^+$ T cells in Chapter 11. Thus, Chapters 9, 10, and 11 together cover the biology of T lymphocyte activation and the functions of T lymphocytes in cell-mediated immunity.

OVERVIEW OF T LYMPHOCYTE ACTIVATION

The initial activation of naive T lymphocytes occurs mainly in peripheral (secondary) lymphoid organs, through which these cells normally circulate and where they may encounter antigens presented by mature dendritic cells (Fig. 9.1). Clones of T lymphocytes, each with a different specificity, are generated in the thymus before antigen exposure. Naive T lymphocytes, which have not previously responded to antigens, circulate throughout the body in a resting state, and they acquire powerful functional capabilities only after they are activated. This activation of naive T lymphocytes occurs in specialized lymphoid organs, the lymph nodes, spleen and mucosal lymphoid tissues, where naive lymphocytes and APCs are brought together (see Chapters 2 and 6).

Naive T lymphocytes move around within lymphoid organs transiently interacting with many dendritic cells and stop when they recognize the antigen for which they express specific receptors. Dendritic cells in lymphoid organs simultaneously present many different antigens. T cells are in constant motion, mainly guided by the fibroblast reticular network, a matrix substratum produced by fibroblastic reticular cells in the T cell zone of the lymphoid organs (see Chapter 2). Antigen recognition results in the generation of biochemical signals that lead to rapid arrest of the T cells. This process stabilizes the contact between the T cells and the relevant antigen-expressing APC and allows the activation program of the T cell to be initiated.

Antigen recognition together with other activating stimuli induce several biological responses in T cells: cytokine secretion; proliferation, leading to an increase in the numbers of cells in the antigen-specific clones (called clonal expansion); and differentiation of the naive cells into effector and memory lymphocytes (Fig. 9.2). In addition, the process of T cell activation is associated with

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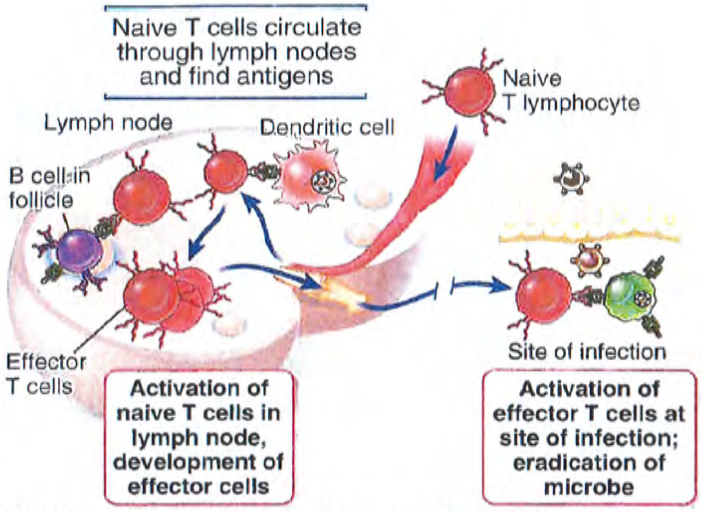


FIGURE 9.1 Activation of naive and effector T cells by antigen. Antigens that are transported by dendritic cells to lymph nodes are recognized by naive T lymphocytes that recirculate through these lymph nodes. The T cells are activated to differentiate into effector cells, which may remain in the lymphoid organs to help B lymphocytes or migrate to sites of infection, where the effector cells are again activated by antigens and perform their various functions, such as macrophage activation.

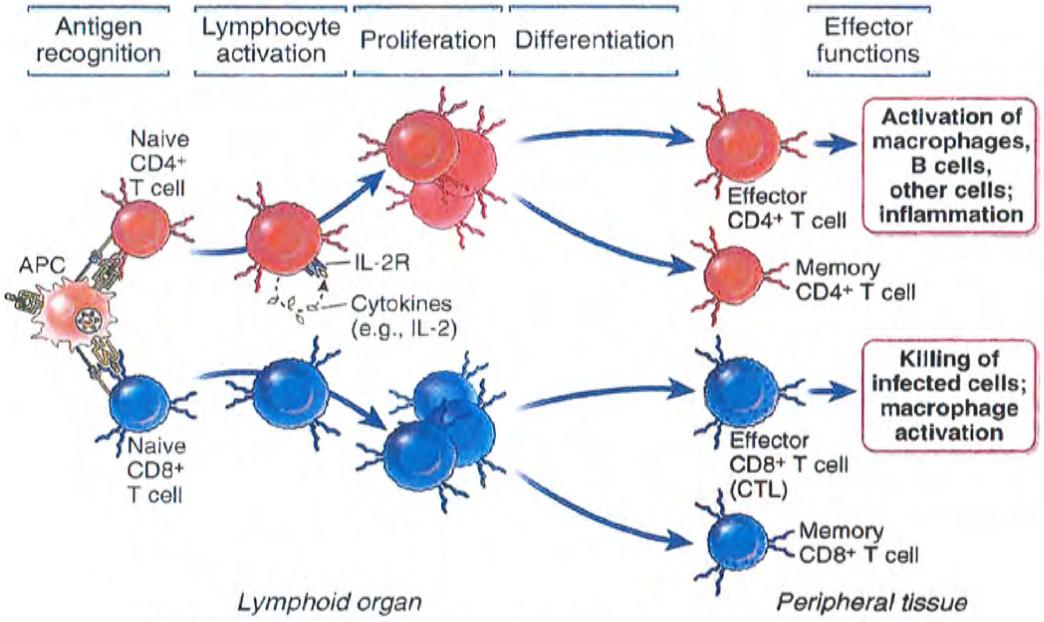


FIGURE 9.2 Sequence of events in T cell responses. Antigen recognition by T cells induces cytokine (e.g., IL-2) secretion, particularly in $CD4^+$ T cells, clonal expansion as a result of cell proliferation, and differentiation of the T cells into effector cells or memory cells. In the effector phase of the response, the effector $CD4^+$ T cells respond to antigen by producing cytokines that have several actions, such as the recruitment and activation of leukocytes and activation of B lymphocytes, while $CD8^+$ CTLs respond by killing other cells and secreting inflammatory cytokines.

changes in the expression of numerous surface molecules, many of which play important roles in inducing and regulating the responses. APCs not only display antigens but also provide the stimuli that guide the magnitude and nature of the T cell response. These stimuli include surface molecules and secreted cytokines. The roles of APCs in instructing T cells how to respond are discussed later in this chapter and in Chapter 10.

Proliferation and differentiation of T cells are regulated by several feedback mechanisms. For example, activated T cells deliver signals back to the APCs, further increasing their ability to activate T cells in a positive feedback loop. At the same time, some surface molecules expressed on activated T cells as well as cytokines secreted by these cells inhibit further activation, and these negative feedback mechanisms serve to establish safe limits to the response.

Effector T cells recognize antigens in lymphoid organs or in peripheral nonlymphoid tissues and are activated to perform functions that are responsible for the elimination of microbes and, in disease states, for tissue damage. Whereas naive cells are activated mainly in secondary lymphoid organs, differentiated effector cells may respond to antigens and carry out their functions in any tissue (see Fig. 9.1). The process of differentiation from naive to effector cells gives the cells the capacity to perform specialized functions and the ability to migrate to any site of infection or inflammation. At these sites, the effector cells again encounter the antigen for which they are specific and respond in ways that serve to eliminate the source of the antigen. Effector T cells of the CD4+ lineage secrete cytokines and express cell surface molecules that can trigger other immune cells. These effector T cells are classified into subpopulations on the basis of their cytokine profiles and functions (see Chapter 10). Some of the $CD4^+$ effector T cells activate macrophages to kill phagocytosed microbes; others secrete cytokines that recruit different types of leukocytes, such as eosinophils and neutrophils, which destroy different types of pathogens; and yet others remain in lymphoid organs and help B cells differentiate into antibody-secreting plasma cells. Cytotoxic T lymphocytes (CTLs), the effector cells of the $CD8^+$ lineage, kill infected cells and tumor cells and also secrete cytokines that activate macrophages and induce inflammation.

Memory T cells that are generated by T cell activation are long-lived cells with an enhanced ability to react against the antigen. These cells are present in the recirculating lymphocyte pool and are abundant in mucosal tissues and the skin as well as in lymphoid organs. After a T cell response wanes, there are many more memory cells of the responding clone than there were naive T cells before the response. These memory cells respond rapidly to subsequent encounters with the antigen and generate new effector cells that eliminate the antigen.

T cell responses decline after the antigen is eliminated. This process of contraction is important for returning the immune system to a state of equilibrium, or homeostasis. It occurs mainly because the majority of antigen-activated effector T cells die by apoptosis. One reason for this is that as the antigen is eliminated, lymphocytes are deprived of survival stimuli that are normally provided by the antigen and by the costimulators and cytokines produced during inflammatory reactions to the antigen. In addition, inhibitory mechanisms activated by antigen recognition function to control the magnitude and duration of the response.

With this overview, we will proceed to a discussion of the signals required for T cell activation and the steps that are common to $CD4^+$ and $CD8^+$ T cells. We will conclude with a discussion of memory cells and the decline of immune responses.

SIGNALS FOR T LYMPHOCYTE ACTIVATION

The proliferation of T lymphocytes and their differentiation into effector and memory cells require antigen recognition, costimulation, and cytokines. In this section, we will summarize the nature of antigens recognized by T cells and discuss specific costimulators and their receptors that contribute to T cell activation. Cytokines are discussed later in this chapter and in Chapter 10.

Recognition of Antigen

Antigen is the necessary first signal for the activation of lymphocytes, ensuring that the resultant immune response is antigen-specific. Because $CD4^+$ and $CD8^+$ T lymphocytes recognize peptide-MHC complexes displayed by APCs, they respond to protein antigens, the natural source of peptides, or to chemicals that bind to and modify proteins. In addition to the T cell receptor (TCR) recognizing peptides displayed by MHC molecules, several other T cell surface proteins participate in the process of T cell activation (see Fig. 7.9). These include adhesion molecules, which stabilize the interaction of the T cells with APCs; coreceptors, which deliver biochemical signals that work in concert with signals from the TCR complex; and costimulators. The biochemical signals delivered by antigen receptors and coreceptors are discussed in Chapter 7.

Activation of naive T cells requires recognition of antigen presented by dendritic cells. This critical role of dendritic cells (DCs) in initiating T cell responses is because these APCs are at the appropriate location to interact with naive T cells (see Chapter 6). In addition, the activation of naive T cells is dependent on signals such as costimulators (discussed later) that are highly expressed by DCs. Protein antigens that cross epithelial barriers or are produced in tissues are captured by DCs and transported to lymph nodes. Antigens that enter the circulation may be captured by DCs in the spleen. Dendritic cells with captured antigens migrate to the T cell zones of draining lymph nodes. As discussed in Chapter 6, both naive T cells and mature DCs are drawn to the T cell zones of secondary lymphoid organs by chemokines produced at these sites that engage the CCR7 chemokine receptor on the cells. By the time the mature DCs reach the T cell areas, they display antigenic peptides on MHC molecules and also express costimulators. Dendritic cells present peptides derived from endocytosed protein antigens mainly in association with class II MHC molecules to naive $CD4^+$ T cells, and peptides derived from cytosolic

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and nuclear proteins displayed by class I MHC molecules to $CD8^+$ T cells (see Chapter 6).

Differentiated effector T cells can respond to antigens presented by cells other than DCs. In humoral immune responses, B cells present antigens to helper T cells and are the recipients of activating signals from the helper cells (see Chapter 12); in cell-mediated immune responses, macrophages present antigens to and respond to $CD4^+$ T cells (see Chapter 10); and virtually any nucleated cell can present antigens to and be killed by $CD8^+$ CTLs (see Chapter 11).

Role of Costimulation in T Cell Activation

The proliferation and differentiation of naive T cells require signals provided by molecules on APCs, called costimulators, in addition to antigen-induced signals (Fig. 9.3). The requirement for costimulatory signals was first suggested by the experimental finding that T cell antigen receptor signaling alone (e.g., induced by anti-CD3 antibodies that cross-link TCR-CD3 complexes, mimicking antigen) resulted in lower responses than those seen with antigens presented by activated APCs. This result indicated that APCs express molecules that work together with antigen for inducing T cell activation. These molecules were called costimulators, and the second signal for T cell activation was called **costimulation**, the first signal being antigen. In the absence of costimulation, T cells that encounter antigens either fail to respond or enter a state of prolonged unresponsiveness (see Chapter 15).

The B7:CD28 Family of Costimulators

The best characterized costimulatory pathway in T cell activation involves the T cell surface receptor CD28, which binds the costimulatory molecules B7-1 (CD80) and B7-2 (CD86) expressed on the surface of activated APCs. CD28 was discovered when stimulatory (agonistic) antibodies against human T cell surface molecules were screened for their ability to enhance T cell responses when added together with an activating anti-CD3 antibody. This was soon followed by the identification of the ligands for CD28, called B7 and later shown to be two homologous proteins, named B7-1 (CD80) and B7-2 (CD86), often collectively called B7. The essential role of CD28 and B7 in T cell activation has been established not only by experiments with cross-linking antibodies but also by the T cell immune deficiency caused by knockout of genes encoding these proteins in mice and by the ability of agents that bind to and block B7 molecules to inhibit T cell responses in experimental animals and in humans. The development of therapeutic agents based on these principles is described later.

B7-1 and B7-2 are structurally similar integral membrane single-chain glycoproteins, each with two extracellular immunoglobulin (Ig)-like domains. CD28 is a disulfide-linked homodimer, each subunit of which has

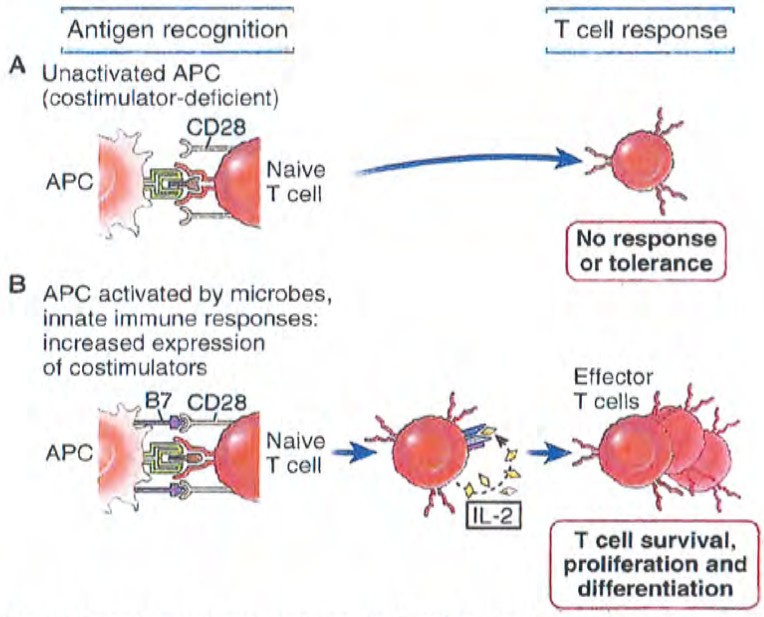


FIGURE 9.3 Functions of costimulators in T cell activation. A, The resting APC (typically dendritic cells presenting self antigens) expresses few or no costimulators and fails to activate naive T cells. (Antigen recognition without costimulation may make T cells unresponsive [tolerant]; we will discuss this phenomenon in Chapter 15.) **B,** Microbes and cytokines produced during innate immune responses activate APCs to express costimulators, such as B7 molecules. The APCs (usually presenting microbial antigens) then become capable of activating naive T cells. Activated APCs also produce cytokines such as IL-12, which stimulate the differentiation of naive T cells into effector cells.

a single extracellular Ig domain. Its cytoplasmic portion contains several tyrosine and proline residues that are involved in binding of adaptor and signaling proteins and in the delivery of activating signals (discussed later). CD28 is expressed on more than 90% of $CD4^+$ T cells and on 50% of $CD8^+$ T cells in humans (and on all naive T cells in mice).

The expression of B7 costimulators is increased by microbial products and during innate immune responses, and ensures that T lymphocytes are activated only when needed. The B7 molecules are expressed mainly on APCs, including dendritic cells, macrophages, and B lymphocytes. They are expressed at low levels on resting APCs and are induced by various stimuli, including microbial products that engage Toll-like receptors and cytokines such as interferon-γ (IFN-γ) produced during innate immune reactions to microbes. The induction of costimulators by microbes and by the cytokines of innate immunity promotes T cell responses to microbial antigens. This illustrates the role of innate immune responses in enhancing adaptive immunity (see Chapter 4). In addition, activated $CD4^+$ T cells themselves enhance the expression of B7 costimulators on the APCs by a pathway dependent on CD40, described later, providing a positive feedback loop that serves to amplify T cell responses. Of all potential APCs, mature dendritic cells express the highest levels of costimulators and, as a result, are the most potent stimulators of naive T cells.

In Chapter 6, we mentioned the essential role of **adjuvants** in inducing primary T cell responses to protein antigens such as vaccines. Many adjuvants are products of microbes, or mimic molecules produced by microbes and necrotic cells, and thus elicit innate immune responses. One of their major functions in T cell activation is to stimulate the expression of costimulators on APCs.

Unactivated, or resting, APCs in normal tissues are capable of presenting self antigens to T cells, but because these tissue APCs express only low levels of costimulators, potentially self-reactive T cells that see the self antigens are not activated and may be rendered permanently unresponsive (see Chapter 15). Regulatory T cells, which are important for tolerance to self antigens (see Chapter 15), are also dependent on B7:CD28-mediated costimulation for their generation and maintenance. It is possible that the low levels of B7 costimulators that are constitutively expressed by resting APCs function together with the self antigens that are displayed by these APCs to maintain regulatory T cells.

CD28 signals work in cooperation with antigen recognition to promote the survival, proliferation, and differentiation of the antigen-specific T cells. Costimulatory signaling via CD28 amplifies signaling pathways that are also induced downstream of the TCR (see Chapter 7) and may trigger additional signals that cooperate with TCR-induced signals (Fig. 9.4). PI3-kinase is recruited to the

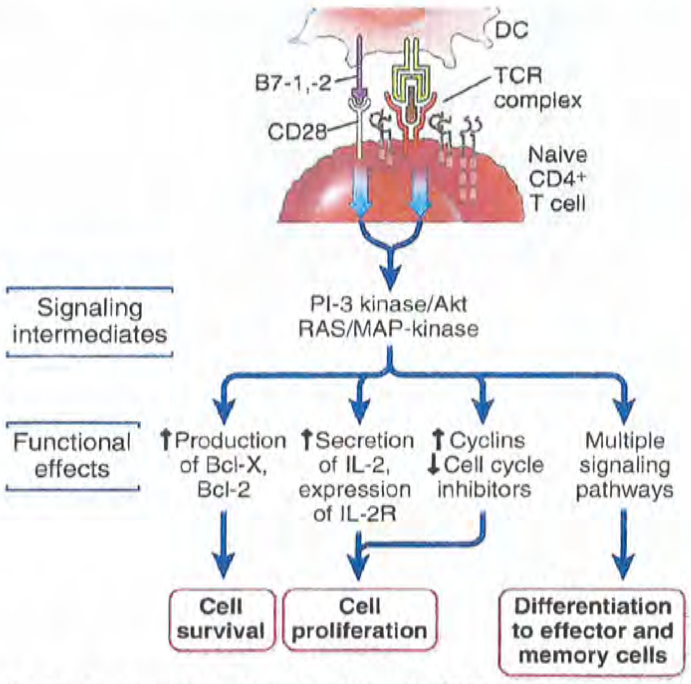


FIGURE 9.4 Mechanisms of T cell costimulation by CD28. CD28 engagement induces signaling pathways that enhance TCR signals or trigger additional signals, all of which stimulate the expression of survival proteins, cytokines, and cytokine receptors; promote cell proliferation; and induce differentiation toward effector and memory cells by activating various transcription factors (not shown, see Chapters 10 and 11).

cytoplasmic tail of CD28, and this in turn activates the downstream prosurvival kinase Akt as well as Itk and PLCγ, which can trigger calcium signaling. CD28 can also contribute to the activation of the JNK MAP kinase via the Rac small G protein and can amplify the activation of the NF-κB pathway. The net results of these signaling pathways in T cells are the increased expression of anti-apoptotic proteins such as Bcl-2 and Bcl-X_L, which promote cell survival; increased metabolic activity; enhanced proliferation; production of cytokines such as IL-2; and differentiation of the naive T cells into effector and memory cells. Previously activated effector and memory T cells are less dependent on costimulation by the B7:CD28 pathway than are naive cells. This property of effector and memory cells enables them to respond to antigens presented by various APCs that may reside in nonlymphoid tissues and may express no or low levels of B7. For instance, the differentiation of $CD8^+$ T cells into effector CTLs requires costimulation, but effector CTLs can kill other cells that do not express costimulators.

Other receptors homologous to CD28 and their ligands homologous to B7 have been identified, and these proteins regulate T cell responses both positively and negatively (Fig. 9.5). Following the demonstration of

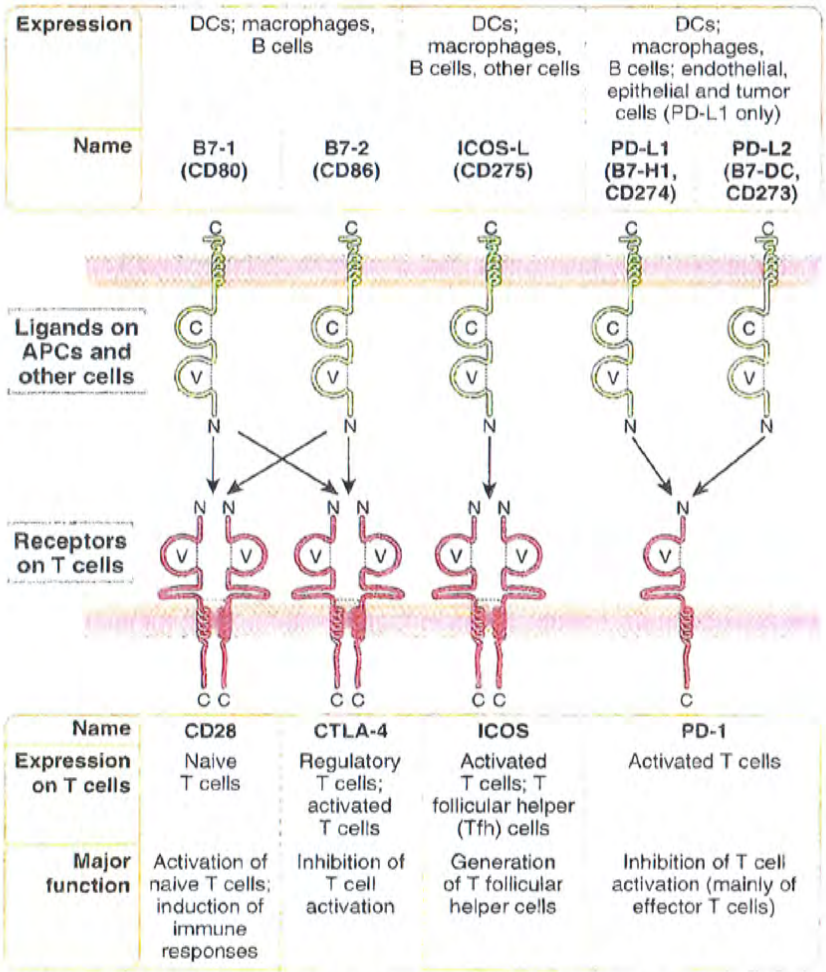


FIGURE 9.5 The major members of the B7 and CD28 families. The known B7 family ligands are expressed on APCs (dendritic cells, macrophages, and B cells), and CD28 family receptors are expressed mainly on T cells. Different CD28 family members stimulate or inhibit different stages and types of T cell responses. The functions of CTLA-4 and PD-1 are discussed in Chapter 15, and the role of ICOS in the generation and function of T follicular helper cells is discussed in Chapter 12. Other widely distributed molecules with limited homology to B7, such as B7-H3 and B7-H4, have been identified, but their physiologic roles are not yet established. Other inhibitory receptors have also been identified, such as BTLA, TIM-3, and TIGIT, but these are not homologous to CD28 and are not shown; some of them are discussed briefly in Chapter 15.

the importance of B7 and CD28, several other proteins structurally related to B7-1 and B7-2 or to CD28 were identified. Some members of the B7:CD28 families are involved in T cell activation (and are thus costimulators) and others are critical inhibitors of T cells (and have been called coinhibitors). The costimulatory receptor other than CD28 whose function is best understood is **ICOS** (inducible costimulator, CD278). Its ligand, called ICOS-L (CD275), is expressed on dendritic cells, B cells, and other cell populations. ICOS plays an essential role in T cell–dependent antibody responses, particularly in the germinal center reaction. It is required for the development and activation of T follicular helper cells, which are essential for the formation of germinal centers and for the generation of high-affinity B cells (see Chapter 12).

The outcome of T cell activation is influenced by a balance between engagement of activating and inhibitory receptors of the CD28 family. The inhibitory receptors of the CD28 family are **CTLA-4** (cytotoxic T lymphocyte antigen 4) and **PD-1** (programmed death 1). (The names of these two proteins do not accurately reflect their distribution or function.) Both receptors are expressed following T cell activation and function to limit immune responses. The concept that a balance between activating and inhibitory receptors controls the magnitude of responses in the immune system was discussed in Chapter 4 in the context of natural killer (NK) cells (see Fig. 4.8). A similar idea is applicable to responses of T and B lymphocytes, although the receptors involved are quite different. Because the inhibitory receptors CTLA-4 and PD-1 are involved in the phenomenon of tolerance, and abnormalities in their expression or function cause autoimmune diseases, we will discuss them in more detail in Chapter 15, when we consider immunological tolerance and autoimmunity. Suffice it to say here that CTLA-4 functions as a competitive inhibitor of CD28 by binding more strongly to B7 molecules, and PD-1 delivers inhibitory signals that block signaling by the antigen receptor and by CD28.

It is likely that the various costimulators and inhibitory receptors of the B7-CD28 family serve distinct roles in different immune responses or at different stages of a response. It is believed that the CD28:B7 interaction is most important for initiating T cell responses by activating naive T cells; ICOS:ICOS-ligand interactions are critical for helper T cell-dependent antibody responses; CTLA-4:B7 interactions inhibit the initial activation of T lymphocytes in secondary lymphoid organs; and PD1:PD-ligand interactions inhibit the activation of effector cells, especially in peripheral tissues. However, these distinctions are not absolute, and there may be overlap in the functions of these pathways.

Other Costimulatory Pathways

Many other T cell surface molecules have been shown to deliver costimulatory signals in vitro, but their physiologic role in promoting T cell activation is less clear than that of the CD28 family. These include proteins of the CD2 family, discussed in Chapter 7, and integrins, discussed in Chapter 3. Several other costimulatory receptors belong to the large tumor necrosis factor (TNF) receptor (TNFR) superfamily, and their ligands are members of the TNF family. Many of the receptors are expressed on activated T cells and regulatory T cells and have been shown to stimulate or to inhibit immune responses under various experimental conditions. Ox40 (CD134) is a TNFR family member, expressed on activated $CD4^+$ and $CD8^+$ T cells, that functions to maintain cell survival and sustained responses. Its ligand, Ox40L, is expressed on activated APCs. 4-1BB (CD137) and CD27 are two other TNFR homologues that are expressed on memory T cells as well as regulatory T cells; their roles in regulating immune responses are not well defined. T cells also express numerous inhibitory receptors in addition to CTLA-4 and PD-1, but their physiologic functions are not well established (see Chapter 15).

The interaction of CD40L on T cells with CD40 on APCs enhances T cell responses by activating the APCs. CD40 ligand (CD40L) is a TNF superfamily membrane protein that is expressed primarily on activated T cells, and CD40 is a member of the TNFR superfamily expressed on B cells, macrophages, and dendritic cells. The functions of CD40 in activating macrophages in cell-mediated immunity and activating B cells in humoral immune responses are described in Chapters 10 and 12, respectively. Activated helper T cells express CD40L, which engages CD40 on the APCs and activates the APCs to make them more potent by enhancing their expression of B7 molecules and secretion of cytokines such as IL-12 that promote T cell differentiation (Fig. 9.6). This phenomenon is sometimes called licensing because activated T cells license APCs to become more powerful stimulators of immune responses. Thus, the CD40 pathway indirectly amplifies T cell responses by inducing costimulators on APCs, but CD40L does not by itself function as a costimulator for T cells.

Therapeutic Costimulatory Blockade

Based on the understanding of costimulatory pathways, therapeutic agents have been developed for controlling injurious immune responses (Fig. 9.7). CTLA-4-Ig, a fusion protein consisting of the extracellular domain of CTLA-4 and the Fc portion of human IgG, binds to B7-1 and B7-2 and blocks the B7:CD28 interaction. The reason for the use of the extracellular domain of CTLA-4 rather than of CD28 to block B7 molecules is that CTLA-4 has a higher affinity for B7 than does CD28. Attachment of the Fc portion of IgG increases the in vivo half-life of the protein (see Chapter 5). CTLA-4-Ig is an approved therapy for rheumatoid arthritis and transplant rejection. Inhibitors of the CD40L:CD40 pathway are in clinical trials for transplant rejection and autoimmune diseases.

Antibodies that block the CTLA-4 and PD-1 inhibitory receptors are approved for the immunotherapy of tumors; they work by preventing CTLA-4 or PD-1 from binding their ligands, thereby reducing inhibition and thus enhancing T cell activation and enabling the cancer-bearing individual to mount more effective antitumor immune responses (see Chapter 18). As one might predict from the role of these inhibitory receptors in maintaining self-tolerance, blocking them for cancer immunotherapy induces autoimmune reactions in many patients.

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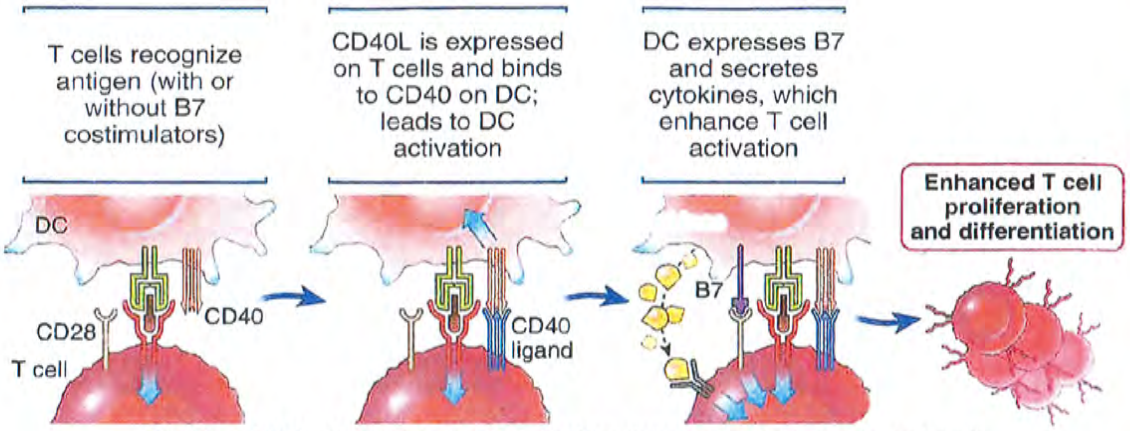


FIGURE 9.6 Role of CD40 in T cell activation. Antigen recognition by T cells together with costimulation *(not shown)* induces the expression of CD40 ligand (CD40L) on the activated T cells. CD40L engages CD40 on APCs and may stimulate the expression of more B7 molecules and the secretion of cytokines that activate T cells. Thus, CD40L on the T cells makes the APCs better at promoting and amplifying T cell activation.

FUNCTIONAL RESPONSES OF T LYMPHOCYTES

The earliest responses of antigen-stimulated T cells consist of changes in the expression of various surface molecules, including cytokine receptors, as well as the secretion of cytokines. These are followed by proliferation of the antigen-specific cells, driven in part by the secreted cytokines, and then by differentiation of the activated cells into effector and memory cells. In the remainder of this chapter, we will describe these steps, their underlying mechanisms, and their functional consequences.

