

JANEWAY'S

8TH EDITION

IMMUNO BIOLOGY

KENNETH MURPHY

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Dynamics of Adaptive Immunity

11

Throughout this book we have examined the separate ways in which the innate and the adaptive immune responses protect the individual from invading microorganisms. In this chapter, we consider how the cells and molecules of the immune system work as an integrated defense system to eliminate or control an infectious agent and how the adaptive immune system provides long-lasting protective immunity. This is the first of several chapters that consider how the immune system functions as a whole in health and disease. The next chapter describes the role and specializations of the mucosal immune system, which forms the front-line defense against most pathogens. Subsequent chapters examine how immune defenses can fail (Chapter 13) or unwanted immune responses occur (Chapters 14 and 15), and how the immune response can be manipulated to benefit the individual (Chapter 16).

In Chapters 2 and 3, we saw how innate immunity is brought into play in the earliest phases of an infection and is probably sufficient to prevent colonization of the body by most of the microorganisms encountered in the environment. However, pathogenic microorganisms, by definition, have developed strategies that allow them to elude or overcome innate immune defenses and to establish a focus of infection from which they can spread. In these circumstances, the innate immune response sets the scene for the induction of an adaptive immune response. In the **primary immune response**, which occurs against a pathogen encountered for the first time, several days are required for the clonal expansion and differentiation of naive lymphocytes into effector T cells and antibody-secreting B cells, as described in Chapters 9 and 10. In most cases, these cells and antibodies will effectively target the pathogen