Changes in Surface Molecules During T Cell Activation

After activation by antigen recognition and costimulation, there are characteristic changes in the expression of various surface molecules in T cells (Fig. 9.8). Many of the molecules that are expressed in activated T cells are also involved in the functional responses of the cells. The functionally important molecules induced after recognition of antigen and costimulators are best defined in $CD4^+$ T cells and include the following:

- ***CD69.*** Within a few hours, T cells increase their expression of CD69. This protein binds to and reduces surface expression of the sphingosine 1-phosphate receptor S1PR1, which we described in Chapter 3 as a receptor that mediates egress of T cells from lymphoid organs. The consequence of decreased S1PR1 expression is that activated T cells are retained in lymphoid organs long enough to receive the signals that initiate their proliferation and differentiation into effector and memory cells. At this time, CD69 expression decreases, the activated T cells reexpress high levels of S1PR1, and therefore the effector and memory cells can exit the lymphoid organs.
- ***CD25 (IL-2Rα).*** The expression of this receptor for the growth factor IL-2 enables activated T cells to respond to this cytokine. This process is described later.
- ***CD40 ligand (CD40L, CD154).*** Within 24 to 48 hours after antigen recognition, $CD4^+$ T cells express high levels of the ligand for CD40. The expression of CD40L enables these activated T cells to mediate their key effector functions, which are to help macrophages and B cells. In addition, as discussed earlier, CD40L on the T cells activates dendritic cells to become better APCs, thus providing a positive feedback mechanism for amplifying T cell responses.
- ***CTLA-4 (CD152).*** CTLA-4 is expressed on T cells within 24 to 48 hours after antigen recognition. The function of CTLA-4 is described in Chapter 15 (see Fig. 15.5).
- ***Adhesion molecules and chemokine receptors.*** During activation, T cells reduce expression of molecules that bring them to the lymphoid organs (such as L-selectin

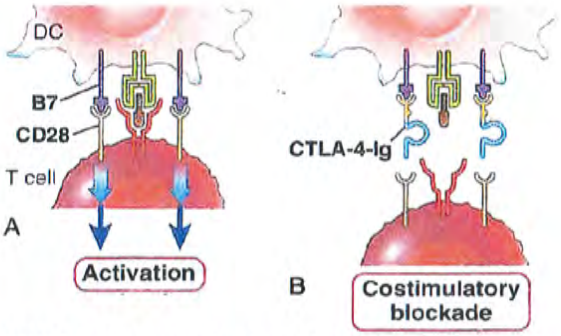


FIGURE 9.7 The mechanism of therapeutic costimulatory blockade. A, The normal T cell response induced by antigen recognition and costimulation mediated by B7-CD28. **B,** A fusion protein consisting of the extracellular portion of CTLA-4 and the Fc tail of an IgG molecule is used to bind to and block B7 molecules, thus preventing their interaction with the activating receptor CD28 and inhibiting T cell activation.

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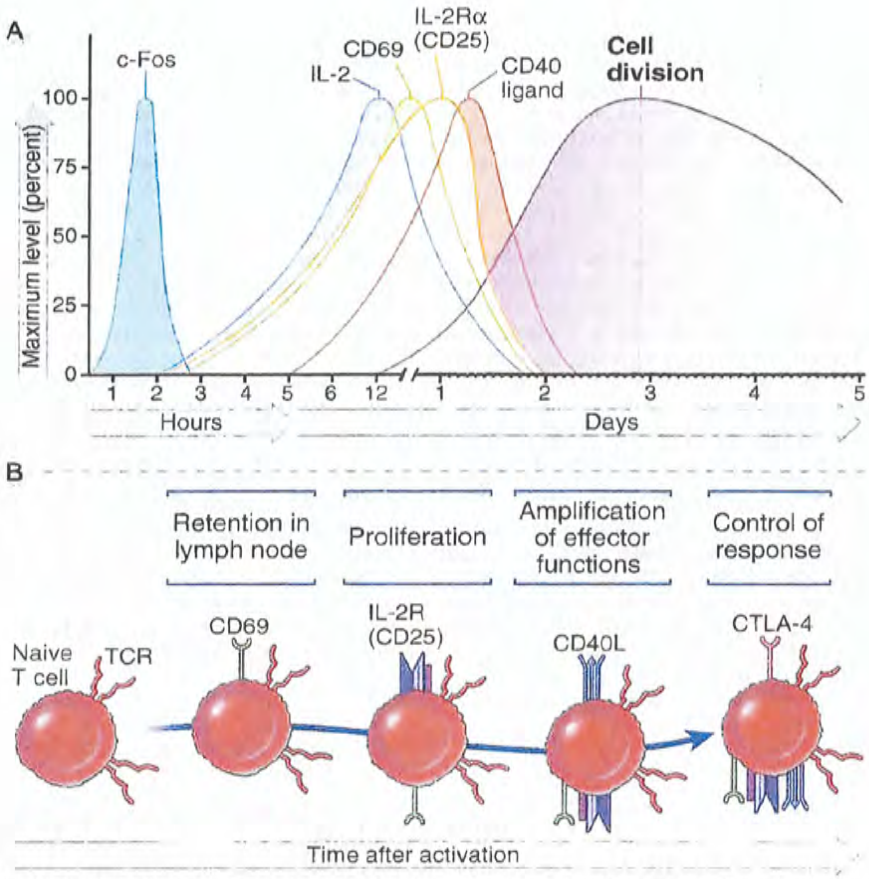


FIGURE 9.8 Changes in surface molecules after T cell activation. A, The approximate kinetics of expression of selected molecules during activation of T cells by antigens and costimulators are shown. The illustrative examples include a transcription factor (c-Fos), a cytokine (IL-2), and surface proteins. These proteins are typically expressed at low levels in naive T cells and are induced by activating signals. CTLA-4 is induced 1 to 2 days after initial activation. The kinetics are estimates and will vary with the nature of the antigen, its dose and persistence, and the type of adjuvant. **B,** The major functions of selected surface molecules are shown and described in the text. *CD40L*, CD40 ligand; *IL-2R*, IL-2 receptor.

[CD62L] and the chemokine receptor CCR7) and increase the expression of molecules that are involved in their migration to peripheral sites of infection and tissue injury (such as the integrins LFA-1 and VLA-4, the ligands for E- and P-selectins, and various chemokine receptors). These molecules and their roles in T cell migration were described in Chapter 3. Activation also increases the expression of CD44, a receptor for the extracellular matrix molecule hyaluronan. Binding of CD44 to its ligand helps retain effector T cells in the tissues at sites of infection and tissue damage.

Cytokines in Adaptive Immune Responses

Numerous cytokines play critical roles in adaptive immune responses. $CD4^+$ helper T cells make the largest amount and variety of these cytokines, but some are also produced by $CD8^+$ T cells and APCs. Cytokines secreted by dendritic cells and other APCs are especially important for the differentiation of naive T cells into effector cells. Different cytokines are involved in the proliferation and differentiation of antigen-stimulated T and B cells and in the effector functions of T cells. Most of these cytokines act on the cells that produce them (autocrine action) or on nearby cells (paracrine action).

The roles of cytokines in the effector functions of T cells are described in Chapters 10 and 11. Here we discuss interleukin-2, the prototype of a T cell-derived cytokine that stimulates T cell responses.

IL-2 Secretion and IL-2 Receptor Expression

Interleukin-2 (IL-2) is a growth, survival, and differentiation factor for T lymphocytes that plays a major role in the proliferation of antigen-stimulated T cells and in the maintenance of functional regulatory T cells. Because

of its ability to support T cell proliferation, IL-2 was originally called T cell growth factor (TCGF). It acts on the same cells that produce it or on adjacent cells (i.e., it functions as an autocrine or paracrine cytokine).

IL-2 is produced mainly by $CD4^+$ T lymphocytes early after recognition of antigen and costimulators. Activation of T cells stimulates transcription of the *Il2* gene and synthesis and secretion of the protein. IL-2 production is rapid and transient, starting within 1 to 2 hours after antigen recognition, peaking at about 8 to 12 hours, and declining by 24 hours. $CD4^+$ T cells secrete IL-2 into the immune synapse formed between the T cell and APC (see Chapter 7). IL-2 receptors (IL-2R) on T cells also tend to localize to the synapse so that the cytokine and its receptor reach sufficiently high local concentrations to initiate cellular responses.

Secreted IL-2 is a 14- to 17-kD globular glycoprotein containing four α helices (Fig. 9.9). It is the prototype of the four-α-helical cytokines that interact with type I cytokine receptors (see Chapter 7).

High-affinity IL-2Rs are transiently expressed on activation of naive and effector T cells; regulatory T cells always express these receptors. The IL-2R consists of three noncovalently associated proteins, IL-2Rα (CD25), IL-2/15Rβ (CD122), and γ_c (CD132). Of the three chains, only IL-2Rα is unique to the IL-2R. The β chain is also part of the IL-15 receptor. The γ chain is shared with a number of cytokine receptors, including those for IL-4, IL-7, IL-9, IL-15, and IL-21, and is therefore called the common γ chain (γ_c). Both the β and γ_c chains engage JAK-STAT signaling pathways (see Chapter 7). IL-2R $\beta\gamma_c$ complexes are expressed at low levels on resting T cells (and on NK cells) and bind IL-2 with a K_d of approximately 10^{-9} M (Fig. 9.10). Expression of IL-2Rα and, to a lesser extent, of IL-2Rβ is increased on activation of naive $CD4^+$ and $CD8^+$ T cells. The α chain associates with the $\beta\gamma_c$ complex to form the complete IL-2R, the IL-2R$\alpha\beta\gamma_c$ complex, which can bind IL-2 more tightly, with a K_d of approximately 10^{-11} M. Therefore, growth stimulation of activated T cells occurs at a similarly low IL-2 concentration. Because both IL-2 secretion and IL-2Rα production occur in response to antigen stimulation, the antigen-activated T cells are the ones that proliferate preferentially in response to the cytokine, compared with bystander cells that have not been activated. IL-2, produced in response to antigen stimulation, is itself a stimulus for induction of IL-2Rα, providing a feedback mechanism by which T cell responses amplify themselves. $CD4^+$ regulatory T cells (see Chapter 15) express the complete IL-2R complex. Chronic T cell stimulation leads to shedding of IL-2Rα, and an increased level of shed IL-2Rα in the serum is a marker of strong antigenic stimulation (e.g., acute rejection of a transplanted organ).



FIGURE 9.9 Structure of IL-2 and its receptor. The crystal structure of IL-2 and its trimeric receptor shows how the cytokine interacts with the three chains of the receptor. *(From Wang X, Rickert M, Garcia KC: Structure of the quaternary complex of interleukin-2 with its α, β, and γc receptors, Science 310:1159–1163, 2005, with the permission of the publishers. Courtesy of Drs. Patrick Lupardus and K. Christopher Garcia, Stanford University School of Medicine, Palo Alto, California.)*

Functions of IL-2

The biology of IL-2 is fascinating because it plays critical roles in both promoting and controlling T cell responses and functions (Fig. 9.11).

- ***IL-2 stimulates the survival, proliferation, and differentiation of antigen-activated T cells.*** IL-2 promotes survival of cells by inducing the antiapoptotic protein Bcl-2. It stimulates cell cycle progression through activation of the mTOR signaling pathway (see Chapter 7), which induces the synthesis of cyclins and relieves a block in cell cycle progression through degradation of the cell cycle inhibitor p27. In addition, IL-2 increases production of effector cytokines, such as IFN-γ and IL-4, by T cells.
- ***IL-2 is required for the survival and function of regulatory T cells,*** which suppress immune responses against self and other antigens. These cells constitutively express the complete IL-2 receptor, including the α chain CD25, and are thus poised to respond to IL-2. Knockout mice lacking IL-2 or IL-2 receptor α or β chains develop uncontrolled T and B cell proliferation and autoimmune disease because of defects in regulatory T cells. This finding suggests that other growth factors can replace IL-2 for expansion of effector T cells, but no other cytokine can replace IL-2 for the maintenance of functional regulatory T cells. We will discuss this role of IL-2 in more detail in Chapter 15, when we describe the properties and functions of regulatory T cells. An interesting feature of this function of IL-2 is that regulatory T cells do not produce significant amounts of the cytokine, implying that they depend for their survival on IL-2 made by other T cells responding to antigens (see Fig. 9.11B).

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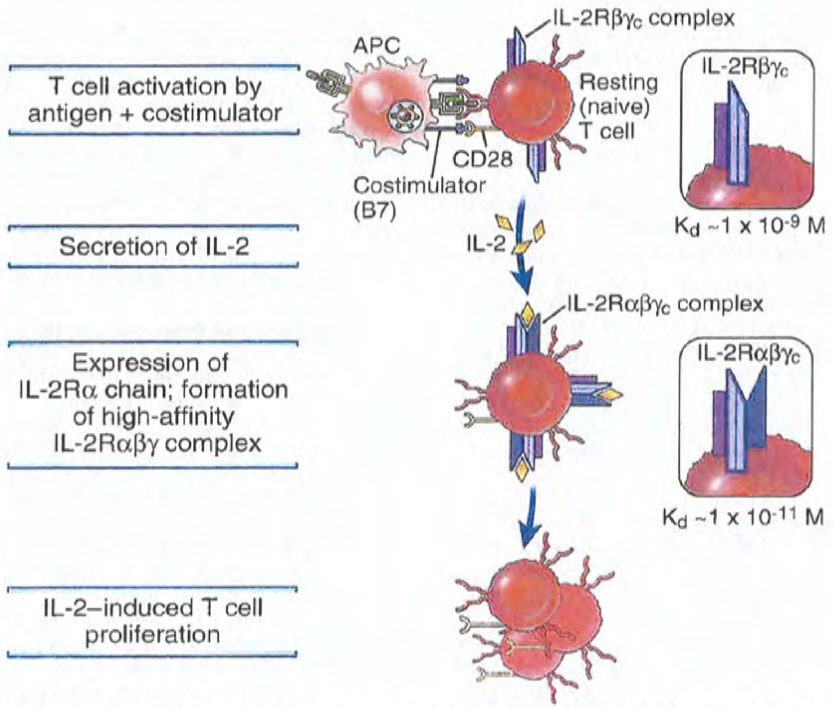


FIGURE 9.10 Regulation of IL-2 receptor expression. Resting (naive) T lymphocytes express the IL-2Rβγc complex, which has a moderate affinity for IL-2. Activation of the T cells by antigen, costimulators, and IL-2 itself leads to expression of the IL-2Rα chain (also called CD25) and increased levels of the high-affinity IL-2Rαβγc complex.

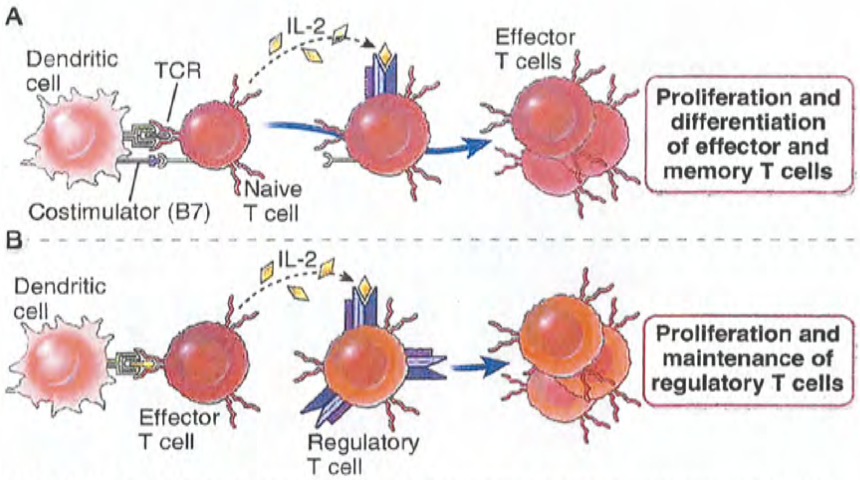


FIGURE 9.11 Biologic actions of IL-2. A, IL-2 stimulates the survival, proliferation, and differentiation of T lymphocytes, acting as an autocrine growth factor, leading to the generation of effector and memory cells. **B,** IL-2 also promotes the survival of regulatory T cells and maintains their functional capability, and thus controls immune responses (e.g., against self antigens).

- IL-2 also has been shown to stimulate the proliferation and differentiation of NK cells and B cells in vitro. The physiologic importance of these actions is not established.

Clonal Expansion of T Cells

T cell proliferation in response to antigen recognition is mediated by a combination of signals from the antigen receptor, costimulators, and autocrine growth factors, primarily IL-2. The expansion of antigen-specific clones that results from this proliferation converts the small pool of naive antigen-specific lymphocytes into the large number of cells required to eliminate the antigen. Before antigen exposure, the frequency of naive T cells specific for any antigen is 1 in 10^5 to 10^6 lymphocytes or less. After microbial antigen exposure, the frequency of $CD8^+$ T cells specific for that microbe may increase to as many as 1 in 3 $CD8^+$ T lymphocytes, representing a greater than 50,000-fold expansion of antigen-specific $CD8^+$ T cells, and the number of specific $CD4^+$ cells increases up to 1 in 100 $CD4^+$ lymphocytes, or a 1000-fold expansion (Fig. 9.12). Studies in mice first showed this tremendous expansion of the antigen-specific population in some acute viral infections, and remarkably it occurred within as little as 1 week after infection. Equally remarkable was the finding that during this massive antigen-specific clonal expansion, bystander T cells not specific for the virus did not proliferate. The expansion of T cells specific for the Epstein-Barr virus and human immunodeficiency virus (HIV) in acutely infected humans is also on this order of magnitude.

Differentiation of Activated T Cells Into Effector Cells

Many of the progeny of the antigen-stimulated T cells differentiate into effector cells. As summarized in the overview of this chapter, effector cells of the $CD4^+$ lineage express surface molecules and secrete cytokines that activate other cells (B lymphocytes, macrophages, and dendritic cells). Whereas naive $CD4^+$ T cells produce mostly IL-2 on activation, effector $CD4^+$ T cells are capable of producing a large number and variety of cytokines that have diverse biologic activities. Effector $CD8^+$ cells are cytotoxic and kill infected cells. Because there are important differences in effector cells of the $CD4^+$ and $CD8^+$ lineages, we will describe their development and functions separately in Chapters 10 and 11.

Development and Properties of Memory T Cells

T cell-mediated immune responses to an antigen usually result in the generation of memory T cells specific for that antigen, which may persist for years, even a lifetime. Memory cells provide effective defense against pathogens that are prevalent in the environment and may be repeatedly encountered. The success of vaccination is attributed in large part to the ability to generate memory cells on initial antigen exposure. Edward Jenner's classic experiment of successful vaccination of a child against smallpox is a demonstration of a memory response. Despite the importance of immunologic memory, many fundamental questions about the generation of memory cells have still not been answered.

Memory cells may develop from effector cells along a linear pathway, or effector and memory populations follow divergent differentiation and are two alternative fates of lymphocytes activated by antigen and other stimuli (Fig. 9.13). The mechanisms that determine whether an individual antigen-stimulated T cell will become a short-lived effector cell or enter the long-lived memory cell pool are not established. The signals that drive the development of memory cells are also not fully understood. One possibility is that the types of

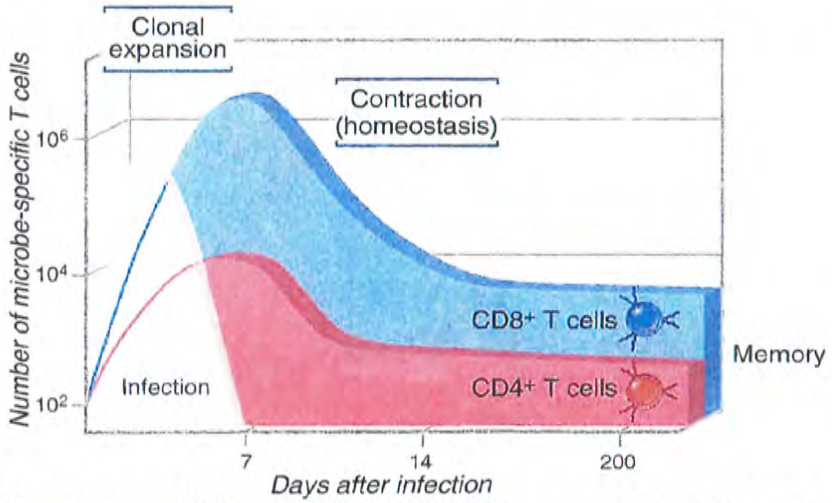


FIGURE 9.12 Clonal expansion of T cells. The numbers of $CD4^+$ and $CD8^+$ T cells specific for microbial antigens and the expansion and decline of the cells during immune responses are illustrated. The numbers are approximations based on studies of model microbial and other antigens in inbred mice.

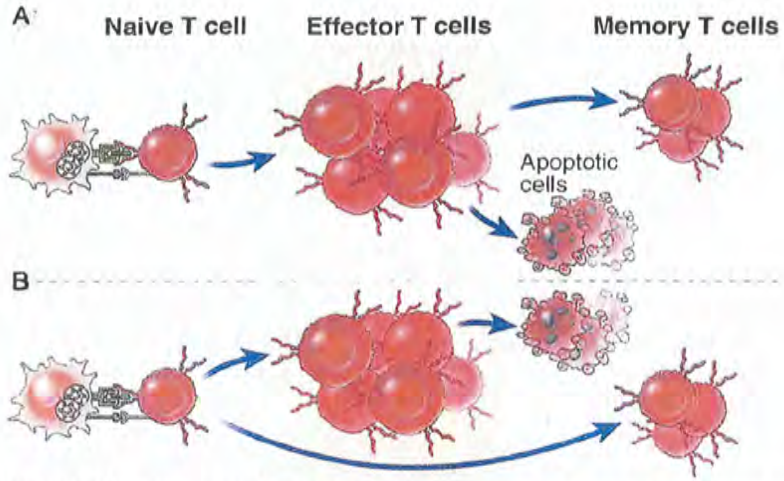


FIGURE 9.13 Development of memory T cells. In response to antigen and costimulation, naive T cells differentiate into effector and memory cells. **A,** According to the linear model of memory T cell differentiation, most effector cells die and some survivors develop into the memory population. **B,** According to the branched differentiation model, effector and memory cells are alternative fates of activated T cells.

transcription factors that are induced during T cell activation influence the choice between the development of effector or memory cells. For instance, expression of the transcription factor T-bet drives differentiation toward effector cells in $CD4^+$ and $CD8^+$ populations, whereas expression of a different transcription factor Blimp-1 promotes the generation of memory cells. Whether induction of these transcription factors is a random (stochastic) process or is influenced by specific external signals is not yet clear.

Properties of Memory T Cells

The defining properties of memory cells are their ability to survive in a quiescent state after antigen is eliminated and to mount larger and more rapid responses to antigens than do naive cells. Several features of memory cells account for these properties.

- ***Memory cells express increased levels of antiapoptotic proteins, which may be responsible for their prolonged survival.*** Whereas naive T cells live for weeks or months and are replaced by mature cells that develop in the thymus, memory T cells may survive for years. Thus, as humans age in an environment in which they are constantly exposed and responding to infectious agents, the proportion of memory cells induced by these microbes compared with naive cells progressively increases. In individuals older than 50 years of age, half or more of circulating T cells may be memory cells (see Fig. 2.10, Chapter 2). The antiapoptotic proteins that promote memory cell survival include Bcl-2 and Bcl-X_L, which block apoptosis induced by a deficiency of survival signals (see Fig. 15.7). The presence of these proteins allows memory cells to survive even after antigen is eliminated and innate immune responses have subsided, when the normal signals for T cell survival and proliferation are no longer present.
- ***Memory cells respond more rapidly to antigen stimulation than do naive cells specific for the same antigen.*** For example, studies in mice have shown that naive T cells differentiate into effector cells in response to antigen in 5 to 7 days, but memory cells acquire effector functions within 1 to 3 days (see Fig. 1.2, Chapter 1). A possible explanation for this accelerated differentiation is that the gene loci for cytokines and other effector molecules are fixed in an accessible chromatin state in memory cells, in part because of changes in methylation and acetylation of histones. These epigenetically modified genes are poised to respond rapidly to antigen challenge.
- ***The number of memory T cells specific for any antigen is greater than the number of naive cells specific for the same antigen.*** As we discussed earlier, proliferation leads to a large clonal expansion in all immune responses and differentiation of naive lymphocytes into effector cells, most of which die after the antigen is eliminated. The memory cells that remain from the expanded clone are typically 10- to 100-fold more numerous than the pool of naive cells before antigen encounter. The increased clone size is one reason that antigen challenge in a previously immunized individual induces a more robust response than the first immunization in a naive individual. As expected, the size of the memory pool is proportional to the size of the naive antigen-specific population.
- ***Memory cells are able to migrate to peripheral tissues and respond to antigens at these sites.*** As we discussed in Chapter 3, naive T cells migrate preferentially to secondary lymphoid organs, but memory cells can migrate to virtually any tissue. These differences are related to differences in the expression of adhesion molecules and chemokine receptors. In addition, memory T cells are less dependent on costimulation than are naive cells, allowing memory cells to respond

to antigens presented by a wide range of APCs in peripheral tissues; in contrast, as we have discussed earlier, naive T cells are dependent on antigen presentation by mature dendritic cells in lymphoid organs.

- ***Memory cells undergo slow proliferation, and this ability to self-renew may contribute to the long life span of the memory pool.*** The cycling of these cells may be driven by cytokines. Because of the capacity for self-renewal, memory cells have been likened to stem cells.
- ***The maintenance of memory cells is dependent on cytokines but does not require antigen recognition.*** The most important cytokine for the maintenance of memory $CD4^+$ and $CD8^+$ T cells is IL-7, which also plays a key role in early lymphocyte development (see Chapter 8) and in the survival of naive T cells (see Chapter 2). Predictably, high expression of the IL-7 receptor (CD127) is characteristic of memory T cells. Memory $CD8^+$ T cells also depend on the related cytokine IL-15 for their survival. IL-7 and IL-15 induce the expression of antiapoptotic proteins and stimulate low-level proliferation, both of which maintain populations of memory T cells for long periods. The ability of memory cells to survive without antigen recognition has been best demonstrated by experiments in mice in which antigen receptors are genetically deleted after mature lymphocytes have developed. In these mice, the number of naive lymphocytes drops rapidly, but memory cells are maintained.

The most reliable phenotypic markers for memory T cells appear to be the surface expression of the IL-7 receptor and a protein of unknown function called CD27, and the absence of markers of naive and recently activated T cells (see Table 2.3). In humans, most naive T cells express the 200-kD isoform of the surface molecule CD45 called CD45RA (for "restricted A"), and most memory T cells express a 180-kD isoform of CD45 called CD45RO (see Chapter 2).

Both $CD4^+$ and $CD8^+$ memory T cells are heterogeneous and can be subdivided into subsets based on their homing properties and functions. **Central memory** T cells express the chemokine receptor CCR7 and the adhesion molecule L-selectin and home mainly to lymph nodes. They have a limited capacity to perform effector functions when they encounter antigen, but they undergo brisk proliferative responses and generate many effector cells on antigen challenge. **Effector memory** T cells, on the other hand, do not express CCR7 or L-selectin and home to peripheral sites, especially mucosal tissues. On antigenic stimulation, effector memory T cells produce effector cytokines such as IFN-γ or rapidly become cytotoxic, but they do not proliferate much. This effector subset, therefore, is poised for a rapid response to repeated exposure to a microbe, but complete eradication of the infection may also require large numbers of effectors generated from the pool of central memory T cells. It is unclear if all memory T cells can be classified into central and effector memory cells.

Some memory T cells migrate into nonlymphoid tissues and survive in these tissues for long periods. These **tissue-resident memory cells** provide rapid responses to recurrent entry of microbes into tissues. The cells express high levels of CD69, the molecule that reduces expression of the sphingosine 1-phosphate receptor S1PR1 (see Chapter 3). As a result, these cells do not respond to the high concentrations of S1P in the lymph and blood, facilitating their retention in tissues.

Memory T cells are also heterogeneous in terms of cytokine profiles. For example, some $CD4^+$ memory T cells may be derived from precursors before commitment to the Th1, Th2, or Th17 phenotype (described in Chapter 10), and when activated by reexposure to antigen and cytokines, they can differentiate into any of these subsets. Other memory T cells may be derived from differentiated Th1, Th2, or Th17 effectors and retain their respective cytokine profiles on reactivation. Memory $CD8^+$ T cells may also exist that have some of the phenotypic characteristics of differentiated CTLs.

DECLINE OF T CELL RESPONSES

Elimination of antigen leads to contraction of the T cell response, and this decline is responsible for maintaining homeostasis in the immune system. There are several reasons that the response declines. As the antigen is eliminated and the innate immune response associated with antigen exposure abates, the signals that normally keep activated lymphocytes alive and proliferating are no longer present. As mentioned earlier, costimulation and growth factors like IL-2 stimulate expression of the antiapoptotic proteins Bcl-2 and Bcl-X_L in the activated lymphocytes, and these proteins keep cells viable. As the level of costimulation and the amount of available IL-2 decrease, the levels of antiapoptotic proteins in the cells drop. At the same time, growth factor deprivation activates sensors of cellular stress (such as the BH3-only protein Bim), which trigger the mitochondrial pathway of apoptosis and are no longer opposed by the antiapoptotic proteins (see Fig. 15.9, Chapter 15). The net result of these changes is that most of the cells that were produced by activation die and the generation of newly activated cells declines, so the pool of antigen-activated lymphocytes contracts.

There has been much interest in the possibility that various regulatory mechanisms contribute to the normal contraction of immune responses against pathogens and other foreign antigens. Such mechanisms might include the inhibitory receptors CTLA-4 and PD-1, apoptosis induced by death receptors of the TNF receptor superfamily (such as TNFRI and Fas), and regulatory T cells. However, the major roles of these inhibitory mechanisms may be to prevent immune responses to self antigens (see Chapter 15).

SUMMARY

- T cell responses are initiated by signals that are generated by TCR recognition of peptide–MHC complexes on the surface of an APC and through signals provided at the same time by costimulators expressed on APCs.

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- The best-defined costimulators are members of the B7 family, which are recognized by receptors of the CD28 family expressed on T cells. The expression of B7 costimulators on APCs is increased by encounter with microbes, providing a mechanism for generating optimal responses against infectious pathogens. Some members of the CD28 family inhibit T cell responses, and the outcome of T cell antigen recognition is determined by the balance between engagement of activating and inhibitory receptors of this family.
- T cell responses to antigen and costimulators include changes in the expression of surface molecules, synthesis of cytokines and cytokine receptors, cellular proliferation, and differentiation into effector and memory cells.
- The surface molecules whose expression is induced on T cell activation include proteins that are involved in retention of T cells in lymphoid organs, growth factors for cytokines, effector and regulatory molecules, and molecules that influence migration of the T cells.
- Shortly after activation, T cells produce the cytokine IL-2 and express high levels of the functional IL-2R. IL-2 drives the proliferation of the cells, which can result in marked expansion of antigen-specific clones.
- Some activated T cells may differentiate into memory cells, which survive for long periods and respond rapidly to antigen challenge. The maintenance of memory cells is dependent on cytokines such as IL-7, which may promote the expression of antiapoptotic proteins and stimulate low-level cycling. Memory T cells are heterogeneous and consist of populations that differ in migration properties and functional responses.
- T cell responses decline after elimination of the antigen, thus returning the system to rest. The decline is largely because the signals for continued lymphocyte activation are also eliminated.

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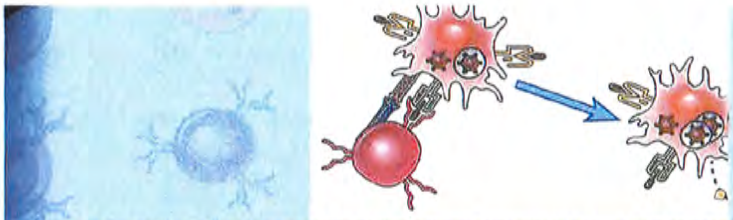
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CHAPTER

11

Differentiation and Functions of CD8⁺ Effector T Cells

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Viruses have evolved to use various cell surface molecules to gain entry into host cells and to use the host cell's genetic and protein synthetic machinery to replicate and disseminate from one cell to another. Viruses can infect and survive in a wide variety of cells. The viruses cannot be destroyed if the infected cells are not phagocytes with intrinsic lysosomal microbicidal mechanisms. Even in phagocytes, if the viruses are in the cytosol, they are inaccessible to these killing mechanisms. In these situations, the only way to eradicate the established infection is to kill the infected cell, crippling the ability of the virus to survive and replicate. In the adaptive immune system, this function of killing cells harboring viruses is mediated by **cytotoxic T lymphocytes (CTLs)**, the effector cells of the CD8⁺ lineage (see Fig. 10.1B). The same mechanism is used to eliminate phagocytes containing ingested bacteria that escape from phagosomes into the cytosol and are no longer susceptible to the killing activity of the phagocytes. In innate immune reactions, the same function of killing infected cells is mediated by natural killer (NK) cells (see Chapter 4).

In addition to their role in defense against microbes, another important function of CD8⁺ CTLs is the eradication of tumors. CTLs also play critical roles in the acute rejection of organ allografts.

In Chapter 6, we discussed the nature of the peptide–MHC complexes that are recognized by CD8⁺ T cells. We discussed the early steps of activation of T cells in Chapter 9. There we mentioned some of the features of activation of CD8⁺ cells, including their remarkable clonal expansion following activation by antigen and other signals. The differentiation of naive CD8⁺ cells, which lack killing ability, into functional CTLs has several special characteristics. In this chapter, we will describe how CTLs are generated from naive CD8⁺ T cells and how they kill other cells, and then discuss the roles of CTLs in host defense.

DIFFERENTIATION OF CD8⁺ T CELLS INTO CYTOTOXIC T LYMPHOCYTES

Differentiation of CD8⁺ T cells into effector CTLs involves acquisition of the machinery to kill target cells. The infected or tumor cell that is killed by CTLs is commonly called the target cell. Naive CD8⁺ cells recognize antigens but need to proliferate and differentiate to generate a sufficiently large pool of CTLs to destroy the source of the antigen. Within the cytoplasm of differentiated CTLs are numerous modified lysosomes (called granules) that contain proteins, including perforin and granzymes, whose function is to kill target cells (described later). In addition, differentiated CTLs are capable of secreting cytokines, mostly interferon-γ (IFN-γ), that activate phagocytes.

The molecular events in CTL differentiation involve transcription of genes encoding these effector molecules. Two transcription factors that are required for this program of new gene expression are T-bet (which we discussed in relationship to Th1 differentiation in Chapter 10) and comesodermin, which is structurally related to T-bet. T-bet and eomesodermin contribute to the high-level expression of perforin, granzymes, and some cytokines, especially IFN-γ.

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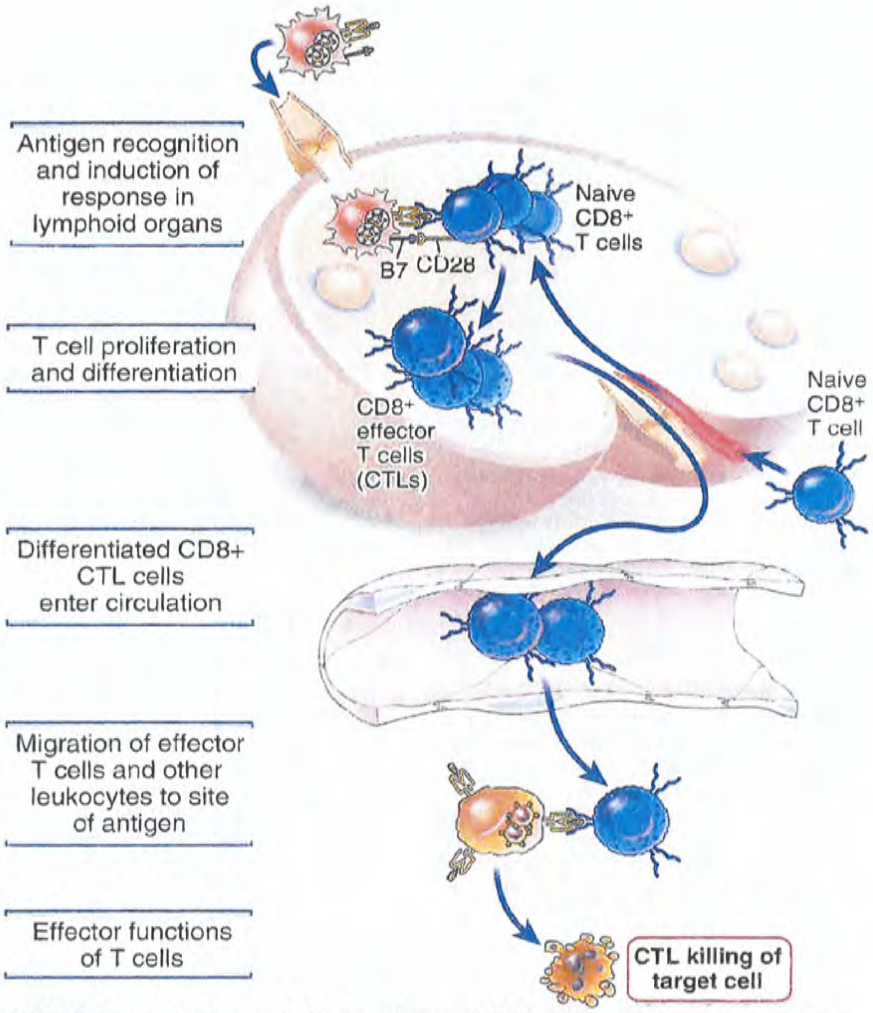


FIGURE 11.1 Induction and effector phases of CD8⁺ T cell responses. CD8⁺ T cells recognize antigens presented by dendritic cells in peripheral lymphoid organs and are stimulated to proliferate and differentiate into effector cells (cytotoxic T lymphocytes, CTLs) and memory cells. The CTLs migrate to tissues at sites of infection, tumor growth, or graft rejection, where they recognize the antigen and respond by killing the cells where the antigen is produced.

The activation of naive CD8⁺ T cells requires antigen recognition and second signals and proceeds in steps similar to those for CD4⁺ T cell responses (Fig. 11.1). However, the activation of naive CD8⁺ T cells has two unique features—it is often dependent on the cross-presentation pathway of antigen presentation by a specialized subset of dendritic cells and may also require help from CD4⁺ T cells.

Nature of Antigen and Antigen-Presenting Cells for Activation of CD8⁺ T Lymphocytes

The activation of naive CD8⁺ T cells, like that of naive CD4+ T cells, is best initiated by antigens presented by dendritic cells. This requirement raises the problem that the antigens recognized by CD8⁺ T cells may be viruses that infect many cell types, including cells other than dendritic cells, or they may be antigens of tumors that are also derived from a variety of cell types. The class I MHC pathway of antigen presentation to CD8⁺ T cells requires that protein antigens be present in the cytosol of the antigen-containing cells so that these proteins can be degraded in proteasomes and can then enter the endoplasmic reticulum via the TAP transporter. When a virus infects a particular cell type such as a liver cell, viral antigens will be presented by class I MHC molecules in that cell. But most viruses are not able to infect DCs, and therefore proteins encoded by those viruses will not be

made in the cytosol of the DCs. Nevertheless, naive T cells respond best to antigens presented by DCs. As we discussed in Chapter 6, the immune system deals with this problem by the process of cross-presentation. In this process, specialized dendritic cells ingest infected cells, tumor cells, or proteins expressed by these cells, transfer the protein antigens into the cytosol, and process the antigens to enter the class I MHC antigen presentation pathway for recognition by $CD8^+$ T cells (see Fig. 6.17). Only some subsets of dendritic cells are efficient at cross-presentation, and, therefore, these dendritic cell subsets are crucial for naive $CD8^+$ T cell activation. Results from experiments in mice suggest that the most efficient cross-presenting APCs are the lymphoid tissue dendritic cells that express CD8 or the peripheral tissue subset that expresses the CD103 integrin (see Chapter 6). The corresponding specialized cross-presenting dendritic cells in human tissues express high levels of CD141, also known as BDCA-3. In addition, plasmacytoid dendritic cells may also cross-present proteins derived from viruses to naive $CD8^+$ T cells.

In addition to presenting antigens in the form of peptide-MHC complexes, dendritic cells likely also provide costimulation via B7 or other molecules (see Chapter 9).

Role of Helper T Cells

The full activation of naive $CD8^+$ T cells and their differentiation into functional CTLs and memory cells may require the participation of $CD4^+$ helper cells. The requirement for helper cells varies according to the type of antigen exposure. In the setting of a strong innate immune response to a microbe, or if APCs are directly infected by the microbe, $CD4^+$ T cell help may not be critical. $CD4^+$ helper T cells are required for $CD8^+$ T cell responses to latent viral infections, organ transplants, and tumors, all of which tend to elicit relatively weak innate immune reactions. The varying importance of $CD4^+$ T cells in the development of CTL responses is illustrated by studies with mice that lack helper T cells. In these mice, some viral infections fail to generate effective CTLs or $CD8^+$ memory cells and are not eradicated, whereas other viruses do stimulate effective CTL responses. A lack of $CD4^+$ T cell helper function accounts for the defects in CTL generation seen in individuals infected with HIV, which infects and eliminates only $CD4^+$ T cells. There is also evidence that $CD4^+$ helper cells are more important for the generation of $CD8^+$ memory T cells than for the differentiation of naive $CD8^+$ T cells into effector CTLs.

Helper T cells promote $CD8^+$ T cell activation by several mechanisms (Fig. 11.2). Helper T cells can secrete cytokines that stimulate the differentiation of $CD8^+$ T cells. The nature of these cytokines is discussed in the section that follows. Activated helper T cells express CD40 ligand (CD40L), which may bind to CD40 on antigen-loaded dendritic cells. This interaction activates the APCs to make them more efficient at stimulating the differentiation of $CD8^+$ T cells, in part by increasing the expression of costimulators. This process has been termed *licensing* of the APCs.

Role of Cytokines

Several cytokines contribute to the differentiation of $CD8^+$ T cells and the maintenance of effector and memory cells of this lineage.

- IL-2 produced by the $CD8^+$ T cells themselves or by $CD4^+$ helper cells promotes proliferation of the $CD8^+$ T cells and their differentiation into CTLs and memory cells. $CD8^+$ cells express the β and γ chains of the IL-2 receptor and may express high levels of the α chain transiently after activation (see Chapter 9).

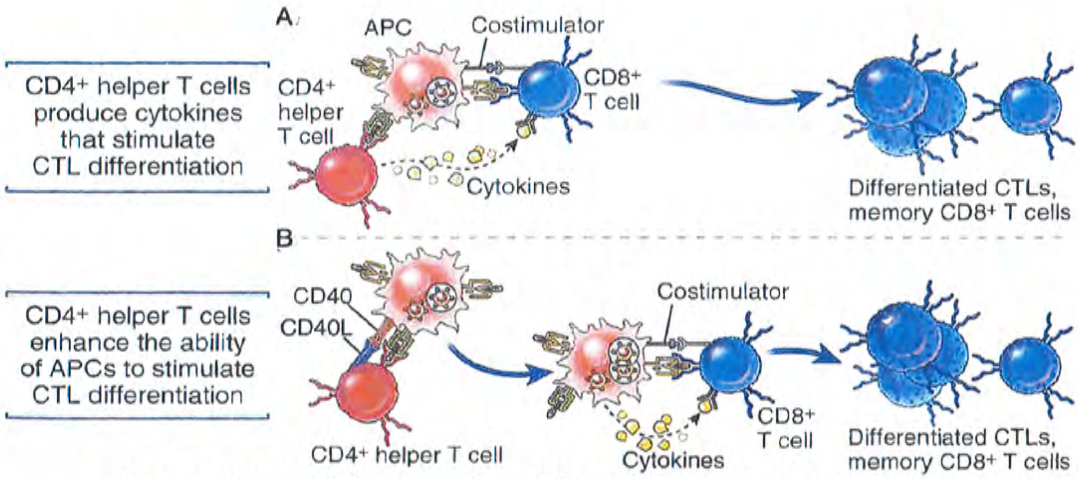


FIGURE 11.2 Role of helper T cells in the differentiation of $CD8^+$ T lymphocytes. $CD4^+$ helper T cells promote the development of $CD8^+$ CTLs and memory cells by secreting cytokines that act directly on the $CD8^+$ cells **(A)** or by activating APCs to become more effective at stimulating the differentiation of the $CD8^+$ T cells, e.g., by increasing the expression of costimulators on the APCs **(B)**.

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- IL-12 and type I IFNs have both been shown to stimulate the differentiation of naive CD8⁺ T cells into effector CTLs. These cytokines may be produced by different dendritic cell populations during the innate immune response to viral and some bacterial infections. Recall that the same cytokines are involved in the differentiation of CD4⁺ T cells into Th1 cells. The cytokines promote development of these two effector populations by stimulating expression of the related transcription factors T-bet (for both Th1 cells and CTLs) and eomesodermin (for CTLs).
- IL-15 is important for the survival of memory CD8⁺ T cells. It may be produced by many cell types, including dendritic cells. Mice lacking IL-15 show a significant loss of memory CD8⁺ T cells.
- IL-21 produced by activated CD4⁺ T cells plays a role in the induction of CD8⁺ effector and memory cells.

Inhibition of CD8⁺ T Cell Responses: T Cell Exhaustion

In some chronic viral infections, CTL effector responses are generated, but they are then gradually extinguished, a phenomenon that is called exhaustion (Fig. 11.3). The term *exhaustion* has been used to imply that the effector response starts but is shut down (unlike in *tolerance*, when lymphocytes fail to develop into effector cells). This phenomenon of exhaustion was first described in a chronic viral infection in mice and was implicated in the prolonged persistence of the virus.

T cell exhaustion develops as a result of persistent antigen exposure. Exhausted CD8⁺ T cells have numerous functional defects, including decreased proliferation, reduced production of IFN-γ, and poor cytotoxic activity, and are thus unable to clear infections. The cells express increased levels of multiple inhibitory receptors, notably PD-1 (see Chapter 9), but also CTLA-4, Tim-3, Lag-3, and others. The cells also express transcription factors associated with effector and memory cells, including T-bet and eomesodermin, but they remain functionally inactive. Blocking PD-1 reverses the inactive state, suggesting that exhaustion may be caused by inhibitory signals through PD-1, and perhaps other inhibitory receptors. The same phenomenon of T cell exhaustion may contribute to the chronicity of some viral infections in humans, such as HIV and hepatitis C virus (HCV), and to the ability of some tumors to evade the immune response (see Chapter 18). The phenomenon of T cell exhaustion may have evolved to attenuate the tissue-damaging consequences of chronic infection.

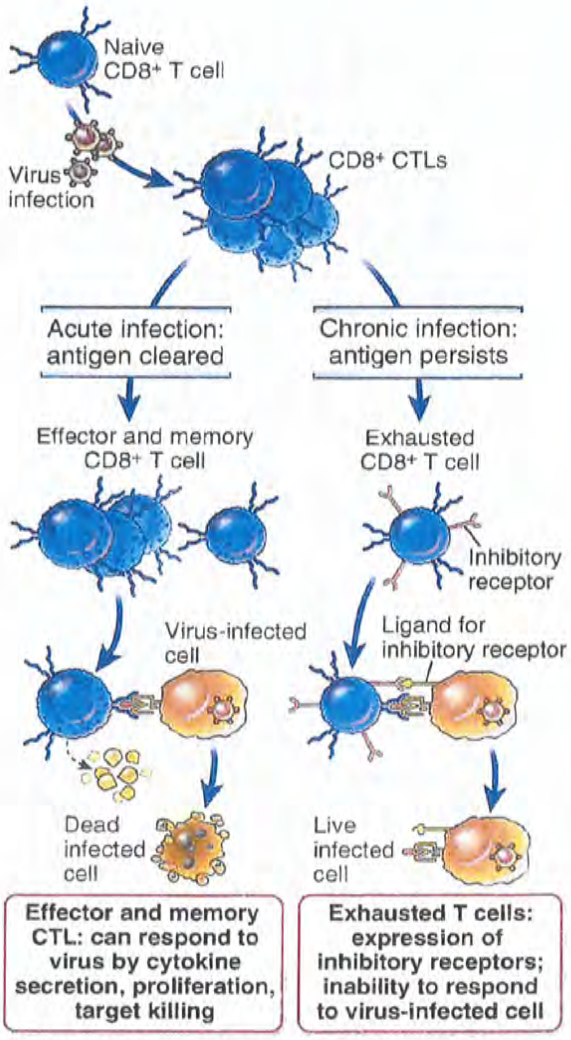


FIGURE 11.3 T cell exhaustion. In acute infections, CD8⁺ T cells differentiate into CTLs that eliminate the infected cells. In situations of persistent or chronic antigen exposure, the response of CD8⁺ T cells is suppressed by the expression and engagement of PD-1 and other inhibitory receptors.

EFFECTOR FUNCTIONS OF CD8⁺ CYTOTOXIC T LYMPHOCYTES

CD8⁺ CTLs eliminate intracellular microbes mainly by killing infected cells (see Fig. 10.1B). In addition to direct cell killing, CD8⁺ T cells secrete IFN-γ and in some cases IL-17 and thus contribute to classical macrophage activation and inflammation in host defense and in hypersensitivity reactions (see Chapter 10). Here we discuss the mechanisms by which differentiated CTLs kill cells harboring microbes.

Mechanisms of CTL-Mediated Cytotoxicity

CTL-mediated killing involves specific recognition of target cells and delivery of proteins that induce cell death. CTLs kill targets that express the same class I MHC–associated antigen that triggered the proliferation and differentiation of naive CD8⁺ T cells from which the CTLs are derived. CTL killing is highly antigen specific, and adjacent uninfected cells that do not express the antigen are not harmed. Killing specificity is achieved because a close region of contact, known as an **immune synapse,**

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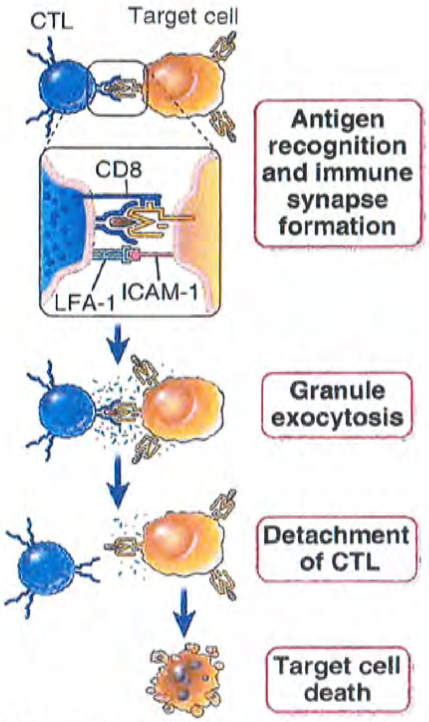


FIGURE 11.4 Steps in CTL-mediated lysis of target cells. A CTL recognizes the antigen-expressing target cell and is activated. Activation results in the release of granule contents from the CTL into the target cell through the area of contact (the immunologic synapse). Granule contents deliver a lethal hit to the target. The CTL may detach and kill other target cells. The formation of conjugates between a CTL and its target and activation of the CTL also require interactions between accessory molecules (LFA-1, CD8) on the CTL and their specific ligands (ICAM-1 and class I MHC, respectively) on the target cell *(not shown)*.

is formed between the CTL and the antigen-expressing target cell (see Chapter 7), and the molecules that actually perform the killing are secreted into the synapse and do not diffuse to other nearby cells.

The process of CTL-mediated killing of targets consists of antigen recognition, activation of the CTLs, delivery of the lethal hit that kills the target cells, and release of the CTLs (Fig. 11.4). Each of these steps is controlled by specific molecular interactions.

Recognition of Antigen and Activation of CTLs

The CTL binds and reacts to the target cell by using its antigen receptor, coreceptor (CD8), and adhesion molecules. To be efficiently recognized by CTLs, target cells must express class I MHC molecules displaying a peptide (the peptide-MHC complex serves as the ligand for the T cell receptor [TCR] and also binds to the CD8 coreceptor). Adhesion of the CTLs to the targets and the formation of the immune synapse are stabilized by a ring of integrins, notably LFA-1 (leukocyte function associated antigen 1) on the CTL binding to its ligand ICAM-1 (intercellular adhesion molecule 1) on the target cell (Fig. 11.5). An enclosed gap is present within the ring between the membranes of the two cells. Distinct regions of the CTL membrane can be observed by immunofluorescence microscopy within the ring, including a signaling patch, which includes the TCR, signaling proteins (such as protein kinase C-θ and the tyrosine kinase Lck), and a secretory region, which appears as a gap on one side of the signaling patch. This interaction results in the initiation of biochemical signals that activate the CTL, which are essentially the same signals as those involved in the activation of helper T cells. Cytokines and costimulators provided by dendritic cells, which are required for the differentiation of naive CD8⁺ T cells into CTLs, are not necessary for triggering the effector function of CTLs (i.e., target cell killing). Therefore, after CD8⁺ T cells specific for an antigen have differentiated into fully functional CTLs, they can kill any nucleated cell that displays that antigen.

In addition to the TCR, CD8⁺ CTLs express receptors that are also expressed by NK cells, which contribute to both regulation and activation of CTLs. Some of these receptors belong to the killer immunoglobulin receptor (KIR) family, discussed in Chapter 4, and recognize class I MHC molecules on target cells but are not specific for a particular peptide-MHC complex. These KIRs transduce inhibitory signals that may serve to prevent CTLs from killing normal cells. In addition, CTLs express the NKG2D receptor, which recognizes the class I MHC–like molecules MIC-A, MIC-B, and ULBP, which are expressed on stressed (infected or transformed) cells. NKG2D may serve to deliver signals that act together with TCR recognition of antigen to enhance killing activity.

Killing of Target Cells by CTLs

The principal mechanism of CTL-mediated target cell killing is the delivery of cytotoxic proteins stored within cytoplasmic granules (also called secretory lysosomes) to the target cell, thereby triggering apoptosis of the target cell (Fig. 11.6). Within a few minutes after a CTL's antigen receptor and coreceptor recognize a peptide-MHC complex on the target cell, the CTL granule proteins enter the target cell, and death occurs during the following 2 to 6 hours, even if the CTL detaches. Thus, the CTL is said to deliver a lethal hit to the target cell. When the CTL recognizes antigen, TCR signals lead to cytoskeleton reorganization. In this process, the microtubule organizing center of the CTL moves to the area of the cytoplasm near the contact with the target cell. The cytoplasmic granules of the CTL are transported along microtubules and become concentrated in the region of the synapse, and the granule membrane fuses with the plasma membrane at the secretory domain. Membrane fusion results in exocytosis of the CTL's granule contents into the confined space within the synaptic ring, between the plasma membranes of the CTL and target cell.

The major cytotoxic proteins in the granules of CTLs (and NK cells) are granzymes and perforin. **Granzymes** A, B, and C are serine proteases. Granzyme B cleaves proteins after aspartate residues and is the only one unequivocally shown to be required for CTL cytotoxicity in vivo. It can cleave and thereby activate caspases, which induce apoptosis. **Perforin** is a membrane-perturbing molecule that is homologous to the C9 complement

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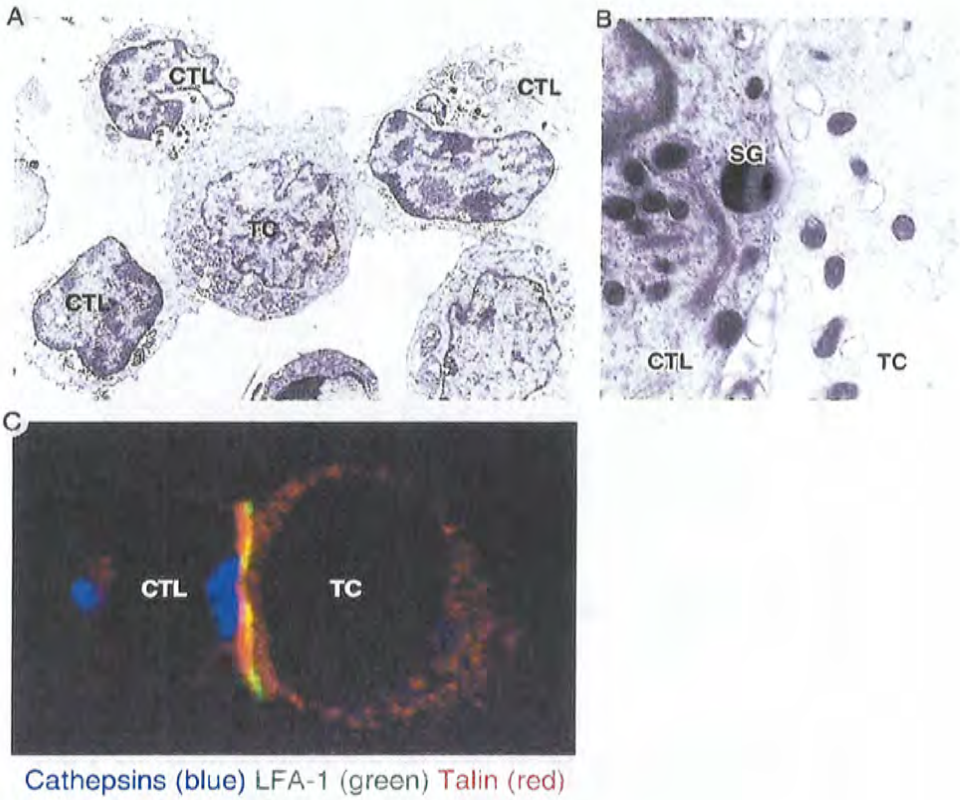


FIGURE 11.5 Formation of conjugates between CTLs and a target cell. **A,** Electron micrograph of three CTLs from a cloned cell line specific for the human MHC molecule HLA-A2 binding to an HLA-A2-expressing target cell (TC) within 1 minute after the CTLs and targets are mixed. Note that in the CTL on the upper left, the granules have been redistributed toward the target cell **B,** Electron micrograph of the point of membrane contact between a CTL *(left)* and target cell *(right)*. Two CTL granules (SG, secretory granules) are near the synapse. Several mitochondria are also visible. **C,** Confocal fluorescence micrograph of an immune synapse between a CTL *(left)* and target cell *(right)* stained with antibodies against cathepsins in a secretory granule *(blue)*, LFA-1 *(green)*, and the cytoskeletal protein talin *(red)*. The image demonstrates the central location of the secretory granule and the peripheral location of the adhesion molecule LFA-1 and associated cytoskeletal protein talin. *(**A** courtesy of Dr. P. Peters, Netherlands Cancer Institute, Amsterdam; **B** from Stinchcombe JC, Bossi G, Booth S, Griffiths GM: The immunological synapse of CTL contains a secretory domain and membrane bridges, Immunity 8:751–761, 2001. Copyright Cell Press, with permission from Elsevier; **C** from Stinchcombe JC, Griffiths GM: The role of the secretory immunological synapse in killing by CD8⁺ CTL, Seminars in Immunology 15:301–205. Copyright 2003 Elsevier Science Ltd.)*

protein. The granules also contain a sulfated proteoglycan, **serglycin**, which serves to hold granzymes and perforin in the granules in an inactive state.

The main function of perforin is to facilitate delivery of the granzymes into the cytosol of the target cell. How this is accomplished is still not well understood. Perforin can polymerize and form aqueous pores in the target cell membrane, but these pores may not be of sufficient size to allow granzymes to enter. According to one model, complexes of granzyme B, perforin, and serglycin are discharged from the CTL onto the target cell, and perforin insertion into the target cell membrane elicits a membrane repair process, which leads to internalization of both the perforin and granzymes into endosomes. Perforin may then act on the endosomal membrane to facilitate the release of the granzymes into the target cell cytosol. Once in the cytosol, the granzymes cleave various substrates, including caspases, and initiate apoptotic death of the cell. For example, granzyme B cleaves and activates caspase-3 as well as the Bcl-2 family member Bid, which triggers the mitochondrial pathway of apoptosis (see Fig. 15.8). Another protein found in human CTL (and NK cell) granules, called **granulysin**, can alter the permeability of target cell and microbial membranes and contributes to killing of infected and tumor cells.

CTLs also use a granule-independent mechanism of killing that is mediated by interactions of membrane molecules on the CTLs and target cells. On activation, CTLs express a membrane protein called **Fas ligand (FasL)** that binds to the death receptor Fas, which is

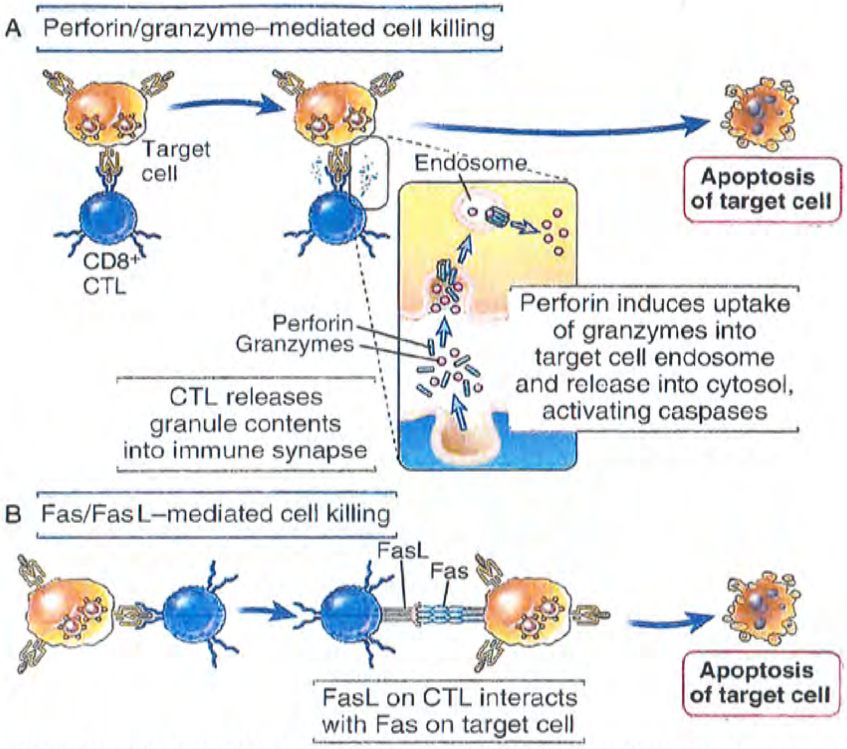


FIGURE 11.6 Mechanisms of CTL-mediated killing of target cells. CTLs kill target cells by two main mechanisms. **A,** Complexes of perforin and granzymes are released from the CTL by granule exocytosis and enter target cells. The granzymes are delivered into the cytoplasm of the target cells by a perforin-dependent mechanism, and they induce apoptosis. **B,** FasL is expressed on activated CTLs, engages Fas on the surface of target cells, and induces apoptosis.

expressed on many cell types. This interaction also results in activation of caspases and apoptosis of Fas-expressing targets (see Fig. 15.9). Studies with knockout mice lacking perforin, granzyme B, or FasL indicate that perforin and granzyme B are the principal mediators of killing by CD8⁺ CTLs.

After delivering the lethal hit, the CTL is released from its target cell, which usually occurs even before the target cell goes on to die. CTLs themselves are not injured during target cell killing, probably because the directed granule exocytosis process during CTL-mediated killing preferentially delivers granule contents into the target cell and away from the CTL. In addition, CTL granules contain a proteolytic enzyme called cathepsin B that is delivered to the CTL surface on granule exocytosis, where it degrades errant perforin molecules that come into the vicinity of the CTL membrane.

Cytokine Production by CD8⁺ Effector T Cells

CD8⁺ T cells produce the macrophage-activating cytokine IFN-γ. In fact, the secretion of IFN-γ in response to specific peptides is a sensitive assay for the frequency of antigen-specific CD8⁺ T cells in a population of lymphocytes. It is likely that both CD4⁺ Th1 cells and CD8⁺ T cells contribute to IFN-γ–induced phagocytic clearance of ingested microbes. CD8⁺ cells may also play a role in some cytokine-induced inflammatory reactions, such as contact sensitivity skin reactions induced by environmental chemicals, where IFN-γ–producing CD8⁺ T cells often arrive earlier than and outnumber CD4⁺ T cells. IL-17 producing CD8⁺ T cells are abundant in some chronic inflammatory diseases of the skin, such as psoriasis.

ROLES OF CD8⁺ CTLs IN HOST DEFENSE

In infections by intracellular microbes, the killing activity of CTLs is important for eradication of the reservoir of infection (see Fig. 10.1B). This is particularly important in two types of situations when cells cannot destroy microbes that infect them. First, most viruses live and replicate in cells that lack the phagosome/lysosome machinery for destroying microbes (such as hepatitis viruses in liver cells). Second, even in phagocytes, some microbes escape from vesicles and live in the cytosol, where microbicidal mechanisms are ineffective because these mechanisms are largely restricted to vesicles (to protect the host cells from damage). Such infections can be eliminated only by destroying the infected cells, and

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in adaptive immune responses. $CD8^+$ CTLs are the principal mechanism for killing infected cells (see Fig. 16.4). Bacteria such as *Mycobacterium tuberculosis* and *Listeria monocytogenes* are examples of microbes that escape from vesicles and enter the cytosol of infected cells. In addition, the caspases that are activated in target cells by granzymes and FasL cleave many substrates and activate enzymes that degrade DNA, but they do not distinguish between host and microbial molecules. Therefore, by activating nucleases in target cells, CTLs can initiate the destruction of microbial DNA as well as the target cell genome, thereby eliminating potentially infectious DNA. The massive expansion of $CD8^+$ T cells that follows infections (see Fig. 9.12) provides a large pool of CTLs to combat these infections. Defects in the development and activity of CTLs result in increased susceptibility to viral and some bacterial infections and reactivation of latent virus infections (such as infection by the Epstein-Barr virus), which are normally kept in check by virus-specific CTLs.

Destruction of infected cells by CTLs is a cause of tissue injury in some infectious diseases. For instance, in infection by hepatitis B and C viruses, the infected liver cells are killed by the host CTL (and NK cell) response and not by the viruses. These viruses are not highly cytopathic, but the host senses and reacts against the infectious microbe and is not able to distinguish microbes that are intrinsically harmful or relatively harmless (see Chapter 19). CTLs may contribute to the immunopathology associated with many other common viral infections, such as influenza.

CTLs are also important mediators of tumor immunity, as discussed in Chapter 18. In addition to their protective roles, $CD8^+$ CTLs contribute to tissue destruction in some autoimmune diseases (see Chapter 19) and to the rejection of tissue grafts (see Chapter 17).

Inherited mutations that interfere with CTL function, such as mutations in perforin, are associated with the familial form of a rare disease called hemophagocytic lymphohistiocytosis. CTLs that are activated by viral antigen secrete IFN-γ, but they do not kill the virus-infected cells because they cannot deliver the lethal hit. Thus, there is persistence of viral antigen, chronic IFN-γ production from the $CD8^+$ T cells, and excessive macrophage activation by the IFN-γ. The severe and prolonged macrophage activation underlies the manifestations of the disease, including enlargement of the spleen caused by increased numbers of activated macrophages ("lymphohistiocytosis") which phagocytose and destroy normal red blood cells ("hemophagocytosis").

SUMMARY

- T cells of the $CD8^+$ subset proliferate and differentiate into cytotoxic T lymphocytes (CTLs), which express cytotoxic granules and can kill infected cells.
- The differentiation of $CD8^+$ T cells into functional CTLs and memory cells requires recognition of antigen presented by dendritic cells, signals from $CD4^+$ helper T cells in some situations, costimulation, and cytokines. Differentiation to CTLs involves the acquisition of the machinery to kill target cells and is driven by various transcription factors.
- In some situations of chronic antigen exposure (such as tumors and chronic viral infections), $CD8^+$ T cells initiate a response but begin to express inhibitory receptors that suppress the response, a process called exhaustion.
- $CD8^+$ CTLs kill cells that express peptides derived from cytosolic antigens (e.g., viral antigens) that are presented in association with class I MHC molecules. CTL-mediated killing is mediated mainly by granule exocytosis, which releases granzymes and perforin. Perforin facilitates granzyme entry into the cytoplasm of target cells, and granzymes initiate the process of apoptosis.
- $CD8^+$ T cells also secrete IFN-γ and thus may participate in defense against phagocytosed microbes and in delayed type hypersensitivity (DTH) reactions.

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CHAPTER

19

Hypersensitivity Disorders

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SUMMARY, 434

Adaptive immunity serves the important function of host defense against microbial infections, but immune responses are also capable of causing tissue injury and disease. Disorders caused by immune responses are called **hypersensitivity diseases**. This term arose from the clinical definition of immunity as sensitivity, which is based on the observation that an individual who has been exposed to an antigen exhibits a detectable reaction, or is sensitive, to subsequent encounters with that antigen. Normally, immune responses eradicate infectious pathogens without serious injury to host tissues. However, these responses are sometimes inadequately controlled, inappropriately targeted to host tissues, or triggered by commensal microorganisms or environmental antigens that are usually harmless. In these situations, the normally beneficial immune response is the cause of disease.

In this chapter, we will describe the pathogenesis of different types of hypersensitivity reactions, with an emphasis on the effector mechanisms that cause tissue injury. We will conclude with a brief consideration of the treatment of immunologic diseases and examples of diseases that illustrate important principles.

CAUSES OF HYPERSENSITIVITY DISEASES

Immune responses that are the cause of hypersensitivity diseases may be specific for antigens from different sources.

- ***Autoimmunity: reactions against self antigens.*** Failure of the normal mechanisms of self-tolerance results in T cell and B cell reactions against one's own cells and tissues that are called **autoimmunity** (see Chapter 15). The diseases caused by autoimmunity are referred to as **autoimmune diseases**. Autoimmune diseases are estimated to affect at least 2% to 5% of the population in developed countries, and the incidence of these disorders is rising. Many of these diseases are common in individuals in the 20- to 40-year age group. They are also more common in women than in men, for reasons that remain obscure. Autoimmune diseases are usually chronic and often debilitating, and an enormous medical and economic burden. Although these disorders have been difficult to treat in the past, many new effective therapies have been developed since the 1990s based on scientific principles. The mechanisms of autoimmunity were described in Chapter 15. In this chapter, we will refer to various autoimmune disorders to illustrate how immune reactions against self can cause disease.
- ***Reactions against microbes.*** Immune responses against microbial antigens may cause disease if the reactions are excessive or the microbes are unusually persistent. T cell responses against persistent microbes may give rise to severe inflammation, sometimes with the formation of granulomas; this is the cause of tissue injury in tuberculosis and some other chronic infections. If antibodies are produced against microbial antigens, the antibodies may bind to the antigens to

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produce immune complexes, which deposit in tissues and trigger inflammation. Rarely, antibodies or T cells against a microbe will cross-react with a host tissue. In some cases involving the intestinal tract (i.e., inflammatory bowel disease), the immune response is directed against commensal bacteria that normally reside in the gut and cause no harm. Sometimes the mechanisms that an immune response uses to eradicate a pathogenic microbe require killing infected cells, and therefore such responses inevitably injure host tissues. For example, in viral hepatitis, the virus that infects liver cells is not cytopathic, but it is recognized as foreign by the immune system. Cytotoxic T lymphocytes (CTLs) eliminate infected cells, and this normal immune response damages the liver. This type of normal reaction is not considered hypersensitivity.

- ***Reactions against nonmicrobial environmental antigens.*** Most healthy individuals do not react against common, generally harmless environmental substances, but almost 20% of the population is abnormally responsive to one or more of these substances. These individuals produce immunoglobulin E (IgE) antibodies that cause allergic diseases (see Chapter 20). Some individuals become sensitized to environmental antigens and chemicals that contact the skin and develop T cell reactions that lead to cytokine-mediated inflammation, resulting in contact sensitivity. Idiosyncratic immunologic reactions against therapeutic drugs are also a frequent clinical problem.

In all of these conditions, the mechanisms of tissue injury are the same as those that normally function to eliminate infectious pathogens. These mechanisms include innate and adaptive immune responses involving phagocytes, antibodies, T lymphocytes, mast cells, and various other effector cells, and mediators of inflammation. The problem in hypersensitivity diseases is that the immune response is not controlled appropriately or is targeted to normal tissues. Because the stimuli for these abnormal immune responses are often impossible to eliminate (e.g., self antigens, commensal microbes, and environmental antigens) and the immune system has many built-in positive feedback loops (amplification mechanisms), once a pathologic immune response starts, it is difficult to control or to terminate it. Therefore, these hypersensitivity diseases tend to be chronic and progressive and pose major therapeutic challenges in clinical medicine.

By convention, and especially in clinical situations, the term *hypersensitivity* refers to harmful immune responses against foreign antigens (environmental antigens, drugs, microbes) and is not used to describe tissue injury in autoimmune diseases. However, in our discussion, we will consider all causes of harmful immune reactions, mainly to emphasize the common pathogenic mechanisms.

MECHANISMS AND CLASSIFICATION OF HYPERSENSITIVITY REACTIONS

Hypersensitivity diseases are commonly classified according to the type of immune response and the effector mechanism responsible for cell and tissue injury (Table 19.1). These mechanisms include some that are predominantly dependent on antibodies and others predominantly dependent on T cells, although a role for both humoral and cell-mediated immunity is often found in many hypersensitivity diseases. We will briefly go over the classification of these diseases, and then consider antibody-mediated and T cell–mediated diseases in greater detail.

- **Immediate (type I) hypersensitivity** is caused by IgE antibodies specific for environmental antigens and is the most prevalent type of hypersensitivity disease:

TABLE 19.1 Classification of Hypersensitivity Diseases

Type of Hypersensitivity	Pathologic Immune Mechanisms	Mechanisms of Tissue Injury and Disease
Immediate: Type I	IgE antibody, Th2 cells	Mast cells, eosinophils, and their mediators (vasoactive amines, lipid mediators, cytokines)
Antibody-mediated: Type II	IgM, IgG antibodies against cell surface or extracellular matrix antigens	Opsonization and phagocytosis of cells Complement- and Fc receptor–mediated recruitment and activation of leukocytes (neutrophils, macrophages) Abnormalities in cellular functions, for example, hormone receptor signaling, neurotransmitter receptor blockade
Immune complex–mediated: Type III	Immune complexes of circulating antigens and IgM or IgG antibodies	Complement- and Fc receptor–mediated recruitment and activation of leukocytes
T cell–mediated: Type IV	1. $CD4^+$ T cells (Th1 and Th17 cells) 2. $CD8^+$ CTLs	1. Cytokine-mediated inflammation and macrophage activation 2. Direct target cell killing, cytokine-mediated inflammation

CTLs, Cytotoxic T lymphocytes; *Ig*, immunoglobulin.

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it will be discussed in detail separately in Chapter 20. Immediate hypersensitivity diseases, commonly grouped under *allergy* or *atopy*, are often caused by activation of interleukin-4 (IL-4), IL-5, and IL-13 producing Th2 cells and the production of IgE antibodies, which activate mast cells and eosinophils and induce inflammation.

- **Antibody-mediated (type II) hypersensitivity.** IgG and IgM antibodies specific for cell surface or extracellular matrix antigens can cause tissue injury by activating the complement system, by recruiting inflammatory cells, and by interfering with normal cellular functions.
- **Immune complex-mediated (type III) hypersensitivity.** IgM and IgG antibodies specific for soluble antigens in the blood form complexes with the antigens, and the immune complexes may deposit in blood vessel walls in various tissues, causing inflammation, thrombosis, and tissue injury.
- **T cell-mediated (type IV) hypersensitivity.** In these disorders, tissue injury may be due to T lymphocytes that induce inflammation or directly kill target cells. In most of these diseases, the major mechanism involves the activation of $CD4^+$ helper T cells, which secrete cytokines that promote inflammation and activate leukocytes, mainly neutrophils and macrophages. CTLs contribute to tissue injury in some diseases.

In the discussion that follows, we will use descriptions that identify the pathogenic mechanisms rather than the less informative numerical designations for types of hypersensitivity. This classification is useful because distinct types of pathologic immune responses show different patterns of tissue injury and may vary in their tissue specificity. As a result, the different immunologic mechanisms cause disorders with distinct clinical and pathologic features. However, immunologic diseases in humans are often complex and caused by combinations of humoral and cell-mediated immune responses and multiple effector mechanisms. This complexity is not surprising, given that a single antigen may normally stimulate both humoral and cell-mediated immune responses in which several types of antibodies and effector T cells are produced.

With this background, we will proceed to a discussion of antibody- and T cell–mediated diseases.

DISEASES CAUSED BY ANTIBODIES

Antibody-mediated diseases are produced either by antibodies that bind to antigens on particular cells or in extracellular tissues or by antigen-antibody complexes that form in the circulation and are deposited in vessel walls (Fig. 19.1). Antibodies against cellular or tissue antigens cause diseases that specifically affect the cells or tissues where these antigens are present, so these diseases are often organ-specific and not systemic. By contrast, the manifestations of diseases caused by immune complexes reflect the site of immune complex deposition and are not determined by the cellular source of the antigen.

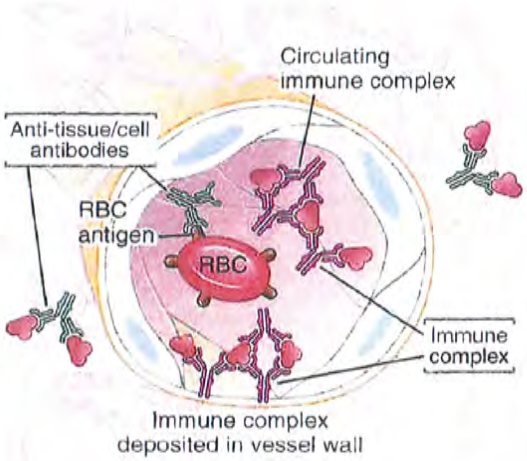


FIGURE 19.1 Types of antibodies that cause disease. This figure illustrates the different forms of antibodies that may cause disease. *Antitissue/cell antibodies:* Antibodies may bind specifically to extracellular tissue antigens and the recruited leukocytes cause tissue injury, or antibodies may bind to cells (in this example, circulating red cells) and promote depletion of these cells. *Immune complexes:* Complexes of antibodies and antigens may be formed in the circulation and deposited in the walls of blood vessels, where the complexes induce inflammation.

Therefore, immune complex–mediated diseases tend to be systemic and affect multiple tissues and organs, although some sites are particularly susceptible, such as kidneys and joints.

To prove that a disease is caused by antibodies, one would need to demonstrate that the lesions can be induced in a normal animal by the adoptive transfer of immunoglobulin purified from the blood or affected tissues of individuals with the disease. An experiment of nature is occasionally seen in children of mothers suffering from antibody-mediated diseases. These infants may be born with transient manifestations of such diseases because of a transplacental passage of antibodies. However, in clinical situations, the diagnosis of diseases caused by antibodies or immune complexes is usually based on the demonstration of antibodies or immune complexes in the circulation or deposited in tissues, as well as clinicopathologic similarities with experimental diseases that are proved to be antibody mediated by adoptive transfer.

Diseases Caused by Antibodies Against Fixed Cell and Tissue Antigens

Antibodies against tissue antigens cause disease by three main mechanisms (Fig. 19.2):

- ***Opsonization and phagocytosis.*** Antibodies that bind to cell surface antigens may directly opsonize cells, or they may activate the complement system, resulting in the production of complement proteins that

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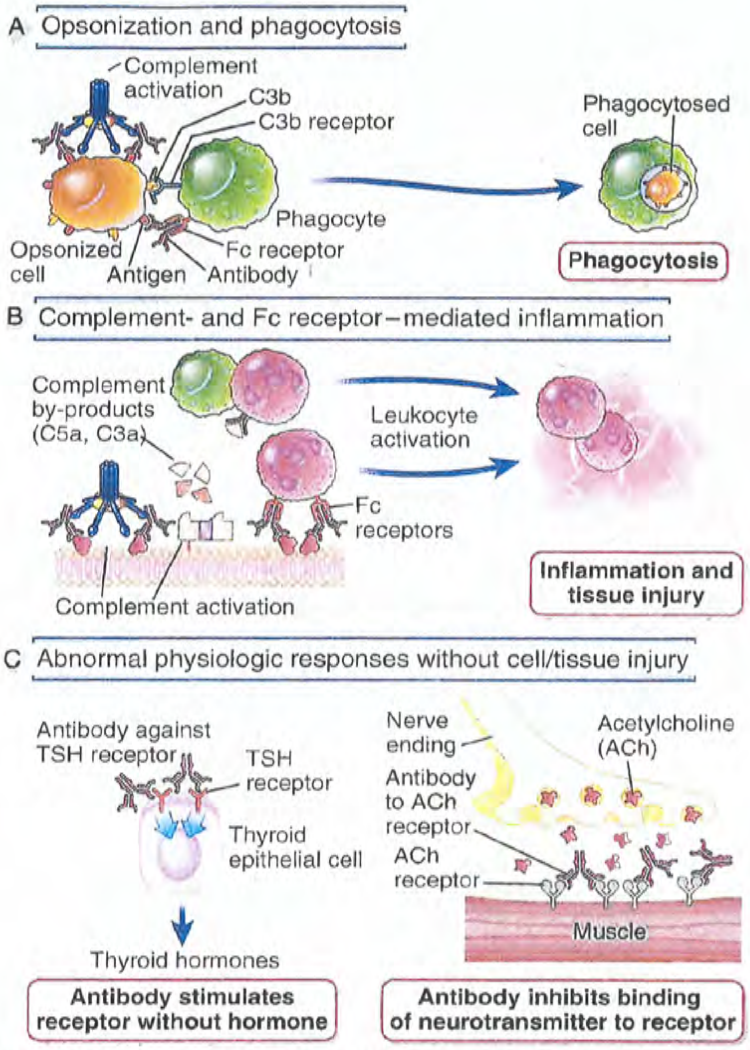


FIGURE 19.2 Effector mechanisms of antibody-mediated disease. A, Antibodies opsonize cells and may activate complement, generating complement products that also opsonize cells, leading to phagocytosis of the cells through phagocyte Fc receptors or C3b receptors. **B,** Antibodies recruit leukocytes by binding to Fc receptors or by activating complement and thereby releasing by-products that are chemotactic for leukocytes. **C,** Antibodies specific for cell surface hormone receptors or neurotransmitter receptors interfere with normal physiology. For example, in Graves' disease *(left panel)* autoantibodies specific for thyroid stimulating hormone (TSH) receptors in the thyroid gland stimulate the activity of the receptors even in the absence of TSH, causing excess thyroid hormone release (hyperthyroidism). In myasthenia gravis *(right panel)*, autoantibodies specific for the acetylcholine receptor on muscle cells block the action of acetylcholine, leading to paralysis.

opsonize cells. These opsonized cells are phagocytosed and destroyed by phagocytes that express receptors for the Fc portions of IgG antibodies and receptors for complement proteins. This is the principal mechanism of cell destruction in autoimmune hemolytic anemia and autoimmune thrombocytopenia, in which antibodies specific for red blood cells or platelets, respectively, lead to the opsonization and removal of these cells from the circulation. The same mechanism is responsible for hemolysis in transfusion reactions (see Chapter 17).

- ***Inflammation.*** Antibodies deposited in tissues activate complement, leading to the liberation of breakdown products such as C5a and C3a, which recruit neutrophils and macrophages. These leukocytes express IgG Fc receptors and complement receptors, which bind the antibodies or attached complement proteins. The leukocytes are activated by signaling from the

receptors (particularly Fc receptors), and leukocyte products (including lysosomal enzymes and reactive oxygen species) are released and cause tissue injury. An example of antibody-mediated inflammation and leukocyte activation causing tissue injury is glomerulonephritis.

- ***Abnormal cellular functions.*** Antibodies that bind to normal cellular receptors or other proteins may interfere with the functions of these receptors or proteins and cause disease without inflammation or tissue damage. For instance, antibodies specific for the thyroid stimulating hormone receptor or the nicotinic acetylcholine receptor cause functional abnormalities that lead to Graves' disease and myasthenia gravis, respectively (see Fig. 19.2C). Antibodies specific for intrinsic factor, required for vitamin B12 absorption, cause pernicious anemia. Antibodies specific for cytokines are rare but known causes of immunodeficiencies.

Antibodies that cause cell- or tissue-specific diseases are usually autoantibodies produced as part of an autoimmune reaction, but sometimes the antibodies are specific for microbes. Examples of autoantibodies against tissue antigens are listed in Table 19.2. Less commonly, the antibodies may be produced against a foreign (e.g., microbial) antigen that is immunologically cross-reactive with a component of self tissues. In a rare sequel to streptococcal infection called rheumatic fever, antibodies produced against the bacteria cross-react with antigens in the heart, deposit in this organ, and cause inflammation and tissue damage. Tissue deposits of antibodies may be detected by morphologic examination in some of these diseases, and the deposition of antibody is often associated with local complement activation, inflammation, and tissue injury (Fig. 19.3A).

Immune Complex–Mediated Diseases

Immune complexes that cause disease may be composed of antibodies bound to either self antigens or foreign antigens.

The occurrence of diseases caused by immune complexes was suspected in the early 1900s by an astute

TABLE 19.2 Examples of Diseases Caused by Cell- or Tissue-Specific Antibodies

Disease	Target Antigen	Mechanisms of Disease	Clinicopathologic Manifestations
Autoimmune hemolytic anemia	Erythrocyte membrane proteins	Opsonization and phagocytosis of erythrocytes, complement-mediated lysis	Hemolysis, anemia
Autoimmune thrombocytopenic purpura	Platelet membrane proteins (gpIIb-IIIa integrin)	Opsonization and phagocytosis of platelets	Bleeding
Pemphigus vulgaris	Proteins in intercellular junctions of epidermal cells (desmoglein)	Antibody-mediated activation of proteases, disruption of intercellular adhesions	Skin blisters (bullae)
Vasculitis caused by ANCA	Neutrophil granule proteins, presumably released from activated neutrophils	Neutrophil degranulation and inflammation	Vasculitis
Goodpasture syndrome	Noncollagenous NC1 protein of basement membrane in glomeruli and lung	Complement- and Fc receptor–mediated inflammation	Nephritis, lung hemorrhage
Acute rheumatic fever	Streptococcal cell wall antigen; antibody cross-reacts with myocardial antigen	Inflammation, macrophage activation	Myocarditis, arthritis
Myasthenia gravis	Acetylcholine receptor	Antibody inhibits acetylcholine binding, down modulates receptors	Muscle weakness, paralysis
Graves' disease (hyperthyroidism)	TSH receptor	Antibody-mediated stimulation of TSH receptors	Hyperthyroidism
Pernicious anemia	Intrinsic factor of gastric parietal cells	Neutralization of intrinsic factor; decreased absorption of vitamin B_{12}	Abnormal erythropoiesis, anemia, neurologic symptoms

ANCA, Anti-neutrophil cytoplasmic antibodies; *TSH*, thyroid-stimulating hormone.

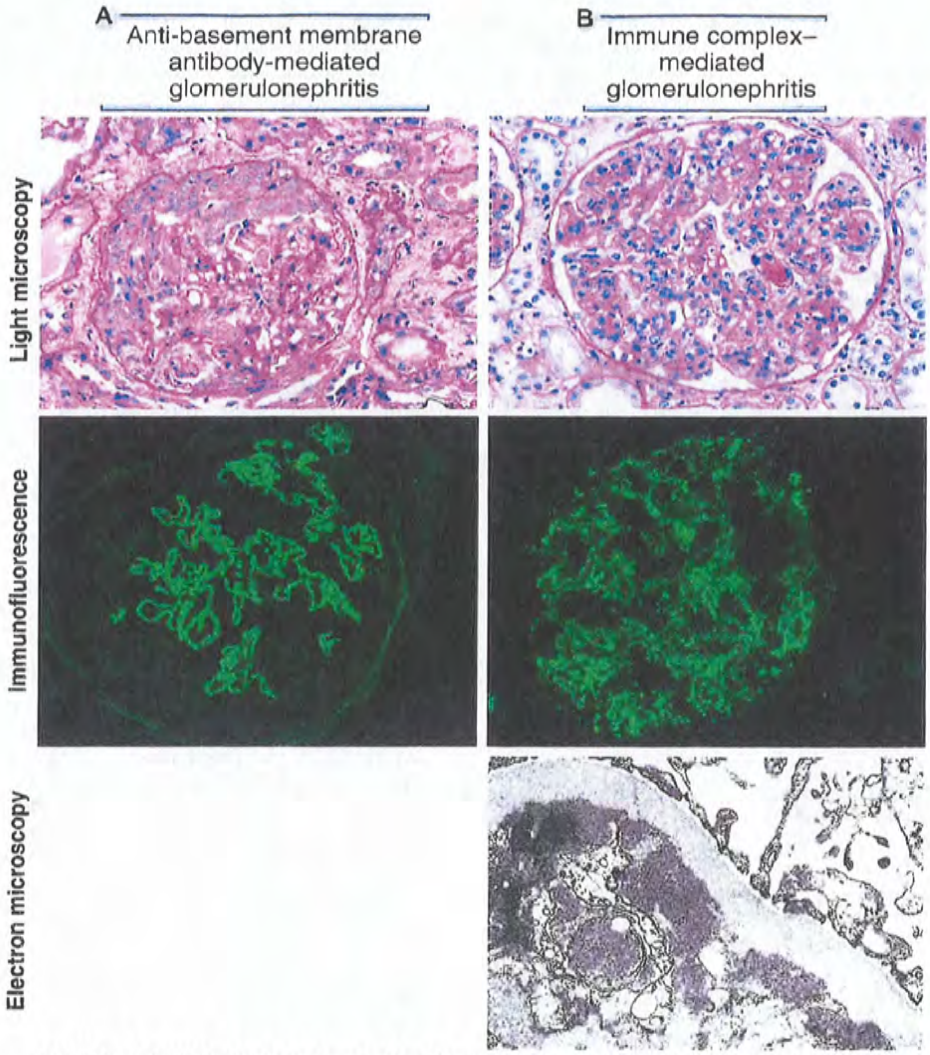


FIGURE 19.3 Pathologic features of antibody-mediated glomerulonephritis. **A,** Glomerulonephritis induced by an antibody against the glomerular basement membrane (Goodpasture syndrome): the light micrograph shows glomerular inflammation and severe damage, and immunofluorescence shows smooth (linear) deposits of antibody along the basement membrane. **B,** Glomerulonephritis induced by the deposition of immune complexes (systemic lupus erythematosus): the light micrograph shows neutrophilic inflammation, and the immunofluorescence and electron micrograph show coarse (granular) deposits of antigen-antibody complexes along the basement membrane. *(Immunofluorescence micrographs are courtesy of Dr. Jean Olson, Department of Pathology, University of California, San Francisco, and the electron micrograph is courtesy of Dr. Helmut Rennke, Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts.)*

physician named Clemens von Pirquet. At the time, diphtheria infections were treated with serum from horses that had been immunized with the diphtheria toxin, which is an example of passive immunization against the toxin by the transfer of serum containing antitoxin antibodies. Von Pirquet noted that joint inflammation (arthritis), rash, and fever developed in patients who were injected with the antitoxin-containing horse serum. Clinical features of this reaction suggested that it was not due to the infection or a toxic component of the serum itself. The symptoms appeared at least 1 week after the first injection of horse serum and more rapidly with each repeated injection. Von Pirquet concluded that this disease was caused by a host response to some component of the serum. He suggested that the host made antibodies to horse serum proteins; these antibodies

formed complexes with the injected proteins, and the disease was due to the antibodies or immune complexes. We now know that his conclusions were entirely accurate. He called this disorder *serum disease*. The same reaction was also observed in humans receiving serum therapy for tetanus, and it is now more commonly known as *serum sickness*. This remains a clinical issue today in individuals who receive therapeutic monoclonal antibodies produced in rodents that contain nonhuman sequences or antisera made in animals that are used to treat snakebites or rabies.

Experimental Models of Immune Complex–Mediated Diseases

Serum Sickness

Much of our current knowledge of immune complex diseases is based on analyses of experimental models of serum sickness. Immunization of an animal such as a rabbit with a large dose of a foreign protein antigen leads to the formation of antibodies against the antigen (Fig. 19.4). These antibodies bind to and form complexes with the circulating antigen, and the complexes are initially cleared by macrophages in the liver and spleen. As more and more antigen-antibody complexes are formed, some of them are deposited in vascular beds. In these tissues, the complexes induce neutrophil-rich inflammation by activating the classical pathway of complement and engaging leukocyte Fc receptors. Because the complexes are often deposited in small arteries, renal glomeruli, and the synovia of joints, the most common clinical and pathologic manifestations are vasculitis, nephritis, and arthritis. The clinical symptoms are usually short-lived, and the lesions heal unless the antigen is injected again. This type of disease is an example of acute serum sickness. A more indolent and prolonged disease, called chronic serum sickness, is produced by multiple injections of antigen, which lead to the formation of smaller complexes that are deposited most often in the kidneys, arteries, and lungs.

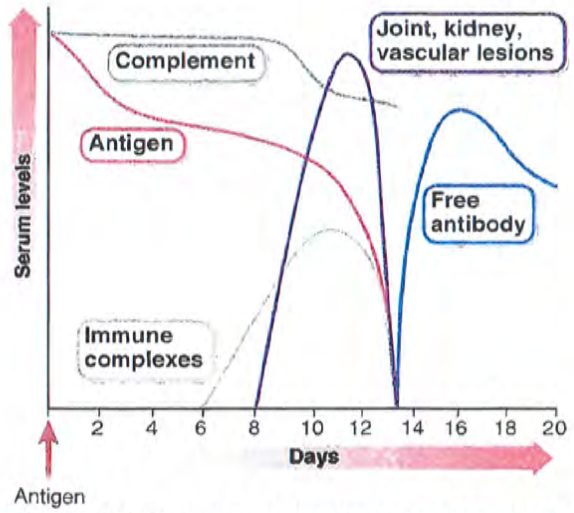


FIGURE 19.4 Sequence of immunologic responses in experimental acute serum sickness. Injection of bovine serum albumin into a rabbit leads to the production of specific antibody and the formation of immune complexes. These complexes are deposited in multiple tissues, activate complement (leading to a decrease in serum complement levels), and cause inflammatory lesions, which resolve as the complexes and the remaining antigen are removed and free antibody (not bound to antigen) appears in the circulation. *(Adapted from Cochrane CG: Immune complex–mediated tissue injury. In Cohen S, Ward PA, McCluskey RT [eds.]: Mechanisms of immunopathology, New York, 1979, Werbel & Peck, pp 29–48. Copyright © 1979, Wiley-Liss, Inc.)*

Arthus Reaction

A localized form of experimental immune complex–mediated vasculitis is called the Arthus reaction. It is induced by subcutaneous injection of an antigen into a previously immunized animal or an animal that has been given an intravenous injection of antibody specific for the antigen. Circulating antibodies rapidly bind to the injected antigen and form immune complexes that are deposited in the walls of small blood vessels at the injection site. This deposition gives rise to a local cutaneous vasculitis, with thrombosis of the affected vessels, leading to tissue necrosis. The clinical relevance of the Arthus reaction is limited; rarely, a subject receiving a booster dose of a vaccine may develop inflammation at the site of injection because of local accumulation of immune complexes, as in an Arthus reaction.

Pathogenesis of Immune Complex–Mediated Diseases

The amount of immune complex deposition in tissues is determined by the nature of the complexes and the characteristics of the blood vessels. Antigen-antibody complexes are produced during normal immune responses, but they cause disease only when they are produced in excessive amounts, are not efficiently cleared, and become deposited in tissues. Small complexes are often not phagocytosed and tend to be deposited in vessels more than large complexes, which are usually cleared by phagocytes. Complexes containing cationic antigens bind avidly to negatively charged components of the basement membranes of blood vessels and kidney glomeruli. Such complexes typically produce severe and long-lasting tissue injury. Capillaries in the renal glomeruli and synovia are sites where plasma is ultrafiltered (to form urine and synovial fluid, respectively) by passing at high pressure through specialized basement membranes, and these locations are among the most common sites of immune complex deposition. However, immune complexes may be deposited in small vessels in virtually any tissue. Deposits of antibody and complement may be detected in the vessels, and if the antigen is known, it is possible to identify antigen molecules in the deposits as well (Fig. 19.3B). Immune complexes deposited in vessel walls and tissues activate leukocytes and mast cells to secrete cytokines and vasoactive mediators. These mediators may cause more immune complex deposition in vessel walls by increasing vascular permeability and blood flow.

The major mechanism of tissue injury in immune complex diseases is inflammation within the walls of blood vessels, resulting from complement activation and binding of leukocyte Fc receptors to the antibodies in

TABLE 19.3 Examples of Human Immune Complex–Mediated Diseases

Disease	Antigen Involved	Clinicopathologic Manifestations
Systemic lupus erythematosus	DNA, nucleoproteins, others	Nephritis, arthritis, vasculitis
Polyarteritis nodosa	Hepatitis B virus surface antigen (in some cases)	Vasculitis
Poststreptococcal glomerulonephritis	Streptococcal cell wall antigens	Nephritis
Serum sickness	Various proteins	Arthritis, vasculitis, nephritis

the deposited complexes. These are the same mechanisms that cause tissue injury in serum sickness, described earlier.

Many systemic immunologic diseases in humans are caused by the deposition of immune complexes in blood vessels (Table 19.3). Systemic lupus erythematosus (SLE) is an autoimmune disease in which complexes consisting of nuclear antigens and antibodies deposit in blood vessels in kidney glomeruli, skin, and many other tissues. In one type of immune complex–mediated vasculitis involving medium-size muscular arteries, called polyarteritis nodosa, the complexes are made up of viral antigen and antibodies, and the disease is a late complication of viral infection, most often with hepatitis B virus. This is also the mechanism of a disease called poststreptococcal glomerulonephritis, which develops in rare cases after streptococcal infection and is caused by complexes of streptococcal antigen and antibodies depositing in the glomeruli of the kidney. In some forms of glomerulonephritis, immune complexes are not detected in the circulation, leading to the postulate that the antigens are first planted in the kidney and the complexes form locally.

DISEASES CAUSED BY T LYMPHOCYTES

T lymphocytes injure tissues either by producing cytokines that induce inflammation or by directly killing target cells (Fig. 19.5). Inflammatory reactions are elicited mainly by $CD4^+$ T cells of the Th1 and Th17 subsets. In some T cell–mediated disorders, the principal mechanism of tissue injury is killing of cells by $CD8^+$ CTLs. The T cells that cause tissue injury may be autoreactive, or they may be specific for foreign protein antigens that are present in or bound to cells or tissues. T lymphocyte–mediated tissue injury may also accompany strong protective immune responses against persistent microbes, especially

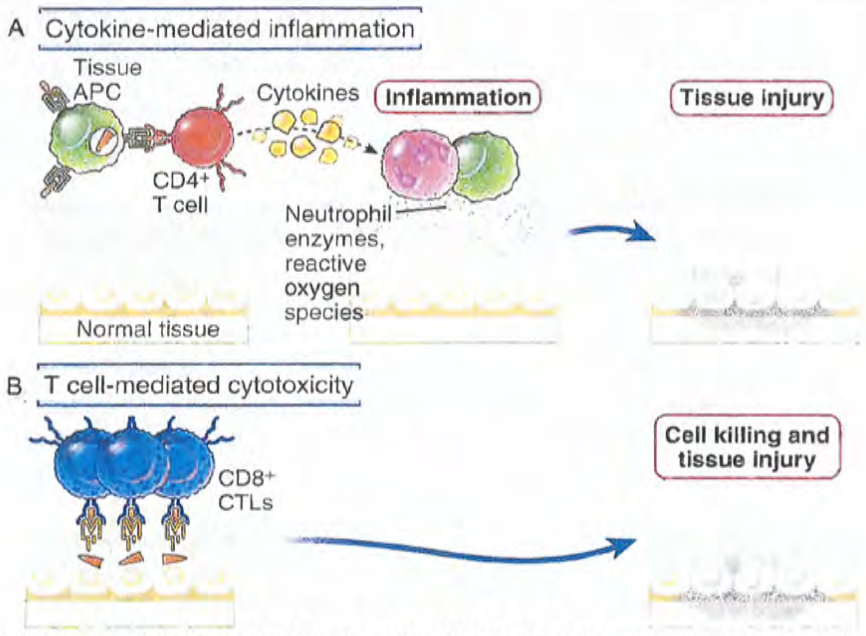


FIGURE 19.5 Mechanisms of T cell–mediated diseases. A, In cytokine-mediated inflammatory reactions, $CD4^+$ T cells (and sometimes $CD8^+$ cells, not shown) respond to tissue antigens by secreting cytokines that stimulate inflammation and activate leukocytes, leading to tissue injury. **B,** In some diseases, $CD8^+$ CTLs directly kill tissue cells. *APC,* Antigen-presenting cell.

intracellular microbes that resist eradication by phagocytes and antibodies.

A role for T cells in causing a particular immunologic disease is suspected largely on the basis of the demonstration of T cells in lesions and the detection of increased levels of cytokines in the blood or tissues that may be derived from T cells. Animal models have been very useful for elucidating the pathogenesis of these disorders.

Diseases Caused by Cytokine-Mediated Inflammation

In immune-mediated inflammation, Th1 and Th17 cells secrete cytokines that recruit and activate leukocytes. IL-17, produced by Th17 cells, promotes neutrophil recruitment; interferon-γ (IFN-γ), produced by Th1 cells, activates macrophages; and tumor necrosis factor (TNF) and chemokines, produced by T lymphocytes and cells of innate immunity (such as dendritic cells and macrophages), are involved in the recruitment and activation of many types of leukocytes. Although we emphasize $CD4^+$ Th1 and Th17 cells as the sources of many of these cytokines, in lesions many other cells may produce the same cytokines. For instance, in some animal models of chronic skin inflammation, the source of IL-17 early in the course of the disease appears to be γδ T cells, and innate lymphoid cells in tissues produce many of the same cytokines as T cells do (see Chapter 4).

Tissue injury results from the products of the recruited and activated neutrophils and macrophages, such as lysosomal enzymes and reactive oxygen species. Cytokines produced by activated lymphocytes and macrophages stimulate more leukocyte recruitment and inflammation, thus propagating the damage (see Chapter 10). Vascular endothelial cells in the lesions may express increased levels of cytokine-regulated surface proteins such as adhesion molecules and class II MHC molecules. The inflammation associated with T cell–mediated diseases is typically chronic, but bouts of acute inflammation may be superimposed on a background of chronic inflammation. Delayed-type hypersensitivity (DTH) is an example of such inflammatory reactions and is described later. Chronic inflammatory reactions often produce fibrosis as a result of the secretion of cytokines and growth factors by the macrophages and T cells.

Many organ-specific autoimmune diseases are caused by activation of autoreactive T cells by self antigens, leading to cytokine release and inflammation. This is believed to be the major mechanism underlying rheumatoid arthritis, multiple sclerosis, type 1 diabetes, psoriasis, and other autoimmune diseases (Table 19.4). Some of these diseases are described in more detail at the end of this chapter.

T cell reactions specific for microbes and other foreign antigens may also lead to inflammation and tissue injury. Intracellular bacteria such as *Mycobacterium tuberculosis* induce strong T cell and macrophage responses that result in granulomatous inflammation and fibrosis (described later); the inflammation and fibrosis may cause extensive tissue destruction and functional impairment, typically in the lungs. Tuberculosis is a good example of an infectious disease in which tissue injury is mainly due to the host immune response (see Chapter 16). T cell responses against intestinal bacteria are believed to underlie some forms of inflammatory bowel disease.

A variety of skin diseases, called contact hypersensitivity, result from topical exposure to chemicals and environmental antigens. These disorders are caused by inflammatory reactions that are likely triggered by neoantigens formed by the binding of the chemicals to self proteins, including MHC molecules. Both $CD4^+$ and $CD8^+$ T cells may be the source of cytokines in contact sensitivity reactions. Examples of contact hypersensitivity include rashes induced by poison ivy and poison oak (in which T cells react against self proteins that are modified by chemicals made by the plants called urushiols) and rashes induced by contact with metals (nickel and beryllium) and a variety of chemicals, such as thiuram, which is used

TABLE 19.4 T Cell–Mediated Diseases

Disease	Specificity of Pathogenic T Cells	Principal Mechanisms of Tissue Injury
Rheumatoid arthritis	Collagen? Citrullinated self proteins?	Inflammation mediated by Th1 and Th17 cytokines. Role of antibodies and immune complexes?
Multiple sclerosis	Protein antigens in myelin (e.g., myelin basic protein)	Inflammation mediated by Th1 and Th17 cytokines; myelin destruction by activated macrophages
Type 1 diabetes mellitus	Antigens of pancreatic islet β cells (insulin, glutamic acid decarboxylase, others)	T cell–mediated inflammation; destruction of islet cells by CTLs
Inflammatory bowel disease	Enteric bacteria. Self antigens?	Inflammation mediated by Th1 and Th17 cytokines
Psoriasis	Unknown skin antigens	Inflammation mediated by T cell–derived cytokines

Examples of human T cell–mediated diseases are listed. In many cases, the specificity of the T cells and the mechanisms of tissue injury are inferred on the basis of the similarity with experimental animal models of the diseases. The roles of Th1 and Th17 cells have been inferred from experimental models and the presence of subset-specific cytokines in human lesions. The cytokines may be produced by cells other than $CD4^+$ T lymphocytes. Ongoing clinical trials targeting these cytokines may provide new information about the contributions of the cytokines in different diseases.

CTLs, Cytotoxic T lymphocytes

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in the manufacture of latex gloves. Some of these reactions become chronic and clinically are called **eczema**. Skin rashes caused by responses to therapeutic drugs are among the most common immune reactions in humans, and also examples of contact sensitivity.

The classical T cell–mediated inflammatory reaction is called **delayed-type hypersensitivity**, described next.

Delayed-Type Hypersensitivity (DTH)

DTH is an injurious cytokine-mediated inflammatory reaction resulting from the activation of T cells, particularly CD4⁺ T cells. The reaction is called delayed because it typically develops 24 to 48 hours after antigen challenge in a previously immunized (sensitized) individual, in contrast to immediate hypersensitivity (allergic) reactions, which develop within minutes (described in Chapter 20).

In the classic animal model of DTH, a guinea pig was first immunized by the administration of a protein antigen in adjuvant; this step is called sensitization. About 2 weeks later, the animal was challenged subcutaneously with the same antigen, and the subsequent reaction was analyzed; this step is called the elicitation phase. Humans may be sensitized for DTH reactions by microbial infection, by contact sensitization with chemicals and environmental antigens, or by intradermal or subcutaneous injection of protein antigens (Fig. 19.6). Subsequent exposure to the same antigen (called challenge) elicits the reaction. For example, purified protein derivative (PPD), a protein antigen of *Mycobacterium tuberculosis*, elicits a DTH reaction, called the tuberculin reaction, when it is injected into individuals who have been exposed to *M. tuberculosis*. A positive tuberculin skin test response is a widely used clinical indicator of previous or active tuberculosis infection.

The characteristic response of DTH evolves over 24 to 48 hours. About 4 hours after the injection of an antigen in a sensitized individual, neutrophils accumulate around the postcapillary venules at the injection site. By about 12 hours, the injection site becomes infiltrated by T cells and blood monocytes, also organized in a perivenular distribution (Fig. 19.7). The endothelial cells lining these venules become enlarged and show increased biosynthetic organelles, and the vessels leak plasma macromolecules. Fibrinogen escapes from the blood vessels into the surrounding tissues, where it is converted into fibrin. The deposition of fibrin, edema, and the accumulation of T cells and monocytes within the extravascular tissue space around the injection site cause the tissue to swell and become firm (indurated). Induration, a diagnostic feature of DTH, is detectable by about 18 hours after the injection of antigen and is maximal by 24 to 48 hours. In clinical practice, loss of DTH responses to universally encountered antigens (e.g., *Candida* antigens) is an indication of deficient T cell function, a condition known as **anergy**. (This general loss of immune responsiveness is different from lymphocyte anergy, a mechanism for maintaining tolerance to specific antigens, discussed in Chapter 15.)

Although DTH has traditionally been considered a Th1-mediated injurious reaction, other T cells may contribute to the inflammation. In some DTH lesions, neutrophils are prominent, suggesting the involvement of Th17 cells. In infections by some helminthic parasites,

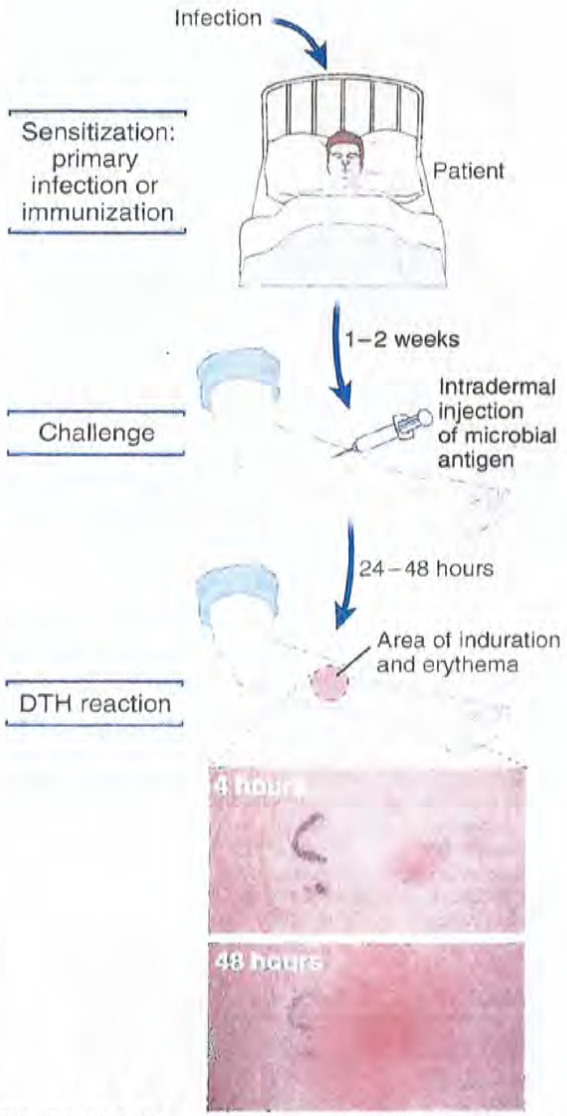


FIGURE 19.6 Delayed-type hypersensitivity (DTH) reaction. Infection or immunization (vaccination) sensitizes an individual, and subsequent challenge with an antigen from the infectious agent elicits a DTH reaction. The reaction is manifested by induration with redness and swelling at the site of the challenge, which peaks at ~48 hours. *(Courtesy of Dr. J. Faix, Department of Pathology, Stanford University School of Medicine, Palo Alto, California.)*

reactions against the parasite eggs elicit DTH with a strong component of eosinophils. In these cases, a role for Th2 cytokines has been demonstrated. $CD8^+$ T cells also produce IFN-γ and contribute to DTH reactions, especially in the skin. In fact, in cutaneous DTH reactions, such as contact hypersensitivity, the dominant T cell population is often $CD8^+$ cells.

Chronic DTH reactions can develop if a Th1 response to an infection activates macrophages but fails to

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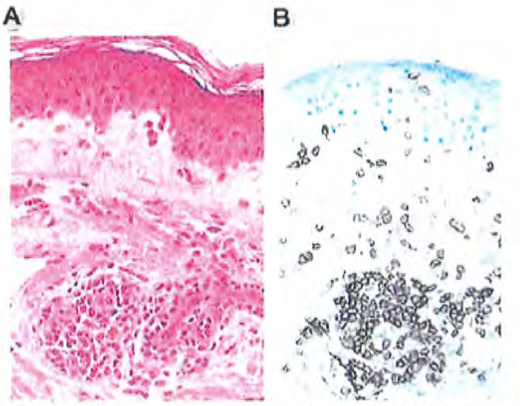


FIGURE 19.7 Morphology of a delayed-type hypersensitivity reaction. A, Histopathologic examination of the reaction in skin illustrated in Fig. 19.6 shows perivascular mononuclear cell infiltrates in the dermis. At higher magnification (not shown), the infiltrate is seen to consist of activated lymphocytes and macrophages surrounding small blood vessels in which the endothelial cells are also activated. **B,** Immunohistochemical staining demonstrates the presence of many $CD4^+$ T lymphocytes. *(Courtesy of Dr. J. Faix, Department of Pathology, Stanford University School of Medicine, Palo Alto, California.)*

eliminate phagocytosed microbes. If the microbes are localized in a small area, the reaction produces nodules of inflammatory tissue called granulomas (Fig. 19.8A). Chronic DTH, as exemplified by granulomatous inflammation, is caused by prolonged cytokine signals (see Fig. 19.8B). In such reactions, the activated T cells and macrophages continue to produce cytokines and growth factors, which amplify the reactions of both cell types and progressively modify the local tissue environment. The result is a cycle of tissue injury and chronic inflammation followed by replacement with connective tissue (fibrosis). In chronic DTH reactions, activated macrophages also undergo changes in response to persistent cytokine signals. These macrophages develop increased cytoplasm and cytoplasmic organelles and histologically may resemble skin epithelial cells, because of which they are sometimes called epithelioid cells. Activated macrophages may fuse to form multinucleate giant cells. Granulomatous inflammation is an attempt to contain the infection but is also the cause of significant tissue injury and functional impairment. This type of inflammation is a characteristic response to some persistent microbes, such as *M. tuberculosis*, and some fungi. Much of the respiratory difficulty associated with tuberculosis or chronic fungal infection of the lungs is caused by replacement of normal lung tissue with fibrotic tissue and is not directly attributable to the microbes.

Diseases Caused by Cytotoxic T Lymphocytes

CTL responses to viral infection can lead to tissue injury by killing infected cells, even if the virus itself has little cytopathic effects. The principal physiologic function of CTLs is to eliminate intracellular microbes, primarily viruses, by killing infected cells. Some viruses directly injure infected cells and are said to be cytopathic, whereas others are not. Because CTLs may not be able to distinguish between cytopathic and noncytopathic viruses, they kill virus-infected cells regardless of whether the infection itself is harmful to the host. Examples of viral infections in which the lesions are mainly due to the host CTL response and not the virus itself include lymphocytic choriomeningitis in mice and certain forms of viral hepatitis in humans (see Chapter 16).

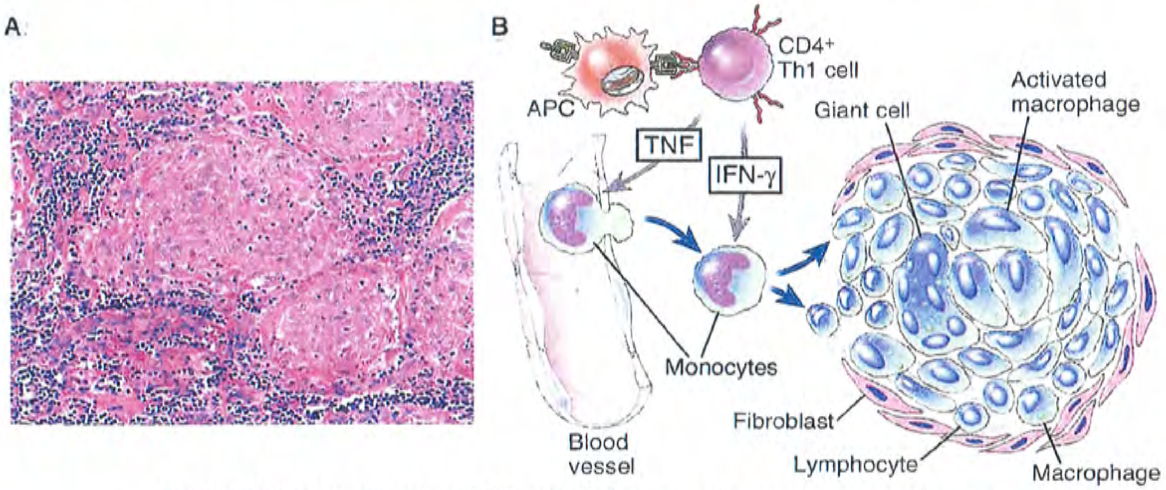


FIGURE 19.8 Granulomatous inflammation. A, Lymph node from a patient with tuberculosis containing granulomas with activated macrophages, multinucleate giant cells, and lymphocytes. In some granulomas, there may be a central area of necrosis (not shown). Immunohistochemical studies would identify the lymphocytes as T cells. **B,** Mechanisms of granuloma formation. Cytokines are involved in the generation of Th1 cells, activation of macrophages, and recruitment of leukocytes. Prolonged reactions of this type lead to the formation of granulomas.

CTLs may contribute to tissue injury in autoimmune disorders in which destruction of particular host cells is a prominent component, such as type 1 diabetes, where insulin-producing β cells in pancreatic islets are destroyed.

THERAPEUTIC APPROACHES FOR IMMUNOLOGIC DISEASES

One of the most impressive recent accomplishments of immunology has been the development of novel therapies for immunologic diseases based on the understanding of basic science (Fig. 19.9). The therapies can be divided into several broad groups.

Broadly Acting Antiinflammatory Agents

The mainstay of therapy for hypersensitivity diseases for many years has been antiinflammatory drugs, particularly corticosteroids. Such drugs inhibit the secretion of cytokines and other mediators of inflammation and thus reduce the inflammation associated with pathologic immune responses. Nonsteroidal antiinflammatory drugs are commonly used to reduce milder inflammatory reactions.

Anticytokine Therapies

A large number of cytokines and their receptors involved in inflammation are being targeted by specific antagonists for the treatment of chronic T cell–mediated inflammatory diseases (Table 19.5). The first success with this class of biologic agents came with a soluble form of the TNF receptor and anti-TNF antibodies, which bind to and neutralize TNF. These agents are of great benefit in many patients with rheumatoid arthritis, Crohn's disease, and psoriasis. Antibodies to the IL-6 receptor have been successfully used for juvenile and adult arthritis. Antagonists of other proinflammatory cytokines, such as IL-1, the p40 chain that is present in both IL-12 and IL-23, and IL-17, are now approved for various inflammatory diseases, and many others are in clinical trials. In addition to these biologic agents, small molecule inhibitors of JAK kinases (important intracellular signaling mediators of a variety of cytokine receptors; see Chapter 7) are also approved to inhibit cytokine actions in rheumatoid arthritis.

Depletion of Cells and Antibodies

Monoclonal antibodies that deplete all lymphoid cells, only B cells, or only T cells are used to treat severe inflammatory diseases. In Chapter 5, we listed some of the depleting antibodies in clinical practice (see Table 5.3). A recent development is the successful use of anti-CD20 antibody (rituximab), which depletes only B cells, to treat diseases that were thought to be caused primarily by T cell–mediated inflammation. This treatment has shown efficacy in patients with rheumatoid

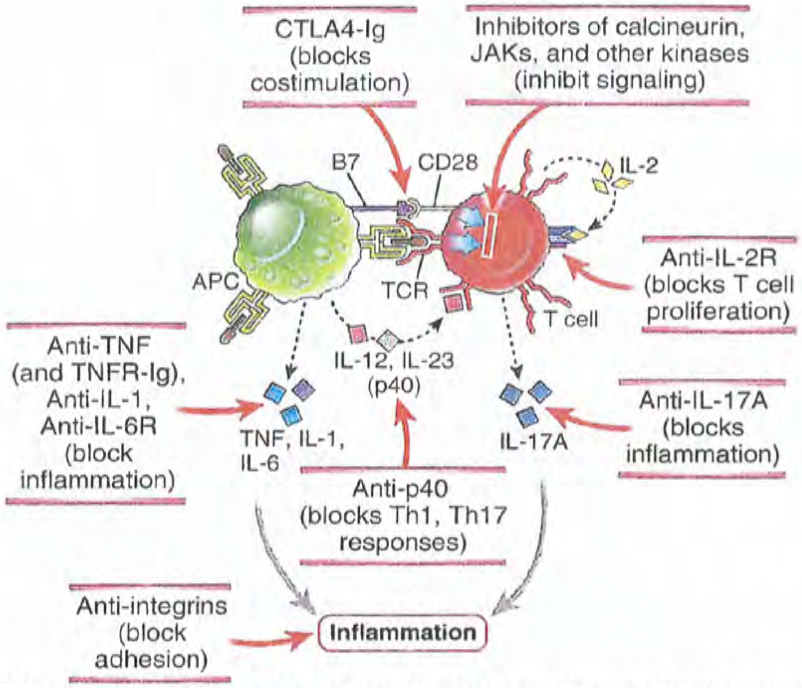


FIGURE 19.9 Novel therapies for inflammatory diseases targeting T cell responses and inflammation. Illustrated are the sites of action of some therapeutic agents that block different components of immune and inflammatory responses. Many of these agents target cytokines and their receptors. B cell depletion by anti-CD20 may also reduce pathologic T cell responses *(not shown)*.

TABLE 19.5 Examples of Cytokine Antagonists in Clinical Use or Trials

Cytokine or Receptor Targeted	Predicted Biologic Effects of Antagonist	Clinical Indications
TNF	Inhibits leukocyte migration into sites of inflammation	Rheumatoid arthritis, psoriasis, inflammatory bowel disease
IL-1	Inhibits leukocyte migration into sites of inflammation	Rare autoinflammatory syndromes, severe gout, rheumatoid arthritis
IL-6 receptor	Inhibits inflammation, antibody responses?	Juvenile idiopathic arthritis, rheumatoid arthritis
IL-17	Inhibits leukocyte recruitment into sites of inflammation	Psoriasis; possibly rheumatoid arthritis (trials ongoing)
p40 chain of IL-12 and IL-23	Inhibits Th1 and Th17 development	Inflammatory bowel disease, psoriasis
IL-2 receptor (CD25)	Inhibits IL-2–mediated T cell proliferation	Acute graft rejection
IFN-α	May be multiple effects on Th1 differentiation, antibody production	Systemic lupus erythematosus
IL-4/IL-13	Inhibits Th2 differentiation and function, IgE production	Asthma
BAFF	Reduces survival of B lymphocytes	Systemic lupus erythematosus

The table lists examples of antagonists against cytokines (antibodies or soluble receptors) that are approved for clinical use or in trials. Monoclonal antibodies specific for each of the listed targets are in clinical use; soluble TNF receptor and IL-1 receptor antagonists are used as well.
IFN, interferon; *IL*, interleukin; *TNF*, tumor necrosis factor.

arthritis and multiple sclerosis. The effectiveness of anti-CD20 may be related to a role of B cells as antigen-presenting cells for T cell responses, especially for the generation and maintenance of memory T cells. Plasmapheresis has been used to eliminate circulating autoantibodies and immune complexes.

Other Biologic Agents

CTLA-4-Ig, the agent that blocks B7 costimulators (see Chapter 9), is approved for treatment of rheumatoid arthritis and graft rejection. Antibodies against integrins have been used to inhibit leukocyte migration into tissues, particularly the central nervous system (CNS) in multiple sclerosis. Antibodies against CD40 ligand block T cell–mediated activation of B cells and macrophages and have been beneficial in patients with multiple sclerosis and inflammatory bowel disease, but some of the treated patients have developed thrombotic complications, apparently because this molecule is expressed on human platelets (where its function is unknown).

Intravenous IgG

Intravenous administration of pooled IgG from healthy donors (IVIG) has beneficial effects in some autoimmune diseases, such as immune thrombocytopenia and hemolytic anemia. It is not clear how this agent, which contains IgG of many unknown specificities, suppresses immune inflammation. One possibility is that the IgG binds to the inhibitory Fc receptor (FcγRIIB) on B lymphocytes (see Chapter 12) and dendritic cells and thus attenuates autoantibody production and inflammatory responses. IVIG may also compete with pathogenic antibodies for binding to the neonatal Fc receptor (FcRn), which functions in adults to protect antibodies from catabolism (see Chapter 5), resulting in reduced half-lives of the pathogenic antibodies.

Tolerance-Inducing Therapies

There are ongoing attempts at more specific treatment, such as inducing tolerance in disease-producing T cells. Multiple sclerosis and type 1 diabetes are two immune diseases in which the target antigens have been defined; in both diseases, clinical trials are underway in which the antigens (peptides of myelin basic protein and insulin, respectively) are administered to patients in ways that inhibit lymphocytes specific for the antigens. A risk of many treatments that block various components of the immune system is that these will interfere with the normal function of the immune system in combating microbes and thus make individuals susceptible to infections. Antigen-specific tolerance avoids this problem by selectively targeting the disease-causing lymphocytes.

There has also been great interest recently in exploiting our knowledge of regulatory T cells (Tregs) to treat inflammatory diseases. Numerous clinical trials are ongoing to purify patients' Tregs, expand and activate them in culture, and transfer them back to the patients. Another approach is to treat patients with low doses of IL-2, which is expected to activate and maintain Tregs more than effector cells, or IL-2 that is mutated to bind preferentially to CD25, the IL-2 receptor chain that is expressed at constant and high levels in Tregs.

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SELECTED IMMUNOLOGIC DISEASES: PATHOGENESIS AND THERAPEUTIC STRATEGIES

In the following section, we will describe the pathogenesis of selected diseases that are caused by antibodies and T cells and novel therapies for these diseases. The goal of this discussion is not to present clinical details but to focus on how diseases illustrate the principles underlying abnormal immune reactions.

Systemic Lupus Erythematosus (SLE): The Prototypic Immune Complex–Mediated Disease

SLE is a chronic, remitting and relapsing, multisystem autoimmune disease that affects predominantly women, with an incidence in the United States of 1 in 700 among women 20 to 60 years of age (about 1 in 250 among black women) and a female-to-male ratio of 10:1. The principal clinical manifestations are rashes, arthritis, and glomerulonephritis, but hemolytic anemia, thrombocytopenia, and central nervous system involvement are also common. Many different autoantibodies are found in patients with SLE. The most frequent are antinuclear, particularly anti-DNA, antibodies; others include antibodies against ribonucleoproteins, histones, and nucleolar antigens. Immune complexes formed from these autoantibodies and their specific antigens deposit in small arteries and capillaries throughout the body and are responsible for glomerulonephritis, arthritis, and vasculitis. Hemolytic anemia and thrombocytopenia are caused by autoantibodies against erythrocytes and platelets, respectively. The principal diagnostic test for the disease is the presence of antinuclear antibodies; antibodies against double-stranded native DNA are specific for SLE.

Pathogenesis of Systemic Lupus Erythematosus

SLE is a complex and incompletely understood disease in which genetic and environmental factors contribute to a breakdown of tolerance in self-reactive B and T lymphocytes. Among the genetic factors is the inheritance of particular HLA alleles. The odds ratio (relative risk) for individuals with HLA-DR2 or HLA-DR3 is 2 to 3, and if both haplotypes are present, the odds ratio is about 5. Genetic deficiencies of classical pathway complement proteins, especially C1q, C2, or C4, are seen in about 5% of patients with SLE. The complement deficiencies may result in defective clearance of immune complexes and apoptotic cells and failure of B cell tolerance. A polymorphism in the inhibitory Fc receptor FcγRIIB has been described in some patients; this may contribute to inadequate control of B cell activation or a failure to attenuate inflammatory responses in innate immune cells. Many other genes have been detected by genome-wide association studies, and the role of some of these, like the phosphatase PTPN22, has been considered in Chapter 15. Environmental factors include exposure to ultraviolet (UV) light. It is postulated that this leads to the apoptotic death of cells and release of nuclear antigens.

Two observations have led to new hypotheses of the pathogenesis of SLE. First, studies in patients have revealed that blood cells show a striking molecular signature (pattern of gene expression) that indicates exposure to IFN-α, a type I interferon that is produced mainly by plasmacytoid dendritic cells. Some studies have shown that plasmacytoid dendritic cells from SLE patients also produce abnormally large amounts of IFN-α. Second, studies in animal models have shown that Toll-like receptors (TLRs) that recognize DNA and RNA, notably the DNA-recognizing TLR9 and the RNA-recognizing TLR7, play a role in the activation of B cells specific for self nuclear antigens. On the basis of these studies, a model for the pathogenesis of SLE has been proposed (Fig. 19.10). According to this model, UV irradiation and other environmental insults lead to the apoptosis of cells. Inadequate clearance of the nuclei of these cells, in part because of defects in clearance mechanisms such as complement proteins and nucleases such as TREX1, results in a large burden of nuclear antigens. Polymorphisms in various susceptibility genes for lupus lead to a defective ability to maintain self-tolerance in B and T lymphocytes, because of which self-reactive lymphocytes remain functional. Failure of B cell tolerance may be due to defects in receptor editing or in deletion of immature B cells in the bone marrow or in peripheral tolerance. Self-reactive B cells that are not rendered tolerant are stimulated by the self nuclear antigens, and antibodies are produced against the antigens. Complexes of the antigens and antibodies bind to Fc receptors on dendritic cells and to the antigen receptor on B cells and may be internalized into endosomes. The nucleic acid components engage endosomal TLRs and stimulate B cells to produce more autoantibodies and activate dendritic cells, particularly plasmacytoid dendritic cells, to produce IFN-α, which further enhances the immune response and may cause more apoptosis. The net result is a cycle of antigen release and immune activation that leads to the production of high-affinity autoantibodies.

New Therapies for Systemic Lupus Erythematosus

The recent advances in our understanding of SLE are leading to novel therapeutic approaches. Clinical trials are underway to test the efficacy of antibodies against IFN-α or its receptor in the disease, and attempts to inhibit TLR signals are being considered. There has been great interest in depleting B cells by use of an antibody against the B cell surface protein CD20. An antibody that blocks the B cell growth factor BAFF is now approved for the treatment of SLE but seems to have only modest efficacy. Clinical trials of B cell depletion using anti-CD20 have had limited success. Additional approaches are to combine B cell depletion with depletion of long-lived plasma cells using proteasome inhibitors (which lead to the accumulation of misfolded proteins and ultimately cell death).

Rheumatoid Arthritis (RA)

RA is an inflammatory disease involving small and large joints of the extremities, including fingers and toes, wrists, shoulders, knees, and ankles. The disease is characterized by inflammation of the synovium associated with destruction of the joint cartilage and bone.

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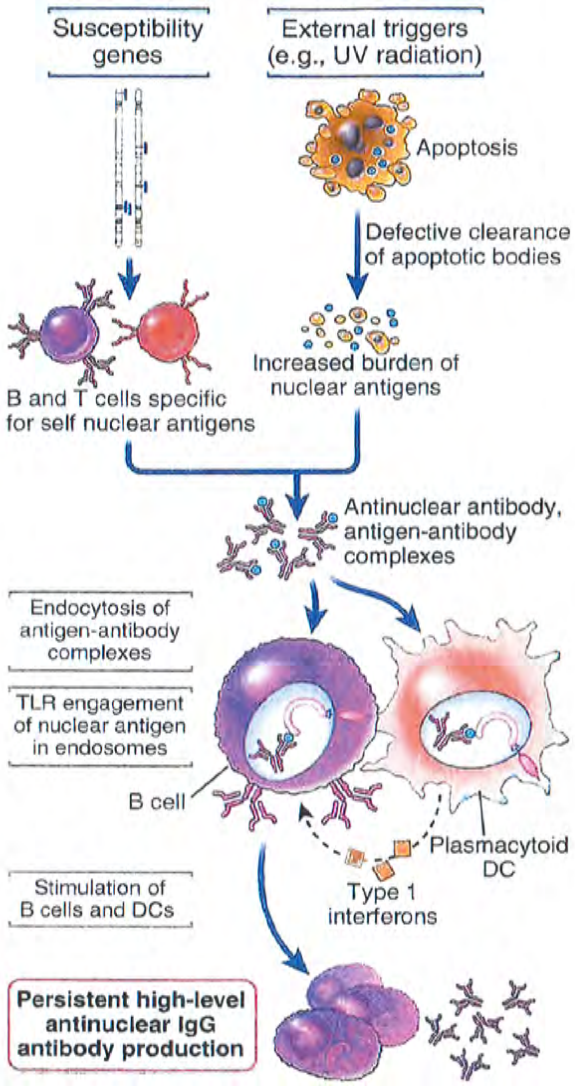


FIGURE 19.10 A model for the pathogenesis of systemic lupus erythematosus. In this hypothetical model, various susceptibility genes interfere with the maintenance of self-tolerance, and external triggers lead to persistence of nuclear antigens. The result is an antibody response against self nuclear antigens, which is amplified by the TLR-dependent activation of dendritic cells and B cells by nucleic acids, and the production of type I interferons.

with a morphologic picture indicative of a local immune response. Both cell-mediated and humoral immune responses may contribute to development of synovitis. $CD4^+$ Th1 and Th17 cells, activated B lymphocytes, plasma cells, and macrophages as well as other inflammatory cells are found in the inflamed synovium, and in severe cases, well-formed lymphoid follicles with germinal centers (so-called tertiary lymphoid organs) may be present. Numerous cytokines, including IL-1, IL-8, TNF, IL-6, IL-17, and IFN-γ, have been detected in the synovial (joint) fluid. Cytokines are believed to recruit leukocytes whose products cause tissue injury and also to activate resident synovial cells to produce proteolytic enzymes, such as collagenase, that mediate destruction of the cartilage, ligaments, and tendons of the joints. Increased osteoclast activity in the joints contributes to the bone destruction in RA, and this may be caused by the production of the TNF family cytokine RANK (receptor activator of nuclear factor κB) ligand by activated T cells. RANK ligand binds to RANK, a member of the TNF receptor family that is expressed on osteoclast precursors, and induces their differentiation and activation. Systemic complications of RA include vasculitis, presumably caused by immune complexes, and lung injury.

Although much of the emphasis in studies of RA has been on the role of T cells, antibodies may also contribute to the joint destruction. Activated B cells and plasma cells are often present in the synovia of affected joints. Patients frequently have circulating IgM or IgG antibodies that react with the Fc (and rarely Fab) portions of their own IgG molecules. These autoantibodies are called rheumatoid factors, and their presence is used as a diagnostic test for RA. Rheumatoid factors may participate in the formation of injurious immune complexes, but their pathogenic role is not established. Another type of antibody that has been detected in at least 70% of patients is specific for cyclic citrullinated peptides (CCP), which are derived from certain proteins that are modified in an inflammatory environment by the enzymatic conversion of arginine residues to citrulline. These anti-CCP antibodies are a specific diagnostic marker for the disease and may be involved in tissue injury.

Pathogenesis of Rheumatoid Arthritis

Like other autoimmune diseases, RA is a complex disorder in which genetic and environmental factors contribute to the breakdown of tolerance to self antigens. The specificity of the pathogenic T and B cells remains unclear, although both B and T cells that recognize citrullinated peptides have been identified. Susceptibility to RA is linked to the HLA-DR4 haplotype and to a few other HLA-DR alleles, all of which share a 5-residue segment (called the shared epitope) in the peptide-binding groove. Recent genome-wide association studies have revealed a large number of genetic polymorphisms associated with RA, including the gene encoding a tyrosine phosphatase, PTPN22, discussed in Chapter 15.

The identification of anti-CCP immune responses has led to new ideas about the pathogenesis of RA (Fig. 19.11). According to one model, environmental insults, such as smoking and some infections, induce the citrullination of self proteins, leading to the creation of new antigenic epitopes. Because these chemically modified epitopes are neoantigens that are not present normally, there may not be tolerance to these antigens. Individuals who have the HLA alleles that are capable of presenting these epitopes may mount T cell and antibody responses against the proteins. If these modified self proteins are also present in joints, the T cells and antibodies attack the joints. Th17 and perhaps Th1 cells secrete cytokines that recruit leukocytes into the joint and activate synovial

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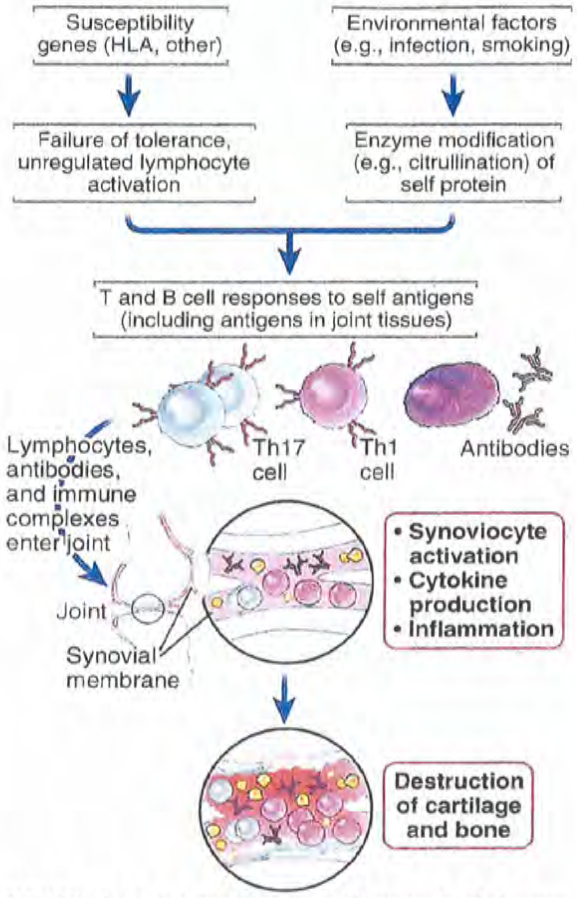


FIGURE 19.11 A model for the pathogenesis of rheumatoid arthritis. According to this model, citrullinated proteins induced by environmental stimuli elicit T cell and antibody responses in genetically susceptible individuals. The T cells and antibodies enter joints, respond to the self proteins, and cause tissue injury mainly by cytokine secretion and perhaps also by antibody-dependent effector mechanisms. Protein modifications other than citrullination may lead to the same result.

cells to produce collagenases and other enzymes. The net result is the progressive destruction of cartilage and bone. The chronic immune responses in the joints may lead to formation of tertiary lymphoid organs in the synovium, and these may maintain and propagate the local immune reaction.

New Therapies for Rheumatoid Arthritis

The realization of the central role of T cells and cytokines in the disease has led to remarkable advances in treatment, in which specific molecules have been targeted on the basis of scientific understanding. Chief among these new therapies are antagonists of TNF, which have transformed the course of the disease in many patients from one of progressive and inexorable joint destruction to one of smoldering but manageable chronic inflammation. Various other targeted therapies have been developed in the past 5 to 10 years; these have provided insight into disease pathogenesis. Blockade of cytokines other than TNF has been effective, including an antibody the blocks the IL-6 receptor, an IL-1 antagonist, and a small molecule that inhibits JAK signaling. Inhibition of T cell activation has been accomplished by blockade of B7:CD28 costimulation with CTLA-4-Ig, a fusion protein made of the extracellular domain of CTLA-4 and the Fc portion of IgG that binds B7 (see Chapter 9). B cell depletion with anti-CD20 antibody has also proven to be efficacious, although the mechanisms underlying this effect are not well understood.

Multiple Sclerosis (MS)

MS is an autoimmune disease of the central nervous system (CNS) in which $CD4^+$ T cells of the Th1 and Th17 subsets react against self myelin antigens, resulting in inflammation with activation of macrophages around nerves in the brain and spinal cord, destruction of the myelin, abnormalities in nerve conduction, and neurologic deficits. It is the most common neurologic disease of young adults. Pathologic examination reveals inflammation in the CNS white matter and demyelination. MS is characterized clinically by weakness, paralysis, and ocular symptoms with exacerbations and remissions; CNS imaging suggests that in patients with active disease, there is frequent new lesion formation.

MS is modeled by experimental autoimmune encephalomyelitis (EAE) in mice, rats, guinea pigs, and nonhuman primates, and this is one of the best characterized experimental models of an organ-specific autoimmune disease mediated mainly by T lymphocytes. EAE is induced by immunizing animals with antigens normally present in CNS myelin, such as myelin basic protein (MBP), proteolipid protein, and myelin oligodendrocyte glycoprotein, together with an adjuvant containing heat-killed mycobacteria, which is necessary to elicit a strong T cell response. About 1 to 2 weeks after immunization, animals develop encephalomyelitis, characterized by perivascular infiltrates of lymphocytes and macrophages in the CNS white matter, followed by demyelination. The neurologic lesions can be mild and self-limited or chronic and relapsing. These lesions result in progressive or remitting and relapsing paralysis. The disease can also be transferred to naive animals with T cells from diseased animals. Although antibodies against myelin antigens have been detected in patients as well as in the animal models, the pathogenic significance of these antibodies is not established.

Pathogenesis of Multiple Sclerosis

There is abundant evidence that EAE is caused by activated $CD4^+$ Th1 and Th17 cells specific for protein antigens in myelin. By analogy with the experimental disease, MS is also thought to be caused by myelin-specific Th1 and Th17 cells, and these cells have been detected in patients and isolated from the blood and CNS. How these cells are activated in patients remains an enigma. One theory is that an infection, most likely a viral infection, activates self myelin–reactive T cells by the phenomenon of molecular mimicry (see Chapter

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15). Self-tolerance may fail because of the inheritance of susceptibility genes. Identical twins have a 25% to 30% concordance rate for development of MS, whereas nonidentical twins have a 6% concordance rate. These observations implicate genetic factors in the development of the disease but also indicate that genetics can contribute only part of the risk. Genetic polymorphisms associated with MS include the HLA locus, with HLA-DRB1*1501 showing the strongest linkage. Genome-wide association studies and other genomic analyses have revealed over 100 genetic variants that contribute to disease risk; most of these map to genes involved in immune function. One interesting association is with a polymorphism in the noncoding region of the gene for the IL-2 receptor α chain, CD25. This polymorphism may alter the generation and maintenance of effector and/or regulatory T cells (Tregs). Other studies have suggested that the peripheral maintenance of Tregs is defective in MS patients, but how much this contributes to a failure of self-tolerance is not known. Once myelin-specific T cells are activated, they migrate into the CNS, where they encounter myelin proteins and release cytokines that recruit and activate macrophages and more T cells, leading to myelin destruction. Studies of EAE suggest that the disease is propagated by a process known as **epitope spreading** (see Chapter 15). The tissue breakdown results in the release of new protein antigens and the expression of new, previously sequestered epitopes that activate more autoreactive T cells.

New Therapies for Multiple Sclerosis

Immunotherapy for MS has, in the past, relied on approaches whose scientific bases are not well understood. These include administration of β-interferon, which may alter cytokine responses, and treatment with a random polymer of four amino acids, which is postulated to bind to HLA molecules and block antigen presentation. Recently, however, several new immune-modifying therapies based on rational principles have been developed. One is an antibody against the VLA-4 integrin (see Chapter 3), which blocks leukocyte migration into the CNS and has shown to be beneficial for patients. However, in a small number of patients, this treatment resulted in the reactivation of a latent JC virus infection, causing a severe and sometimes fatal CNS disease. Another recently approved drug to treat MS also interferes with leukocyte migration. The drug, called fingolimod (FTY720), blocks the sphingosine 1-phosphate–mediated pathway of T cell egress from lymphoid tissues (see Chapter 3). In a large subset of patients, B cell depletion with anti-CD20 antibody is beneficial. These results suggest an important role of B cells, presumably as APCs, in the activation of pathogenic T cells. Because MBP is known to be an important self antigen that is the target of the immune response in MS, it is hoped that the administration of MBP peptides will induce antigen-specific tolerance or generate Tregs specific for the relevant antigen, and early clinical trial results are promising. It is also striking that most of the therapies are more effective in early MS, which is characterized by inflammation, than in progressive MS, which is characterized by neurodegeneration and is the major cause of permanent disability. This realization is leading to new attempts to restore myelination and repair damaged axons and neurons.

Type 1 Diabetes

Type 1 diabetes, previously called insulin-dependent diabetes, is a multisystem metabolic disease resulting from impaired insulin production that affects about 0.2% of the US population, with a peak onset at 11 to 12 years of age. The incidence of the disease appears to be increasing in North America and Europe. The disease is characterized by hyperglycemia and ketoacidosis. Chronic complications of diabetes include progressive atherosclerosis of arteries, which can lead to ischemic necrosis of limbs and internal organs, and microvascular obstruction causing damage to the retina, renal glomeruli, and peripheral nerves. Type 1 diabetes is caused by a deficiency of insulin resulting from immune-mediated destruction of the insulin-producing β cells of the islets of Langerhans in the pancreas, and continuous hormone replacement therapy is needed. There is usually a long lag of many years between the initiation of autoimmunity and overt clinical disease because 90% or more of the islets have to be destroyed before clinical manifestations are seen.

Pathogenesis of Type 1 Diabetes

Several mechanisms may contribute to β cell destruction, including inflammation mediated by $CD4^+$ Th1 cells reactive with islet antigens (including insulin), CTL-mediated lysis of islet cells, local production of cytokines (TNF and IL-1) that damage islet cells, and autoantibodies against islet cells. In the few cases in which the pancreatic lesions have been examined at the early active stages of the disease, the islets show cellular necrosis and lymphocytic infiltration consisting of both $CD4^+$ and $CD8^+$ T cells. This lesion is called insulitis. Autoantibodies against islet cells and insulin are also detected in the blood of these patients. In susceptible children who have not developed diabetes (such as relatives of patients), the presence of antibodies against islet cells is predictive of the development of type 1 diabetes. An informative animal model of the disease is the nonobese diabetic (NOD) mouse, which develops spontaneous diabetes. In this model, there is evidence for defective survival and function of Tregs as well as resistance of effector T cells to suppression by Tregs. Another interesting idea that has emerged mostly from the mouse model is that post-translational modification of islet antigens may lead to the creation of new epitopes that elicit immune responses, similar to the neoantigens in RA, discussed previously.

Multiple genes are associated with type 1 diabetes. Most attention has been focused on the role of HLA genes. Between 90% and 95% of Caucasians with type 1 diabetes have HLA-DR3 or DR4, or both, in contrast to about 40% of normal subjects, and 40% to 50% of patients are DR3/DR4 heterozygotes, in contrast to 5% of normal subjects. Several non-HLA genes also contribute to the disease. The first of these to be identified is insulin, with tandem repeats in the promoter region being associated with disease susceptibility. The mechanism of this association is unknown: it may be related to

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the level of expression of insulin in the thymus, which determines whether insulin-specific T cells will be deleted (negatively selected) during their maturation. Several other polymorphisms have been identified in patients and in NOD mice, including in the *IL2* and *CD25* genes. Different polymorphisms in these genes may increase or decrease the risk of developing the disease, but how these polymorphisms affect T cell responses is not fully established. Some studies have suggested that viral infections (e.g., with coxsackievirus B4) may precede the onset of type 1 diabetes, perhaps by initiating cell injury, inducing inflammation and the expression of costimulators, and triggering an autoimmune response. However, epidemiologic data suggest that repeated infections protect against type 1 diabetes, and this is similar in the NOD model. In fact, it has been postulated that one reason for the increasing incidence of type 1 diabetes in developed countries is the control of infectious diseases.

New Therapies for Type 1 Diabetes

The most interesting new therapeutic strategies for type 1 diabetes are focused on inducing tolerance with diabetogenic peptides from islet antigens (such as insulin) and inducing or giving Tregs to patients. These clinical trials are in their early stages.

Inflammatory Bowel Disease

Inflammatory bowel disease consists of two disorders, Crohn's disease and ulcerative colitis, in which T cell–mediated inflammation causes intestinal injury. Crohn's disease is characterized by chronic inflammation and destruction of the intestinal wall, with frequent formation of fistulas. In ulcerative colitis, the lesions are largely confined to the mucosa and consist of ulcers with underlying foci of inflammation. The pathogenesis of inflammatory bowel disease was described in Chapter 14. New therapies for these diseases include antibodies against TNF, the p40 chain of IL-12 and IL-23, and integrins.

SUMMARY

- Disorders caused by abnormal immune responses are called hypersensitivity diseases. Pathologic immune responses may be autoimmune responses directed against self antigens or uncontrolled and excessive responses to foreign (e.g., microbial) antigens.
- Hypersensitivity diseases may result from antibodies that bind to cells or tissues (type II hypersensitivity), circulating immune complexes that are deposited in tissues (type III), or T lymphocytes reactive with antigens in tissues (type IV). Immediate hypersensitivity (type I) reactions are the cause of allergic diseases and are described in Chapter 20.
- The effector mechanisms of antibody-mediated tissue injury are complement activation and Fc receptor–mediated inflammation. Some antibodies cause disease by opsonizing host cells for phagocytosis or by interfering with normal cellular functions without producing tissue injury.
- The effector mechanisms of T cell–mediated tissue injury are inflammatory reactions induced by cytokines secreted mainly by $CD4^+$ Th1 and Th17 cells and cell lysis by CTLs. The classical T cell–mediated reaction is delayed-type hypersensitivity, induced by activation of previously primed T cells and the production of cytokines that recruit and activate various leukocytes, predominantly macrophages.
- The current treatment of autoimmune diseases is targeted at reducing immune activation and the injurious consequences of the autoimmune reaction. Agents include those that block inflammation, such as antibodies against cytokines and integrins, and those that block lymphocyte activation or destroy lymphocytes. A future goal of therapy is to inhibit the responses of lymphocytes specific for self antigens and to induce tolerance in these cells.
- Autoimmune diseases such as SLE, RA, MS, and type 1 diabetes illustrate many of the effector mechanisms that cause tissue injury in hypersensitivity reactions and the roles of susceptibility genes and environmental factors in the development of autoimmunity.

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APPENDIX

I

CYTOKINES

Cytokine and Subunits	Principal Cell Source	Cytokine Receptor and Subunits*	Principal Cellular Targets and Biologic Effects
Type I Cytokine Family Members			
Interleukin-2 (IL-2)	T cells	CD25 (IL-2Rα) CD122 (IL-2Rβ) CD132 (γc)	T cells: proliferation and differentiation into effector and memory cells; promotes regulatory T cell development, survival, and function NK cells: proliferation, activation B cells: proliferation, antibody synthesis (in vitro)
Interleukin-3 (IL-3)	T cells	CD123 (IL-3Rα) CD131 (βc)	Immature hematopoietic progenitors: induced maturation of all hematopoietic lineages
Interleukin-4 (IL-4)	$CD4^+$ T cells (Th2, Tfh), mast cells	CD124 (IL-4Rα) CD132 (γc)	B cells: isotype switching to IgE T cells: Th2 differentiation, proliferation Macrophages: alternative activation and inhibition of IFN-γ–mediated classical activation
Interleukin-5 (IL-5)	$CD4^+$ T cells (Th2), group 2 ILCs	CD125 (IL-5Rα) CD131 (βc)	Eosinophils: activation, increased generation
Interleukin-6 (IL-6)	Macrophages, endothelial cells, T cells	CD126 (IL-6Rα) CD130 (gp130)	Liver: synthesis of acute-phase protein B cells: proliferation of antibody-producing cells T cells: Th17 differentiation
Interleukin-7 (IL-7)	Fibroblasts, bone marrow stromal cells	CD127 (IL-7R) CD132 (γc)	Immature lymphoid progenitors: proliferation of early T and B cell progenitors T lymphocytes: survival of naive and memory cells
Interleukin-9 (IL-9)	$CD4^+$ T cells	CD129 (IL-9R) CD132 (γc)	Mast cells, B cells, T cells, and tissue cells: survival and activation
Interleukin-11 (IL-11)	Bone marrow stromal cells	IL-11Rα CD130 (gp130)	Production of platelets
Interleukin-12 (IL-12): IL-12A (p35) IL-12B (p40)	Macrophages, dendritic cells	CD212 (IL-12Rβ1) IL-12Rβ2	T cells: Th1 differentiation NK cells and T cells: IFN-γ synthesis, increased cytotoxic activity

Continued

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Cytokine and Subunits	Principal Cell Source	Cytokine Receptor and Subunits*	Principal Cellular Targets and Biologic Effects
Interleukin-13 (IL-13)	$CD4^+$ T cells (Th2), NKT cells, group 2 ILCs, mast cells	CD213a1 (IL-13Rα1) CD213a2 (IL-13Rα2) CD132 (γc)	B cells: isotype switching to IgE Epithelial cells: increased mucus production Macrophages: alternative activation
Interleukin-15 (IL-15)	Macrophages, other cell types	IL-15Rα CD122 (IL-2Rβ) CD132 (γc)	NK cells: proliferation T cells: survival and proliferation of memory $CD8^+$ cells
Interleukin-17A (IL-17A) Interleukin-17F (IL-17F)	$CD4^+$ T cells (Th17), group 3 ILCs	CD217 (IL-17RA) IL-17RC	Epithelial cells, macrophages and other cell types: increased chemokine and cytokine production; GM-CSF and G-CSF production
Interleukin-21 (IL-21)	Th2 cells, Th17 cells, Tfh cells	CD360 (IL-21R) CD132 (γc)	B cells: activation, proliferation, differentiation Tfh cells: development Th17 cells: increased generation
Interleukin-23 (IL-23): IL-23A (p19) IL-12B (p40)	Macrophages, dendritic cells	IL-23R CD212 (IL-12Rβ1)	T cells: differentiation and expansion of Th17 cells
Interleukin-25 (IL-25; IL-17E)	T cells, mast cells, eosinophils, macrophages, mucosal epithelial cells	IL-17RB	T cells and various other cell types: expression of IL-4, IL-5, IL-13
Interleukin-27 (IL-27): IL-27 (p28) EBI3 (IL-27B)	Macrophages, dendritic cells	IL-27Rα CD130 (gp130)	T cells: enhancement of Th1 differentiation; inhibition of Th17 differentiation NK cells: IFN-γ synthesis?
Stem cell factor (c-Kit ligand)	Bone marrow stromal cells	CD117 (KIT)	Pluripotent hematopoietic stem cells: induced maturation of all hematopoietic lineages
Granulocyte-monocyte CSF (GM-CSF)	T cells, macrophages, endothelial cells, fibroblasts	CD116 (GM-CSFRα) CD131 (βc)	Immature and committed progenitors, mature macrophages: induced maturation of granulocytes and monocytes, macrophage activation
Monocyte CSF (M-CSF, CSF1)	Macrophages, endothelial cells, bone marrow cells, fibroblasts	CD115 (CSF1R)	Committed hematopoietic progenitors: induced maturation of monocytes
Granulocyte CSF (G-CSF, CSF3)	Macrophages, fibroblasts, endothelial cells	CD114 (CSF3R)	Committed hematopoietic progenitors: induced maturation of granulocytes
Thymic stromal lymphopoietin (TSLP)	Keratinocytes, bronchial epithelial cells, fibroblasts, smooth muscle cells, endothelial cells, mast cells, macrophages, granulocytes and dendritic cells	TSLP-receptor CD127 (IL-7R)	Dendritic cells: activation Eosinophils: activation Mast cells: cytokine production T cells: Th2 differentiation

Cytokine and Subunits	Principal Cell Source	Cytokine Receptor and Subunits*	Principal Cellular Targets and Biologic Effects
Type II Cytokine Family Members			
IFN-α (multiple proteins)	Plasmacytoid dendritic cells, macrophages	IFNAR1 CD118 (IFNAR2)	All cells: antiviral state, increased class I MHC expression NK cells: activation
IFN-β	Fibroblasts, plasmacytoid dendritic cells	IFNAR1 CD118 (IFNAR2)	All cells: antiviral state, increased class I MHC expression NK cells: activation
Interferon-γ (IFN-γ)	T cells (Th1, $CD8^+$ T cells), NK cells	CD119 (IFNGR1) IFNGR2	Macrophages: classical activation (increased microbicidal functions) B cells: isotype switching to opsonizing and complement-fixing IgG subclasses (established in mice) T cells: Th1 differentiation Various cells: increased expression of class I and class II MHC molecules, increased antigen processing and presentation to T cells
Interleukin-10 (IL-10)	Macrophages, T cells (mainly regulatory T cells)	CD210 (IL-10Rα) IL-10Rβ	Macrophages, dendritic cells: inhibition of expression of IL-12, costimulators, and class II MHC
Interleukin-22 (IL-22)	Th17 cells	IL-22Rα1 *or* IL-22Rα2 IL-10Rβ2	Epithelial cells: production of defensins, increased barrier function Hepatocytes: survival
Interleukin-26 (IL-26)	T cells, monocytes	IL-20R1IL-10R2	Not established
Interferon-λs (type III interferons)	Dendritic cells	IFNLR1 (IL-28Rα) CD210B (IL-10Rβ2)	Epithelial cells: antiviral state
Leukemia inhibitory factor (LIF)	Embryonic trophectoderm, bone marrow stromal cells	CD118 (LIFR) CD130 (gp130)	Stem cells: block in differentiation
Oncostatin M	Bone marrow stromal cells	OSMR CD130 (gp130)	Endothelial cells: regulation of hematopoietic cytokine production Cancer cells: inhibition of proliferation
TNF Superfamily Cytokines†			
Tumor necrosis factor (TNF, TNFSF1)	Macrophages, NK cells, T cells	CD120a (TNFRSF1) *or* CD120b (TNFRSF2)	Endothelial cells: activation (inflammation, coagulation) Neutrophils: activation Hypothalamus: fever Muscle, fat: catabolism (cachexia)
Lymphotoxin-α (LTα, TNFSF1)	T cells, B cells	CD120a (TNFRSF1) or CD120b (TNFRSF2)	Same as TNF
Lymphotoxin-αβ (LTαβ)	T cells, NK cells, follicular B cells, lymphoid inducer cells	LTβR	Lymphoid tissue stromal cells and follicular dendritic cells: chemokine expression and lymphoid organogenesis
BAFF (CD257, TNFSF13B)	Dendritic cells, monocytes, follicular dendritic cells, B cells	BAFF-R (TNFRSF13C) *or* TACI (TNFRSF13B) *or* BCMA (TNFRSF17)	B cells: survival, proliferation

Continued

Cytokine and Subunits	Principal Cell Source	Cytokine Receptor and Subunits*	Principal Cellular Targets and Biologic Effects
APRIL (CD256, TNFSF13)	T cells, dendritic cells, monocytes, follicular dendritic cells	TACI (TNFRSF13B) *or* BCMA (TNFRSF17)	B cells: survival, proliferation
Osteoprotegerin (OPG, TNFRSF11B)	Osteoblasts	RANKL	Osteoclast precursor cells: inhibits osteoclast differentiation
IL-1 Family Cytokines			
Interleukin-1α (IL-1α)	Macrophages, dendritic cells, fibroblasts, endothelial cells, keratinocytes, hepatocytes	CD121a (IL-1R1) IL-1RAP *or* CD121b (IL-1R2)	Endothelial cells: activation (inflammation, coagulation) Hypothalamus: fever
Interleukin-1β (IL-1β)	Macrophages, dendritic cells, fibroblasts, endothelial cells, keratinocyte	CD121a (IL-1R1) IL-1RAP *or* CD121b (IL-1R2)	Endothelial cells: activation (inflammation, coagulation) Hypothalamus: fever Liver: synthesis of acute-phase proteins T cells: Th17 differentiation
Interleukin-1 receptor antagonist (IL-1RA)	Macrophages	CD121a (IL-1R1) IL-1RAP	Various cells: competitive antagonist of IL-1
Interleukin-18 (IL-18)	Monocytes, macrophages, dendritic cells, Kupffer cells, keratinocytes, chondrocytes, synovial fibroblasts, osteoblasts	CD218a (IL-18Rα) CD218b (IL-18Rβ)	NK cells and T cells: IFN-γ synthesis Monocytes: expression of GM-CSF, TNF, IL-1β Neutrophils: activation, cytokine release
Interleukin-33 (IL-33)	Endothelial cells, smooth muscle cells, keratinocytes, fibroblasts	ST2 (IL1RL1) IL-1 Receptor Accessory Protein (IL1RAP)	T cells: Th2 development ILCs: activation of group 2 ILCs
Other Cytokines			
Transforming growth factor-β (TGF-β)	T cells (mainly Tregs), macrophages, other cell types	TGF-β R1 TGF-β R2 TGF-β R3	T cells: inhibition of proliferation and effector functions; differentiation of Th17 and Treg B cells: inhibition of proliferation; IgA production Macrophages: inhibition of activation; stimulation of angiogenic factors Fibroblasts: increased collagen synthesis

APRIL, A proliferation-inducing ligand; *BAFF*, B cell–activating factor belonging to the TNF family; *BCMA*, B cell maturation protein; *CSF*, colony-stimulating factor; *IFN*, interferon; *IgE*, immunoglobulin E; *ILCs*, innate lymphoid cells; *MHC*, major histocompatibility complex; *NK* cell, natural killer cell; *OSMR*, oncostatin M receptor; *RANK*, receptor activator for nuclear factor κB ligand; *RANKL*, RANK ligand; *TACI*, transmembrane activator and calcium modulator and cyclophilin ligand interactor; *TNF*, tumor necrosis factor; *TNFSF*, TNF superfamily; *TNFRSF*, TNF receptor superfamily.

*Most cytokine receptors are dimers or trimers composed of different polypeptide chains, some of which are shared between receptors for different cytokines. The set of polypeptides that compose a functional receptor (cytokine binding plus signaling) for each cytokine is listed. The functions of each subunit polypeptide are not listed.

†All TNF superfamily (TNFSF) members are expressed as cell surface transmembrane proteins, but only the subsets that are predominantly active as proteolytically released soluble cytokines are listed in the table. Other TNFSF members that function predominantly in the membrane-bound form and are not, strictly speaking, cytokines are not listed in the table. These membrane-bound proteins and the TNFRSF receptors they bind to include OX40L (CD252, TNFSF4):OX40 (CD134, TNFRSF4); CD40L (CD154, TNFSF5):CD40 (TNFRSF5); FasL (CD178, TNFSF6):Fas (CD95, TNFRSF6); CD70 (TNFSF7):CD27 (TNFRSF27); CD153 (TNFSF8):CD30 (TNFRSF8); TRAIL (CD253, TNFSF10):TRAIL-R (TNFRSF10A-D); RANKL (TNFSF11):RANK (TNFRSF11); TWEAK (CD257, TNFSF12):TWEAKR (CD266, TNFRSF12); LIGHT (CD258, TNFSF14):HVEM (TNFRSF14); GITRL (TNFSF18):GITR (CD357 TNFRSF18); 4-1BBL:4-1BB (CD137).



APPENDIX

III

LABORATORY TECHNIQUES COMMONLY USED IN IMMUNOLOGY

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Immunologic techniques are used widely both in research laboratories and clinical settings, and many of these are based on the use of antibodies. In addition, many of the techniques of modern molecular biology have provided invaluable information about the immune system and are also now being used to analyze immune features of diseases for diagnostic purposes. We have mentioned these techniques often throughout the book. In this appendix, we will describe the principles underlying some of the most commonly used laboratory methods in immunology. In addition, we will summarize how B and T lymphocyte responses are studied with use of laboratory techniques. Details of how to carry out various assays may be found in laboratory manuals and research papers.

LABORATORY METHODS USING ANTIBODIES

The exquisite specificity of antibodies for particular antigens makes antibodies valuable reagents for detecting, purifying, and quantitating antigens. Because antibodies can be produced against virtually any type of macromolecule and small chemical, antibody-based techniques may be used to study virtually any type of molecule in solution or in cells. The method for producing monoclonal antibodies (see Chapter 5) has greatly increased our ability to generate antibodies of almost any desired specificity. Historically, many of the uses of antibody depended on the ability of antibody and specific antigen to form large immune complexes, either in solution or in gels, that could be detected by various optical methods. These methods were of great importance in early studies but have now been replaced almost entirely by simpler methods based on immobilized antibodies or antigens.

Quantitation of Antigen by Immunoassays

Immunologic methods of quantifying antigen concentration provide exquisite sensitivity and specificity and have become standard techniques for both research and clinical applications. All modern immunochemical methods of quantitation are based on having a pure antigen or antibody whose quantity can be measured by an indicator molecule (or a label). When the antigen or antibody is labeled with a radioisotope, as first introduced by Rosalyn Yalow and colleagues, it may be quantified by instruments that detect radioactive decay events:

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the assay is called a **radioimmunoassay (RIA)**. More commonly now, the antigen or antibody is covalently coupled to an enzyme and is quantified by determining with a spectrophotometer the rate at which the enzyme converts a clear substrate to a colored product; the assay is called an **enzyme-linked immunosorbent assay (ELISA)**. Several variations of RIA and ELISA exist, but the most commonly used version is the sandwich assay (Fig. A.1). The sandwich assay uses two different antibodies reactive with different epitopes on the antigen whose concentration needs to be determined. A fixed quantity of one antibody is attached to a series of replicate solid supports, such as plastic microtiter wells. Test solutions containing antigen at an unknown concentration or a series of standard solutions with known concentrations of antigen are added to the wells and allowed to bind. Unbound antigen is removed by washing, and the second antibody, which is enzyme linked or radiolabeled, is allowed to bind. The antigen serves as a bridge, so the more antigen in the test or standard solutions, the more enzyme-linked or radiolabeled second antibody will bind. The results from the standard solutions are used to construct a binding curve for the second antibody as a function of antigen concentration, from which the quantities of antigen in the test solutions may be inferred. When this test is performed with two monoclonal antibodies, it is essential that these antibodies see nonoverlapping determinants on the antigen; otherwise, the second antibody cannot bind. The sandwich ELISA approach has been adapted to many point-of-care and home tests,

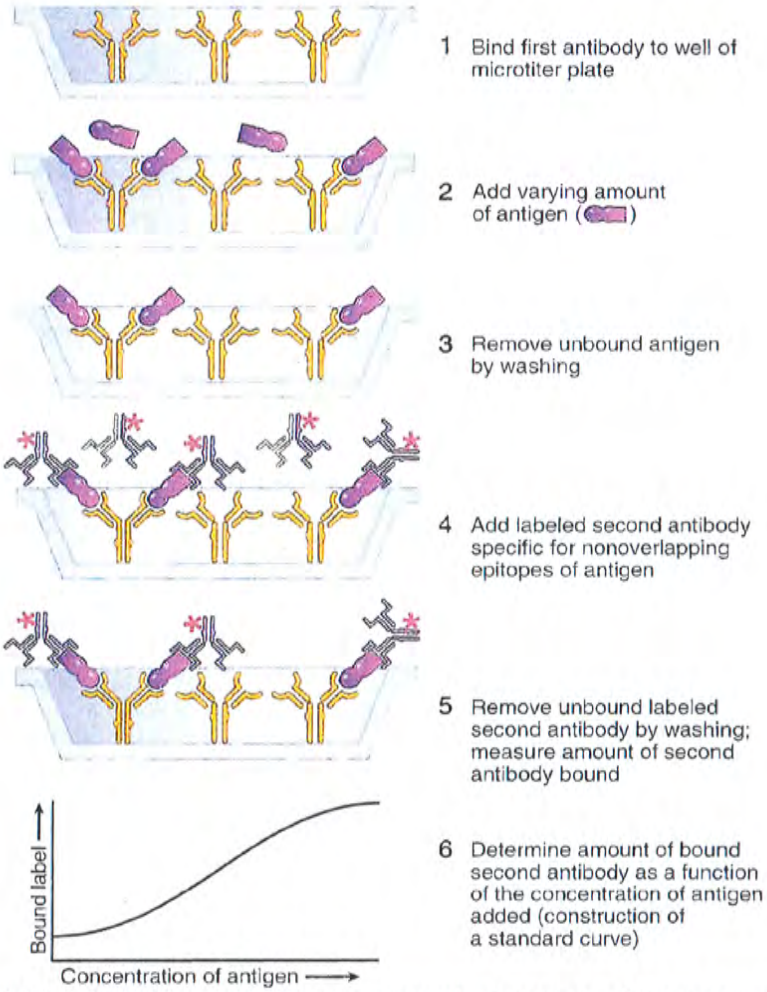


FIGURE A.1 Sandwich enzyme-linked immunosorbent assay or radioimmunoassay. A fixed amount of one immobilized antibody is used to capture an antigen. The binding of a second, labeled antibody that recognizes a nonoverlapping determinant on the antigen will increase as the concentration of antigen increases and thus allow quantification of the antigen.

in which the enzyme generation of a colored product is readily determined by portable spectrophotometers, or in the case of commonly used pregnancy tests, by eye.

In an important clinical variant of immunobinding assays, samples from patients may be tested for the presence of antibodies that are specific for a microbial antigen (e.g., antibodies reactive with proteins from human immunodeficiency virus [HIV] or hepatitis B virus) as indicators of infection. In this case, a saturating quantity of antigen is added to replicate wells containing plate-bound antibody, or the antigen is attached directly to the plate, and serial dilutions of the patient's serum are then allowed to bind. The amount of the patient's antibody bound to the immobilized antigen is determined by use of an enzyme-linked second anti-human immunoglobulin (Ig) antibody.

Identification and Purification of Proteins

Antibodies can be used to identify and characterize proteins and to purify specific proteins from mixtures. Two commonly used methods to identify and purify proteins are immunoprecipitation and immuno-affinity chromatography. Western blotting is a widely used technique to determine the presence and size of a protein in a biologic sample.

Immunoprecipitation and Immuno-Affinity Chromatography

Immunoprecipitation is a technique in which an antibody specific for one protein antigen in a mixture of proteins is used to identify this specific antigen (Fig. A.2A). The antibody is typically added to a protein mixture (usually a detergent lysate of specific cells), and staphylococcal protein A (or protein G) covalently attached to agarose beads is added to the mixture. The Fab portions of the antibody bind to the target protein, and the Fc portion of the antibody is captured by the protein A or protein G on the beads. Unwanted proteins that do not bind to the antibody are then removed by washing the beads (by repeated detergent addition and centrifugation). The specific protein that is recognized by and now bound to the antibody may be eluted from the beads and dissociated from the antibody by use of a harsh denaturant (such as sodium dodecyl sulfate), and the proteins are separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). Proteins may be detected after electrophoresis by staining the polyacrylamide gel with a protein stain or by a Western blot analysis (described later). If the original mixture contained radioactively labeled proteins, specific proteins immunoprecipitated by the antibody may be revealed by autofluorography or autoradiography, with protein bands being captured on x-ray film placed on the dried SDS–polyacrylamide gel containing separated proteins.

Immuno-affinity chromatography, a variant of affinity chromatography, is a purification method that relies on antibodies attached to an insoluble support to purify antigens from a solution (see Fig. A.2B). Antibodies specific for the desired antigen are typically covalently attached to a solid support, such as agarose beads, and packed into a column. A complex mixture of antigens is passed through the beads to allow the antigen that is recognized by the antibody to bind. Unbound molecules are washed away, and the bound antigen is eluted by changing the pH or by exposure to high salt or other chaotropic conditions that break antigen-antibody interactions. A similar method may be used to purify antibodies from culture supernatants or natural fluids, such as serum, by first attaching the antigen to beads and passing the supernatants or serum through.

Western Blotting

Western blotting (Fig. A.3) is used to identify and determine the relative quantity and molecular weight of a protein within a mixture of proteins or other molecules. The mixture is first subjected to analytical separation, typically by SDS-PAGE, so that the final positions of different proteins in the gel are a function of their molecular size. The array of separated proteins is then transferred from the separating polyacrylamide gel to a support membrane by electrophoresis such that the membrane acquires a replica of the array of separated macromolecules present in the gel. SDS is displaced from the protein during the transfer process, and native antigenic determinants are often regained as the protein refolds. The position of the protein antigen on the membrane can then be detected by binding of an unlabeled antibody specific for that protein (the primary antibody) followed by a labeled second antibody that binds to the primary antibody. This approach provides information about antigen size and quantity. In general, second antibody probes are labeled with enzymes that generate chemiluminescent signals and leave images on photographic film. Near-infrared fluorophores can also be used to label antibodies, and light produced by the excitation of the fluorophore provides more accurate antigen quantitation compared with enzyme-linked second antibodies. The sensitivity and specificity of this technique can be increased by starting with immunoprecipitated proteins instead of crude protein mixtures. This sequential procedure is especially useful for detection of protein-protein interactions. For example, the physical association of two different proteins in the membrane of a lymphocyte can be established by immunoprecipitating a membrane extract by use of an antibody specific for one of the proteins and probing a Western blot of the immunoprecipitate using an antibody specific for the second protein that may have been co-immunoprecipitated along with the first protein.

The technique of transferring proteins from a gel to a membrane is called Western blotting as a biochemist's joke. Southern is the last name of the scientist who first blotted DNA from a separating gel to a membrane by capillary transfer, a technique since called Southern blotting. By analogy, Northern blotting was the term applied to the technique of transferring RNA from a gel to a membrane, and Western blotting is the term used to describe the transfer of proteins to a membrane.

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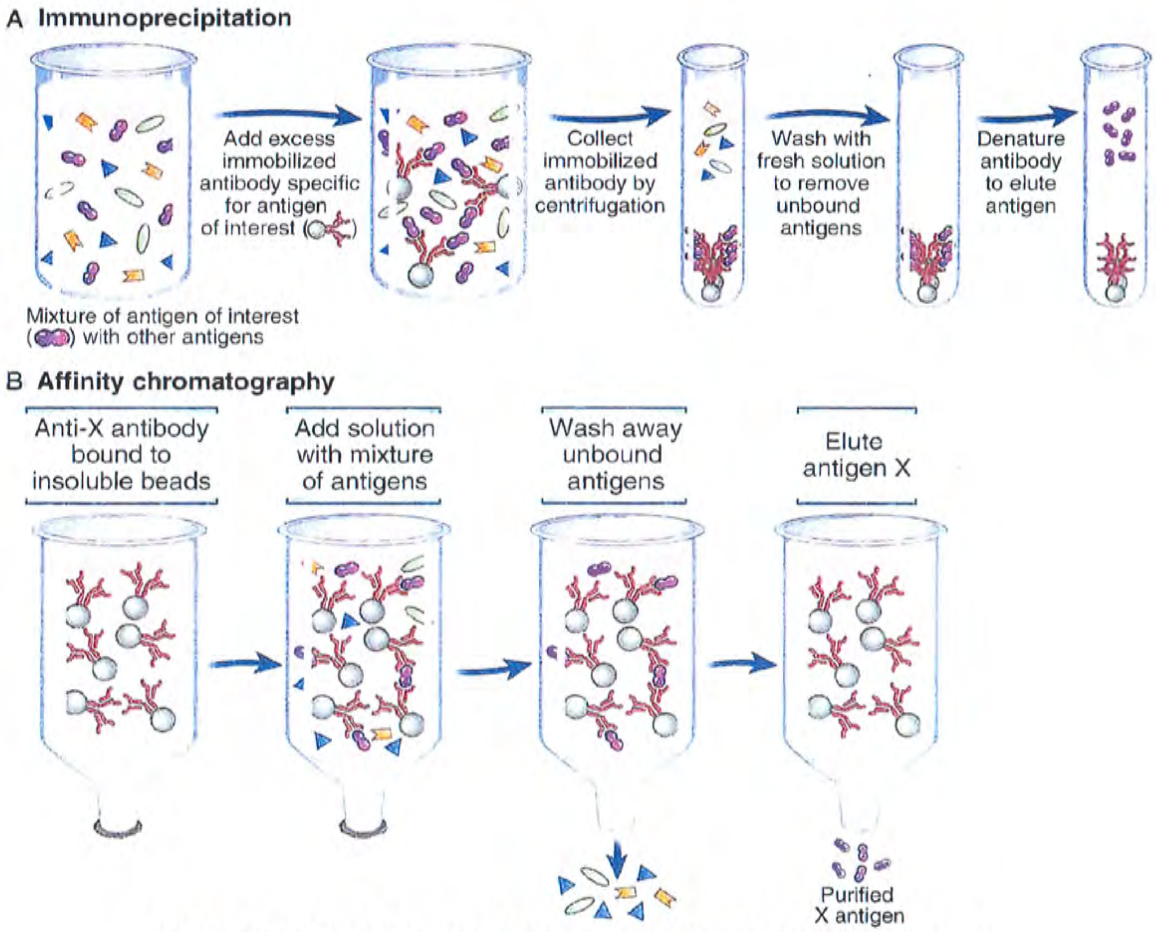


FIGURE A.2 Isolation of an antigen by immunoprecipitation or affinity chromatography. A, A particular antigen can be purified from a mixture of antigens in serum or other solutions by adding antibodies specific to the antigen that are bound to insoluble beads. Unbound antigens are then washed away, and the desired antigen is recovered by changing the pH or ionic strength of the solution so that the affinity of antibody-antigen binding is lowered. Immunoprecipitation can be used as a means of purification, as a means of quantification, or as a means of identification of an antigen. Antigens purified by immunoprecipitation are often analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. **B,** Affinity chromatography is based on the same principle as immunoprecipitation, except that the antibody is fixed to an insoluble matrix or beads, usually in a column. The method is often used to isolate soluble antigens *(shown)* or antibodies specific for an immobilized antigen.

Labeling and Detection of Antigens in Cells and Tissues

Antibodies specific for antigens expressed on or in particular cell types are commonly used to identify these cells in tissues or cell suspensions and to separate these cells from mixed populations. In these methods, the antibody can be radiolabeled, enzyme linked, or, most commonly, fluorescently labeled, and a detection system is used that can identify the bound antibody. Antibodies attached to magnetic beads can be used to physically isolate cells expressing specific antigens.

Flow Cytometry

The tissue lineage, maturation stage, or activation status of a cell can often be determined by analyzing the cell surface or intracellular expression of different molecules. This is commonly done by staining the cell with fluorescently labeled probes that are specific for those molecules and measuring the quantity of fluorescence emitted by the cell (Fig. A.4). The flow cytometer is a specialized instrument that can detect fluorescence on individual cells in a suspension and thereby determine the number of cells expressing the molecule to which a fluorescent

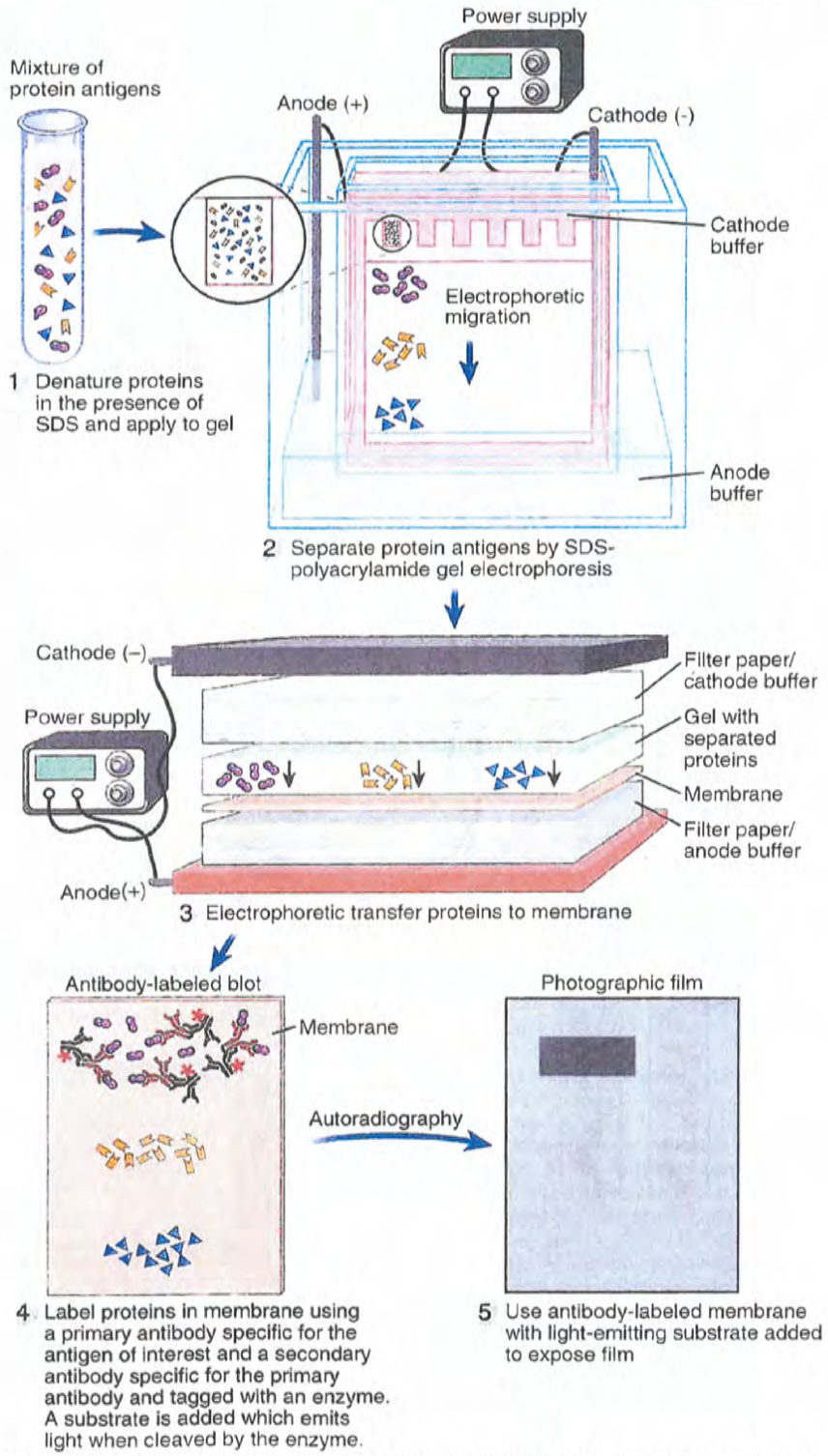


FIGURE A.3 Characterization of antigens by western blotting. Protein antigens, separated by sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis and transferred to a membrane, can be detected by an antibody that is in turn revealed by a second antibody that may be conjugated to an enzyme such as horseradish peroxidase or to a fluorophore.

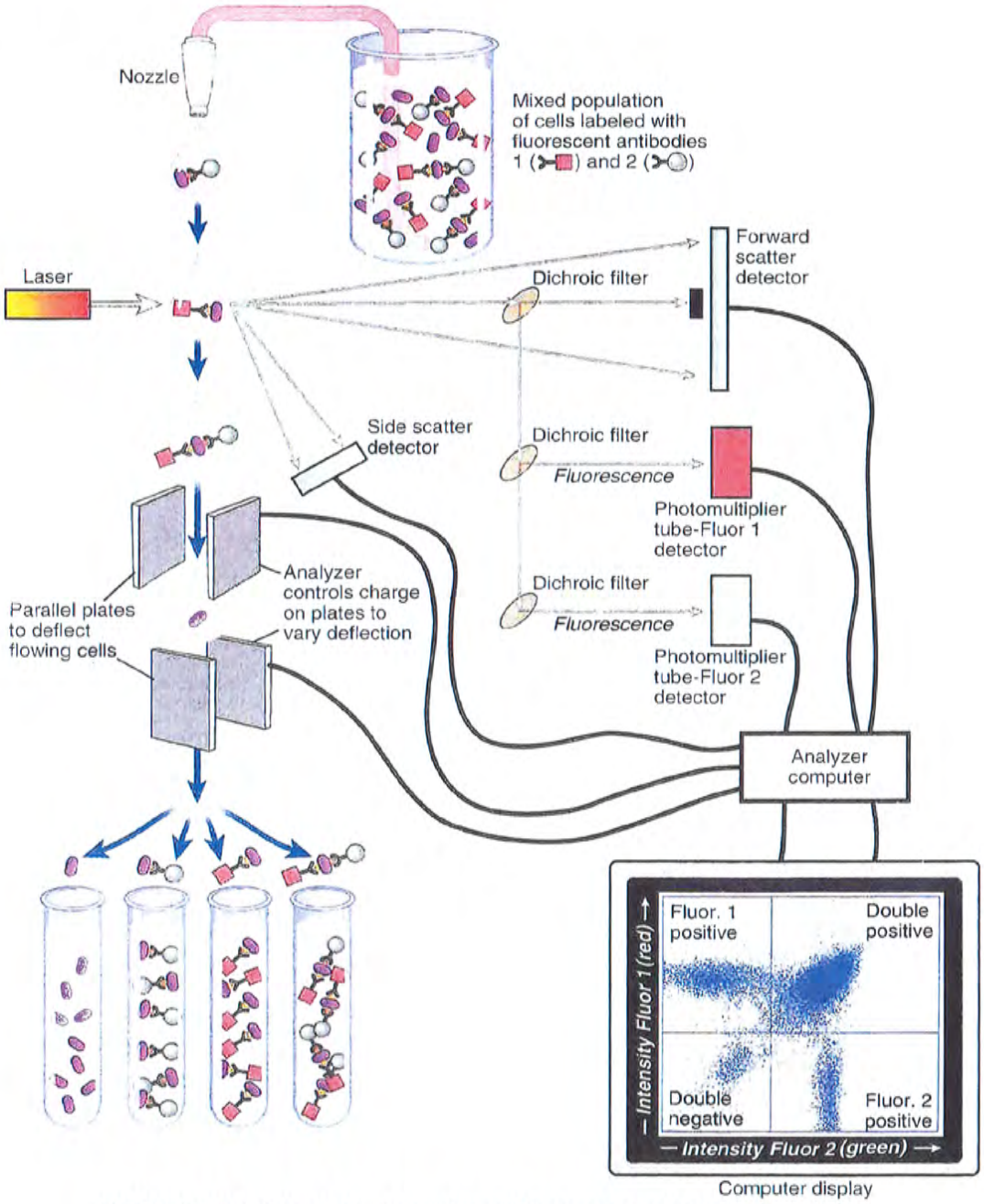


FIGURE A.4 Principle of flow cytometry and fluorescence-activated cell sorting. The incident laser beam is of a designated wavelength, and the light that emerges from the sample is analyzed for forward and side scatter as well as fluorescent light of two or more wavelengths that depend on the fluorochrome labels attached to the antibodies. The separation depicted here is based on two antigenic markers (two-color sorting). Modern instruments can routinely analyze and separate cell populations on the basis of three or more different-colored probes.

probe binds. Suspensions of cells are incubated with fluorescently labeled probes, and the amount of probe bound by each cell in the population is measured by passing the cells one at a time through a fluorimeter with a laser-generated incident beam. The relative amounts of a particular molecule on different cell populations can be compared by staining each population with the same probe and determining the amount of fluorescence emitted. In preparation for flow cytometric analysis, cell suspensions are stained with the fluorescent probes of choice. Most often, these probes are fluorochrome-labeled antibodies specific for a cell surface molecule. Alternatively, cytoplasmic molecules can be stained by temporarily permeabilizing cells and permitting the labeled antibodies to enter through the plasma membrane. In addition to antibodies, various fluorescent indicators of cytoplasmic ion concentrations and reduction-oxidation potential can be detected by flow cytometry. Cell cycle studies can be performed by flow cytometric analysis of cells stained with fluorescent DNA-binding probes, such as propidium iodide. Apoptotic cells can be identified with fluorescent probes, such as annexin V, that bind to abnormally exposed phospholipids on the surface of the dying cells. Modern flow cytometers can routinely detect three or more different-colored fluorescent signals, each attached to a different antibody or other probe. This technique permits simultaneous analysis of the expression of many different combinations of molecules by a cell. In addition to detecting fluorescent signals, flow cytometers also measure the forward and side light-scattering properties of cells, which reflect cell size and internal complexity, respectively. This information is often used to distinguish different cell types. For example, compared with lymphocytes, neutrophils cause greater side scatter because of their cytoplasmic granules, and monocytes cause greater forward scatter because of their size.

A newly developed antibody-based technology called mass cytometry combines the single-cell flow technology of flow cytometers with mass spectrometry. The commercially available device used for this purpose is called **CyTOF**, with "TOF" indicating that it is a time-of-flight–type of mass cytometer. Antibodies specific for molecules of interest are labeled with any one of a large number of heavy metals, using a different metal for each antibody specificity. These antibodies are incubated with the cell population being studied, and the cells are analyzed by a CyTOF instrument that performs mass spectrometry on individual cells. Unlike fluorescence labels, many different heavy metal labels can be resolved by mass spectrometry without overlap, allowing for the detection of as many as 100 different molecules on a single cell.

Cytokine Bead Assays

In these assays, the concentration of many different cytokines in a single solution can be determined simultaneously. Microscopic-sized beads of different sizes are labeled with different amounts of a fluorochrome, such as allophycocyanin (APC), and the beads are pre-coated with a cytokine-specific antibody. The beads with each anti-cytokine specificity can be distinguished from one another based on size and APC fluorescence intensity. These beads are mixed with the test solution that contains multiple cytokines, such as serum or supernatants of lymphocyte cultures. Each cytokine will bind only to beads of one particular size and fluorescence intensity. Biotinylated detection antibodies specific for each cytokine are added to form antibody-antigen sandwiches, and phycoerythrin (PE)-conjugated streptavidin is added to detect these sandwiches. The beads are simultaneously analyzed by a two-laser flow-based detection instrument. One laser identifies the bead and determines the cytokine being detected. The other laser measures the intensity of the PE-fluorescence signal, which is directly related to the amount of cytokine bound. Standard solutions with known concentrations of the cytokines are used to calibrate the results.

Purification of Cells

A **fluorescent-activated cell sorter** (FACS) is an adaptation of the flow cytometer that allows one to separate cell populations according to which and how much fluorescent probe they bind. This technique is accomplished by differentially deflecting the cells with electromagnetic fields whose strength and direction are varied according to the measured intensity of the fluorescent signal (see Fig. A.4). The cells may be labeled with fluorescently tagged antibodies ex vivo, or, in the case of experimental animal studies, labeling may be accomplished in vivo by expression of transgenes that encode fluorescent proteins, such as green fluorescent protein. (Transgenic technology is described later in this appendix.)

Another commonly used technique to purify cells with a particular phenotype relies on antibodies that are attached to magnetic beads. These "immunomagnetic reagents" will bind to certain cells, depending on the specificity of the antibody used, and the bound cells can then be pulled out of suspension by a strong magnet.

Immunofluorescence and Immunohistochemistry

Antibodies can be used to identify the anatomic distribution of an antigen within a tissue or within compartments of a cell. To do so, the tissue or cell is incubated with an antibody that is labeled with a fluorochrome or enzyme, and the position of the label, determined with a suitable microscope, is used to infer the position of the antigen. In the earliest version of this method, called immunofluorescence, the antibody was labeled with a fluorescent dye and allowed to bind to a monolayer of cells or to a frozen section of a tissue. The stained cells or tissues were examined with a fluorescence microscope to locate the antibody. Although sensitive, the fluorescence microscope is not an ideal tool to identify the detailed structures of the cell or tissue because of a low signal-to-noise ratio. This problem has been overcome by new technologies including confocal microscopy, which uses optical sectioning technology to filter out unfocused fluorescent light, and two-photon microscopy, which prevents out-of-focus light from forming. Alternatively, antibodies may be coupled to enzymes that convert colorless substrates to colored insoluble substances that precipitate at

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the position of the enzyme. A conventional light microscope may then be used to localize the antibody in a stained cell or tissue. The most common variant of this method uses the enzyme horseradish peroxidase, and the method is commonly referred to as the immunoperoxidase technique. Another commonly used enzyme is alkaline phosphatase. Different antibodies coupled to different enzymes may be used in conjunction to produce simultaneous two-color localizations of different antigens. In other variations, antibody can be coupled to an electron-dense probe such as colloidal gold, and the location of antibody can be determined subcellularly by means of an electron microscope, a technique called immunoelectron microscopy. Different-sized gold particles have been used for simultaneous localization of different antigens at the ultrastructural level.

In all immunomicroscopic methods, signals may be enhanced by use of sandwich techniques. For example, instead of attaching horseradish peroxidase to a specific mouse antibody directed against the antigen of interest, it can be attached to a second anti-antibody (e.g., rabbit anti-mouse Ig antibody) that is used to bind to the first, unlabeled antibody. When the label is attached directly to the specific, primary antibody, the method is referred to as direct; when the label is attached to a secondary or even tertiary antibody, the method is indirect. In some cases, molecules other than antibody can be used in indirect methods. For example, staphylococcal protein A, which binds to IgG, or avidin, which binds to primary antibodies labeled with biotin, can be coupled to fluorochromes or enzymes.

Measurement of Antigen-Antibody Interactions

In many situations, it is important to know the affinity of an antibody for an antigen. For example, the usefulness of a monoclonal antibody as an experimental or therapeutic reagent depends on its affinity. Antibody affinities for antigen can be measured directly for small antigens (e.g., haptens) by a method called equilibrium dialysis (Fig. A.5). In this method, a solution of antibody is confined within a "semipermeable" membrane of porous cellulose and immersed in a solution containing the antigen. (*Semipermeable* in this context means that small molecules, such as antigen, can pass freely through the membrane pores but that macromolecules, such as antibody, cannot.) If no antibody is present within the membrane-bound compartment, the antigen in the bathing solution enters until the concentration of antigen within the membrane-bound compartment becomes exactly the same as that outside. Another way to view the system is that at dynamic equilibrium, antigen enters and leaves the membrane-bound compartment at exactly the same rate. However, when antibody is present inside the membrane, the net amount of antigen inside the membrane at equilibrium increases by the quantity that is bound to antibody. This phenomenon occurs because only unbound antigen can diffuse across the membrane, and at equilibrium, it is the unbound concentration of antigen that must be identical inside and outside the membrane. The extent of the increase in antigen inside the membrane depends on the antigen concentration, on the antibody concentration, and on the dissociation constant (K_d) of the binding interaction. K_d can be calculated by measurement of antigen and antibody concentrations, by spectroscopy, or by other means.

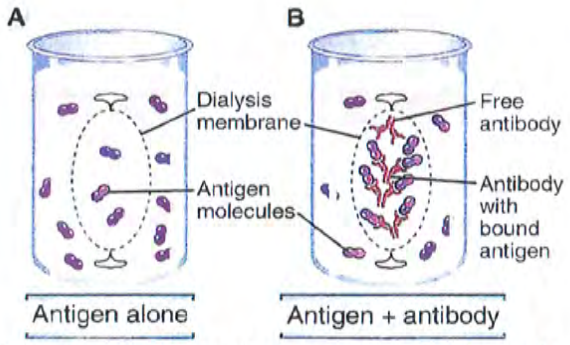


FIGURE A.5 Analysis of antigen-antibody binding by equilibrium dialysis. In the presence of antibody **(B)**, the amount of antigen within the dialysis membrane is increased compared with the absence of antibody **(A)**. As described in the text, this difference, caused by antibody binding of antigen, can be used to measure the affinity of the antibody for the antigen. This experiment can be performed only when the antigen is a small molecule (e.g., a hapten) capable of freely crossing the dialysis membrane.

An alternative way to determine K_d is by measurement of the rates of antigen-antibody complex formation and dissociation. These rates depend, in part, on the concentrations of antibody and antigen and on the affinity of the interaction. All parameters except the concentrations can be summarized as rate constants, and both the on-rate constant (K_{on}) and the off-rate constant (K_{off}) can be calculated experimentally by determining the concentrations and the actual rates of association or dissociation, respectively. The ratio of K_{off}/K_{on} allows one to cancel out all the parameters not related to affinity and is exactly equal to the dissociation constant K_d. Thus, one can measure K_d at equilibrium by equilibrium dialysis or calculate K_d from rate constants measured under non-equilibrium conditions.

Another method, more commonly used today, to measure the kinetics of antigen-antibody interactions depends on surface plasmon resonance. In this method, a specialized biosensing instrument (such as the Biacore, Uppsala, Sweden) uses an optical approach to measure the affinity of an antibody that is passed over an antigen that is immobilized over a metal film. A light source is focused on this film through a prism at a specific angle (resonance), and the reflected light provides a surface plasmon resonance readout. Adsorption of an antibody to the antigen alters the surface plasmon resonance readout, and this alteration can provide information on affinity.

TRANSGENIC MICE AND GENE TARGETING

Three important and related methods for studying the functional effects of specific gene products in vivo are the creation of conventional transgenic mice that ectopically

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express a particular gene in a defined tissue; the creation of gene "knockout" mice, in which a targeted disruption is used to ablate the function of a particular gene; and the generation of "knockin" mice, in which an existing gene in the germline is replaced with a modified version of the same. A knockin approach could either replace a normal version of a gene with a mutant version or, in principle, "correct" an existing mutant gene with a "normal" version. These techniques involving genetically engineered mice have been widely used to analyze many biologic phenomena, including the development, activation, and tolerance of lymphocytes.

For the creation of conventional transgenic mice, foreign DNA sequences, called transgenes, are introduced into the pronuclei of fertilized mouse eggs, and the eggs are implanted into the oviducts of pseudopregnant females. Usually, if a few hundred copies of a gene are injected into pronuclei, about 25% of the mice that are born are transgenic. One to 50 copies of the transgene insert in tandem into a random site of breakage in a chromosome and are subsequently inherited as a simple mendelian trait. Because integration usually occurs before DNA replication, most (approximately 75%) of the transgenic pups carry the transgene in all of their cells, including germ cells. In most cases, integration of the foreign DNA does not disrupt endogenous gene function. Also, each founder mouse carrying the transgene is a heterozygote, from which homozygous lines can be bred.

The great value of transgenic technology is that it can be used to express genes in particular tissues by attaching coding sequences of the gene to regulatory sequences that normally drive the expression of genes selectively in that tissue. For instance, lymphoid promoters and enhancers can be used to overexpress genes, such as rearranged antigen receptor genes, in lymphocytes, and the insulin promoter can be used to express genes in the β cells of pancreatic islets. Examples of the utility of these methods for study of the immune system are mentioned in many chapters of this book. Transgenes can also be expressed under the control of promoter elements that respond to drugs or hormones, such as tetracycline or estrogens. In these cases, transcription of the transgene can be controlled at will by administration of the inducing agent.

A powerful method for development of animal models of single-gene disorders, and the most definitive way to establish the obligatory function of a gene in vivo, is the creation of knockout mice by targeted mutation or disruption of the gene. This technique has mostly relied on the phenomenon of homologous recombination. If an exogenous gene is inserted into a cell, for instance, by electroporation, it can integrate randomly into the cell's genome. However, if the gene contains sequences that are homologous to an endogenous gene, it will preferentially recombine with and replace endogenous sequences. To select for cells that have undergone homologous recombination, a drug-based selection strategy is used. The fragment of homologous DNA to be inserted into a cell is placed in a vector typically containing a neomycin resistance *(neo)* gene and a viral thymidine kinase *(tk)* gene (Fig. A.6A). This targeting vector is constructed in such a way that the *neo* gene is always inserted into the chromosomal DNA, but the *tk* gene is lost whenever homologous recombination (as opposed to random insertion) occurs. The vector is introduced into cells, and the

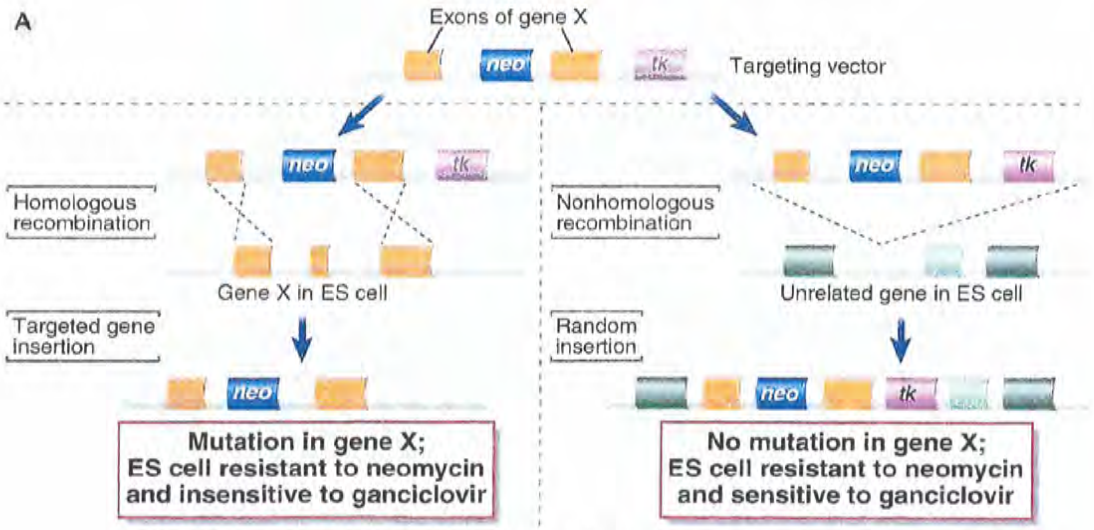


FIGURE A.6 Generation of gene knockout. A, The disruption of gene X in an embryonic stem (ES) cell is accomplished by homologous recombination. A population of ES cells is transfected with a targeting vector that contains sequences homologous to two exons of gene X flanking a neomycin resistance *(neo)* gene. The *neo* gene replaces or disrupts one of the exons of gene X on homologous recombination. The thymidine kinase *(tk)* gene in the vector will be inserted into the genome only if random, nonhomologous recombination occurs.

Continued

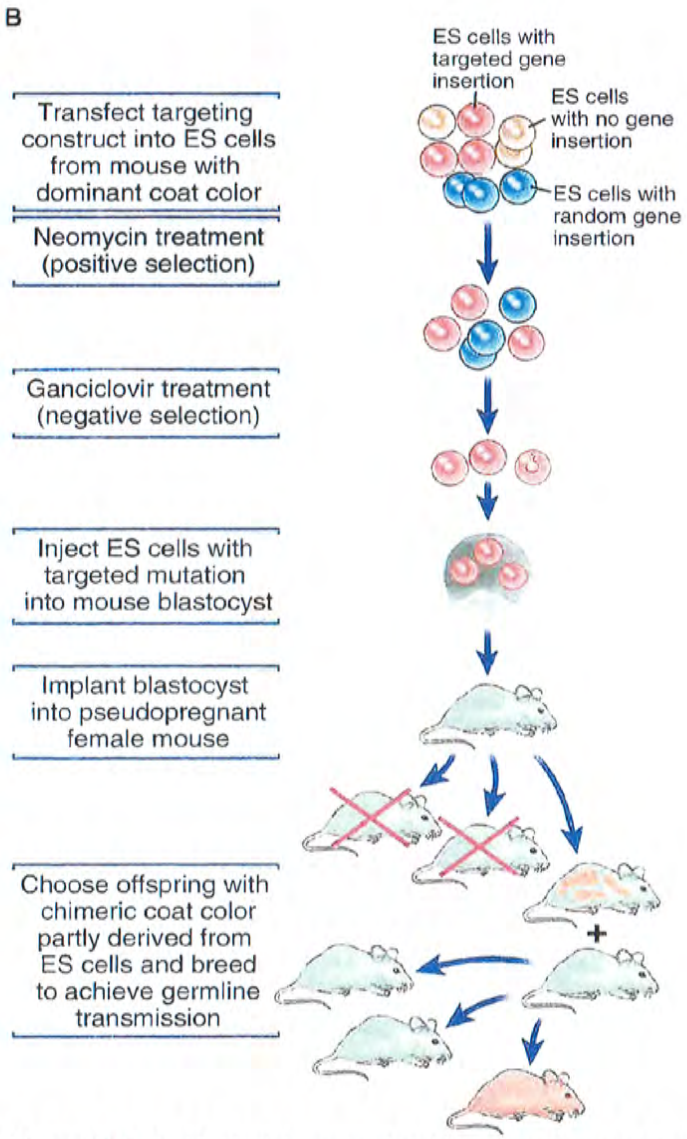


FIGURE A.6, cont'd B, The ES cells that were transfected by the targeting vector are selected by neomycin and ganciclovir so that only those cells with targeted insertion (homologous recombination) survive. These cells are then injected into a blastocyst, which is then implanted into the uterus of a pseudopregnant mouse. A chimeric mouse will develop in which some of the tissues are derived from the ES cell carrying the targeted mutation in gene X. These chimeric mice are identified by a mixed-color coat, including the color of the mouse strain from which the ES cells were derived and the color of the mouse strain from which the blastocyst was derived. If the mutation is present in germ cells, it can be propagated by further breeding.

cells are grown in neomycin and ganciclovir, a drug that is metabolized by *tk* to generate a lethal product. Cells in which the gene is integrated randomly will be resistant to neomycin but will be killed by ganciclovir, whereas cells in which homologous recombination has occurred will be resistant to both drugs because the *tk* gene will not be incorporated. This positive-negative selection ensures that the inserted gene in surviving cells has undergone homologous recombination with endogenous sequences. The presence of the inserted DNA in the middle of an endogenous gene usually disrupts the coding sequences and ablates the expression or function

of that gene. In addition, targeting vectors can be designed such that homologous recombination will lead to the deletion of one or more exons of the endogenous gene.

To generate a mouse carrying a targeted gene disruption or mutation, a targeting vector is used to first disrupt the gene in a murine embryonic stem (ES) cell line. ES cells are pluripotent cells derived from mouse embryos that can be propagated and induced to differentiate in culture or that can be incorporated into a mouse blastocyst, which may be implanted in a pseudopregnant mother and carried to term. Importantly, the progeny of the ES cells develop normally into mature tissues that will express the exogenous genes that have been transfected into the ES cells. Thus, the targeting vector designed to disrupt a particular gene is inserted into ES cells, and colonies in which homologous recombination has occurred (on one chromosome) are selected with drugs, as described earlier (see Fig. A.6B). The presence of the desired recombination is verified by analysis of DNA with techniques such as Southern blot hybridization or polymerase chain reaction. The selected ES cells are injected into blastocysts, which are implanted into pseudopregnant females. Mice that develop will be chimeric for a heterozygous disruption or mutation, that is, some of the tissues will be derived from the ES cells and others from the remainder of the normal blastocyst. The germ cells are also usually chimeric, but because these cells are haploid, only some will contain the chromosome copy with the disrupted (mutated) gene. If chimeric mice are mated with normal (wild-type) animals and either sperm or eggs containing the chromosome with the mutation fuse with the wild-type partner, all cells in the offspring derived from such a zygote will be heterozygous for the mutation (so-called germline transmission). Such heterozygous mice can be mated to yield animals that will be homozygous for the mutation with a frequency that is predictable by simple mendelian segregation. Such knockout mice are deficient in expression of the targeted gene.

Homologous recombination can also be used to replace a normal gene sequence with a modified version of the same gene (or of another gene), thereby creating a knockin mouse strain. Knockin mice can be used to assess the biologic consequences of a change in a single base, for instance, as opposed to the deletion of a gene. A knockin approach could, in principle, also be used to replace a defective gene with a normal one. In certain circumstances, a different gene may be placed at a defined site in the genome by use of a knockin strategy rather than in a random site as in conventional transgenic mice. Knockin approaches are used when it is desirable to have the expression of the transgene regulated by certain endogenous DNA sequences, such as a particular enhancer or promoter region. In this case, the targeting vector contains an exogenous gene encoding a desired product as well as sequences homologous to an endogenous gene that are needed to target the site of recombination.

Although the conventional gene-targeting strategy has proved to be of great usefulness in immunology research, the approach has some limitations. First, the mutation of one gene during development may be compensated for by altered expression of other gene products, and therefore the function of the targeted gene may be obscured. Second, in a conventional gene knockout mouse, the importance of a gene in only one tissue or at only one time during development cannot be easily assessed. Third, a functional selection marker gene, such as the *neo* gene, is permanently introduced into the animal genome, and this alteration may have unpredictable results on the phenotype of the animal. An important refinement of gene knockout technology that can overcome many of these drawbacks is a "conditional" targeting approach.

A commonly used conditional strategy takes advantage of the bacteriophage-derived Cre/*loxP* recombination system. The Cre enzyme is a DNA recombinase that recognizes a 34-bp sequence motif called ***loxP***, and the enzyme mediates the deletion of gene segments flanked by two *loxP* sites in the same orientation. To generate mice with *loxP*-tagged genes, targeting vectors are constructed with one *loxP* site flanking the *neo* gene at one end and a second *loxP* site flanking the sequences homologous to the target at the other end. These vectors are transfected into ES cells, and mice carrying the *loxP*-flanked but still functional target gene are generated as described for conventional knockout mice. A second strain of mice carrying a *cre* transgene is then bred with the strain carrying the *loxP*-flanked ("floxed") target gene. In the offspring, expression of Cre recombinase will mediate deletion of the target gene. Both the normal gene sequences and the *neo* gene will be deleted. Importantly, expression of the *cre* gene, and therefore deletion of the targeted gene, can be restricted to certain tissues or specified times by the use of *cre* transgene constructs with different promoters. For example, selective deletion of a gene only in macrophages and granulocytes can be accomplished by using a *cre* transgenic mouse in which *cre* is driven by a lysozyme promoter, or the selective loss of a gene only in regulatory T cells can be accomplished using a *foxp3* promoter driving a *cre* transgene. Alternatively, a steroid-inducible promoter can be used so that Cre expression and subsequent gene deletion occur only after mice are given a dose of dexamethasone. Many other variations on this technology have been devised to create conditional mutants. Cre/*loxP* technology can also be used to create knockin mice. In this case, *loxP* sites are placed in the targeting vector to flank the *neo* gene and the homologous sequences, but they do not flank the replacement (knockin) gene sequences. Therefore, after *cre*-mediated deletion, the exogenous gene remains in the genome at the targeted site.

Gene knock in technology has been applied to create "reporter" mice in which cells that would normally express a particular protein will express a fluorescent molecule at the same time as the native protein. This is accomplished by replacing the native gene with a transgene that encodes the fluorescent reporter protein and the native protein, both under the control of the native promoter and enhancer. Reporter mice have been developed that allow the visualization of immune cells of particular subsets in vivo, such as mice in which IL-17–producing Th17 cells also express a fluorescent protein. These cells can be detected using intravital fluorescence

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microscopy. The cells expressing the reporter genes can also be isolated alive and subjected to functional studies ex vivo, even if the native gene reported is a nuclear transcription factor whose expression would otherwise only be detectable by methods that kill the cells. For example, live regulatory T cells can be isolated by FACS-sorting lymph nodes from a reporter mouse that expresses green fluorescent protein simultaneously with the transcription factor FoxP3.

A new approach to generating mutations in cell lines, as well as in ES cells, utilizes a modification of a bacterial defense system against foreign DNA called CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) Cas9 (CRISPR-associated nuclease 9) system. In the gene editing variation of this, a guide RNA hybridizes with a chosen target DNA sequence and allows the Cas9 nuclease to generate a targeted double-stranded break. Although such a break can disrupt a gene, co-transfecting a plasmid with a mutated version of the target sequence allows efficient homologous recombination and the creation of a targeted knockin mutation. This is the most rapid approach available for the generation of knockout or knockin mutations in cell lines or in the germlines of experimental animals.

METHODS FOR STUDYING T LYMPHOCYTE RESPONSES

Our current knowledge of the cellular events in T cell activation is based on a variety of experimental techniques in which different populations of T cells are activated by defined stimuli, and functional responses are measured. In vitro experiments have provided a great deal of information on the changes that occur in a T cell when it is stimulated by antigen. More recently, several techniques have been developed to study T cell proliferation, cytokine expression, and anatomic redistribution in response to antigen activation in vivo. The new experimental approaches have been particularly useful for the study of naive T cell activation and the localization of antigen-specific memory T cells after an immune response has waned.

Polyclonal Activation of T Cells

Polyclonal activators of T cells bind to many or all T cell receptor (TCR) complexes regardless of specificity and activate the T cells in ways similar to peptide-MHC complexes on antigen-presenting cells (APCs). Polyclonal activators are mostly used in vitro to activate T cells isolated from human blood or the lymphoid tissues of experimental animals. Polyclonal activators can also be used to activate T cells with unknown antigen specificities, and they can evoke a detectable response from mixed populations of naive T cells, even though the frequency of cells specific for any one antigen would be too low to elicit a detectable response. The polymeric carbohydrate-binding plant proteins called lectins, such as concanavalin-A and phytohemagglutinin (PHA), are one commonly used group of polyclonal T cell activator. These lectins bind specifically to certain sugar residues on T cell surface glycoproteins, including the TCR and CD3 proteins, and thereby stimulate the T cells. Antibodies specific for invariant framework epitopes on TCR or CD3 proteins also function as polyclonal activators of T cells. Often, these antibodies need to be immobilized on solid surfaces or beads or cross-linked with secondary anti-antibodies to induce optimal activation responses. Because soluble polyclonal activators do not provide costimulatory signals that are normally provided by APCs, they are often used together with stimulatory antibodies to receptors for costimulators, such as anti-CD28 or anti-CD2. Superantigens, another kind of polyclonal stimulus, bind to and activate all T cells that express particular types of TCR β chain (see Chapter 16, Fig. 16.3). T cells of any antigen specificity can also be stimulated with pharmacologic reagents, such as the combination of the phorbol ester PMA and the calcium ionophore ionomycin, that mimic signals generated by the TCR complex.

Antigen-Induced Activation of Polyclonal T Cell Populations

Polyclonal populations of normal T cells that are enriched for T cells specific for a particular antigen can be derived from the blood and peripheral lymphoid organs of individuals after immunization with the antigen. The immunization serves to expand the number of antigen-specific T cells, which can then be restimulated in vitro by adding antigen and MHC-matched APCs to the T cells. This approach can be used to study antigen-induced activation of a mixed population of previously activated ("primed") T cells expressing many different TCRs, but the method does not permit analysis of responses of naive T cells.

Antigen-Induced Activation of T Cell Populations With a Single Antigen Specificity

Monoclonal populations of T cells, which express identical TCRs, have been useful for functional, biochemical, and molecular analyses. The limitation of these monoclonal populations is that they are maintained as long-term tissue culture lines and therefore may have phenotypically diverged from normal T cells in vivo. One type of monoclonal T cell population that is frequently used in experimental immunology is an antigen-specific T cell clone. Such clones are derived by isolating T cells from immunized individuals, as described for polyclonal T cells, followed by repetitive in vitro stimulation with the immunizing antigen plus MHC-matched APCs and cloning of single antigen-responsive cells in semisolid media or in liquid media by limiting dilution. Antigen-specific responses can easily be measured in these populations because all the cells in a cloned cell line have the same receptors and have been selected for growth in response to a known antigen-MHC complex. Both helper and cytotoxic T lymphocyte clones have been established from mice and humans. Other monoclonal T cell populations used in the study of T cell activation include antigen-specific T cell hybridomas, which are produced like B cell hybridomas (see Fig. 5.9, Chapter 5), and

tumor lines derived from T cells have been established in vitro after removal of malignant T cells from animals or humans with T cell leukemias or lymphomas. Although some tumor-derived lines express functional TCR complexes, their antigen specificities are not known, and the cells are usually stimulated with polyclonal activators for experimental purposes. The Jurkat line, derived from a human T cell leukemia cell, is an example of a tumor line that is widely used as a model to study T cell signal transduction.

TCR transgenic mice are a source of homogeneous, phenotypically normal T cells with identical antigen specificities that are widely used for in vitro and in vivo experimental analyses. If the rearranged α and β chain genes of a single TCR of known specificity are expressed as a transgene in mice, a majority of the mature T cells in the mice will express that TCR. If the TCR transgene is crossed onto a RAG-1– or RAG-2–deficient background, no endogenous *TCR* gene expression occurs, and 100% of the T cells will express only the transgenic TCR. TCR transgenic T cells can be activated in vitro or in vivo with a single peptide antigen, and they can be identified by antibodies specific for the transgenic TCR. One of the unique advantages of TCR transgenic mice is that they permit the isolation of sufficient numbers of naive T cells of defined specificity to allow one to study functional responses to the first exposure to antigen. This advantage has allowed investigators to study the in vitro conditions under which antigen activation of naive T cells leads to differentiation into functional subsets such as T_H1 and T_H2 cells (see Chapter 9). Naive T cells from TCR transgenic mice can also be injected into normal syngeneic recipient mice, where they home to lymphoid tissues. The recipient mouse is then exposed to the antigen for which the transgenic TCR is specific. By use of antibodies that label the TCR transgenic T cells, it is possible to follow their expansion and differentiation in vivo and to isolate them for analysis of recall (secondary) responses to antigen ex vivo.

Methods to Enumerate and Study Functional Responses of T Cells

Proliferation assays for T lymphocytes, like those of other cells, are conducted in vitro by determining the amount of ^{3}H-labeled thymidine incorporated into the replicating DNA of cultured cells. Thymidine incorporation provides a quantitative measure of the rate of DNA synthesis, which is usually directly proportional to the rate of cell division. Cellular proliferation in vivo can be measured by injecting the thymidine analogue bromodeoxyuridine (BrdU) into animals and staining cells with anti-BrdU antibody to identify and enumerate nuclei that have incorporated BrdU into their DNA during DNA replication.

Fluorescent dyes can be used to study proliferation of T cells in vivo. T cells are first labeled with chemically reactive lipophilic fluorescent esters and then adoptively transferred into experimental animals. The dyes enter cells, form covalent bonds with cytoplasmic proteins, and then cannot leave the cells. One commonly used dye of this type is 5,6-carboxyfluorescein diacetate succinimidyl ester (CFSE), which can be detected in cells by standard flow cytometric techniques. Every time a T cell divides, its dye content is halved, and therefore it is possible to determine whether the adoptively transferred T cells present in lymphoid tissues of the recipient mouse have divided in vivo and to estimate the number of doublings each T cell has gone through.

Peptide-MHC tetramers are used to enumerate T cells with a single antigen specificity isolated from blood or lymphoid tissues of experimental animals or humans. These tetramers contain four of the peptide-MHC complexes that the T cell would normally recognize on the surface of APCs. The tetramer is made by producing a class I MHC molecule to which is attached a small molecule called biotin by use of recombinant DNA technology. Biotin binds with high affinity to a protein called avidin, and each avidin molecule binds four biotin molecules. Thus, avidin forms a substrate for assembly of four biotin-conjugated MHC proteins. The MHC molecules can be loaded with a peptide of interest and thus stabilized, and the avidin molecule is labeled with a fluorochrome, such as FITC. This tetramer binds to T cells specific for the peptide-MHC complex with high enough avidity to label the T cells, even in suspension. This method is the only feasible approach for identification of antigen-specific T cells in humans. For instance, it is possible to identify and enumerate circulating HLA-A2–restricted T cells specific for an HIV peptide by staining blood cells with a tetramer of HLA-A2 molecules loaded with the peptide. The same technique is being used to enumerate and isolate T cells specific for self antigens in normal individuals and in patients with autoimmune diseases. Peptide-MHC tetramers that bind to a particular transgenic TCR can also be used to quantify the transgenic T cells in different tissues after adoptive transfer and antigen stimulation. The technique is now widely used with class I MHC molecules; in class I molecules, only one polypeptide is polymorphic, and stable molecules can be produced in vitro. This is more difficult for class II molecules because both chains are polymorphic and required for proper assembly, but class II-peptide tetramers are also being produced.

Cytokine secretion assays can be used to quantify cytokine-secreting effector T cells within lymphoid tissues. The most commonly used methods are cytoplasmic staining of cytokines with analysis of the stained cells by flow cytometry, and single-cell enzyme-linked immunosorbent assays (ELISpots). In these types of studies, antigen-induced activation and differentiation of T cells take place in vivo, and then T cells are isolated, restimulated with antigen or polyclonal activators, and tested for cytokine expression in vitro. Cytoplasmic staining of cytokines requires permeabilizing of the cells so that fluorochrome-labeled antibodies specific for a particular cytokine can gain entry into the cell, and the stained cells are analyzed by flow cytometry. Cytokine expression by T cells specific for a particular antigen can be determined by additionally staining T cells with peptide-MHC tetramers or, in the case of TCR transgenic T cells, antibodies specific for the transgenic TCR. By use of a combination of CFSE and anti-cytokine antibodies, it is possible to examine the relationship between cell division and

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cytokine expression. In the ELISpot assay, T cells freshly isolated from blood or lymphoid tissues are cultured in plastic wells coated with antibody specific for a particular cytokine. As cytokines are secreted from individual T cells, they bind to the antibodies in discrete spots corresponding to the location of individual T cells. The spots are visualized by adding secondary enzyme-linked anti-Ig, as in a standard ELISA (see earlier), and the number of spots is counted to determine the number of cytokine-secreting T cells.

METHODS FOR STUDYING B LYMPHOCYTE RESPONSES

Activation of Polyclonal B Cell Populations

It is technically difficult to study the effects of antigens on normal B cells because, as the clonal selection hypothesis predicted, very few lymphocytes in an individual are specific for any one antigen. An approach to circumventing this problem is to use anti-Ig antibodies as analogues of antigens, with the assumption that anti-Ig will bind to constant (C) regions of membrane Ig molecules on all B cells and will have the same biologic effects as an antigen that binds to the hypervariable regions of membrane Ig molecules on only the antigen-specific B cells. To the extent that precise comparisons are feasible, this assumption appears generally correct, indicating that anti-Ig antibody is a valid model for antigens. Thus, anti-Ig antibody is frequently used as a polyclonal activator of B lymphocytes, similar to the use of anti-CD3 antibodies as polyclonal activators of T lymphocytes, discussed earlier.

Antigen-Induced Activation of B Cell Populations With a Single Antigen Specificity

To examine the effects of antigen binding to B cells, investigators have attempted to isolate antigen-specific B cells from complex populations of normal lymphocytes or to produce cloned B cell lines with defined antigenic specificities. These efforts have met with little success. However, transgenic mice have been developed in which virtually all B cells express a transgenic Ig of known specificity, so that most of the B cells in these mice respond to the same antigen. A somewhat more sophisticated approach has been to generate antigen receptor knockin mice, in which rearranged Ig H and L chain genes have been homologously recombined into their endogenous loci. Such knockin animals have proved particularly useful in the examination of receptor editing.

Assays to Measure B Cell Proliferation and Antibody Production

Much of our knowledge of B cell activation is based on in vitro experiments, in which different stimuli are used to activate B cells and their proliferation and differentiation can be measured accurately. The same assays may be done with B cells recovered from mice exposed to different antigens or with homogeneous B cells expressing transgene-encoded antigen receptors.

B cell proliferation is measured by use of CFSE labeling or ^{3}H-labeled thymidine incorporation in vitro and BrdU labeling in vivo, as described earlier for T cell proliferation.

Antibody production is measured in two different ways: with assays for cumulative Ig secretion, which measure the amount of Ig that accumulates in the supernatant of cultured lymphocytes or in the serum of an immunized individual; and with single-cell assays, which determine the number of cells in an immune population that secrete Ig of a particular specificity or isotype. The most accurate, quantitative, and widely used technique to measure the total amount of Ig in a culture supernatant or serum sample is ELISA. By use of antigens bound to solid supports, it is possible to use ELISA to quantify the amount of antibody in a sample specific for a particular antigen. In addition, the availability of anti-Ig antibodies that detect Igs of different heavy or light chain classes allows measurement of the quantities of different isotypes in a sample. Other techniques to measure antibody levels include hemagglutination for anti-erythrocyte antibodies and complement-dependent lysis for antibodies specific for known cell types. Both assays are based on the demonstration that if the amount of antigen (i.e., cells) is constant, the concentration of antibody determines the amount of antibody bound to cells, and this is reflected in the degree of cell agglutination or subsequent binding of complement and cell lysis. Results from these assays are usually expressed as antibody titers, which are the dilution of the sample giving half-maximal effects or the dilution at which the endpoint of the assay is reached.

A single-cell assay for antibody secretion is the ELISpot assay. In this method, antigen is bound to the bottom of a well, antibody-secreting cells are added, and antibodies that have been secreted and are bound to the antigen are detected by an enzyme-linked anti-Ig antibody, as in an ELISA, in a semisolid medium. Each spot represents the location of an antibody-secreting cell. Single-cell assays provide a measure of the numbers of Ig-secreting cells, but they cannot accurately quantify the amount of Ig secreted by each cell or by the total population. The ELISA and ELISpot techniques can be adapted to assess affinity of antibodies, by the use of antigens with differing numbers of hapten moieties. In this way, affinity maturation can be assessed by testing serum or B cells sampled at different times during an immune response.

CLINICAL DIAGNOSTIC APPLICATIONS OF IMMUNOLOGIC ASSAYS

Many of the techniques discussed above are used in clinical laboratories to determine the status of the immune system of patients. Here we will summarize some of the most common laboratory approaches commonly used for initial diagnosis of immunologic abnormalities. In many cases, abnormalities found by these approaches are followed up with highly specialized tests, including molecular genetic analyses.

Flow Cytometry to Determine Numbers of Subsets of Circulating Immune Cells

This approach is routinely used to determine total numbers of B cells, T cells, NK cells, and T cells subsets ($CD4^+$ and $CD8^+$) in circulation. Follow-up approaches include looking at populations of naive and memory T cell subsets ($CD45RA^+/RO^+$), γδ T cells, isotype switched memory B cells ($CD27^+$ IgM^- IgD^-), and even subsets of T helper cells (Th1, Th2, Th17, Treg) depending on the context.

Assays for Innate Immunity

The **neutrophil oxidative burst assay** is commonly performed using a dihydrorhodamine (DHR) flow cytometric analysis and can be used to detect both overt chronic granulomatous disease as well as X-linked carriers of the disease.

NK cell cytotoxicity assays evaluate ex vivo NK cell killing of a target cell population (e.g., cells lacking MHC). A low value suggests NK cell dysfunction and is useful in the evaluation of patients with recurrent infections (primarily viral), as well as in patients with suspected primary cause of hemophagocytic lymphohistiocytosis (HLH).

Assays for Humoral Immunity

Serum protein electrophoresis can reveal decreased gamma globulins in immunodeficiency as well as monoclonal Ig peaks associated with malignant and premalignant clonal expansions of plasma cells.

Serum levels of different antibody classes, including IgG, IgA, IgM, and IgE as well as IgG subclasses, are usually determined by automated nephelometry, which involves mixing a dilution of patient's serum with antibodies specific for different Ig heavy chain classes, forming small immune complexes that are detected and quantified by measuring light scattering. Evaluation of the levels of IgG subclasses in serum is most helpful in patients who have normal to borderline low total IgG (≤400 mg/dL in the adult population).

Complement levels and function are quantified in several clinical contexts including recurrent infections, recurrent angioedema, and/or autoimmune disease. In the setting of recurrent infections (particularly with encapsulated organisms like Neisseria), a CH50 level is recommended as the initial screening test followed more detailed pathway analysis if the setting of a low/absent CH50. The CH50 is a screening test for deficiency of classical or terminal pathways, and is determined by measuring the ability of a patent's serum to cause hemolysis of sheep erythrocytes precoated with complement-fixing antibodies. The dilution of the serum that results in 50% hemolysis is of the erythrocytes is the CH50. Analysis of individual complement proteins is performed by nephelometry or variations of ELISAs. In the context of recurrent angioedema, a C4 level is often recommended as the initial screening test, followed by a C1 inhibitor level and function in the setting of a low C4 and/or a high level of clinical suspicion of an underlying C1 inhibitor deficiency. In the context of autoimmune disease, a low C3 and/or C4 level can be useful measures of ongoing immune complex formation.

Autoantibody screening for a range of specificities may be performed depending on the clinical context, using various techniques in which a patents serum is tested for the presence of Ig that binds to purified antigens or to cells.

Vaccine responses are routinely measured to assess humoral immune function. Responses are determined by measuring serum levels of IgG specific to both T cell-dependent antigens (proteins or glycoproteins, e.g., vaccination against tetanus toxoid, diphtheria toxoid, and *Haemophilus influenza* type B) and T cell–independent antigens (polysaccharides, e.g., Pneumovax). Titers are most commonly measured approximately 6 weeks post-vaccination, and low titers may warrant further evaluation for an underlying B cell immunodeficiency.

Assays for Cellular Immunity

T cell receptor excision circles (TRECs), which are formed by V-D-J recombination during T cell maturation, are measured in a newborn blood screening assay that is now mandatory in most states in the United States. TREC levels are used to assess recent T cell output from the thymus, and a low level warrants further evaluation for severe combined immunodeficiency (SCID).

T cell proliferation assays are performed to assess T cell function, and evaluated by stimulating cells ex vivo with mitogens such as pokeweed mitogen (PWM) and PHA, specific antigens (*Candida* and tetanus toxoid are commonly used), or following ligation by antibodies to CD3 and CD28. Robust cellular proliferation to these stimuli suggests intact T cell function.

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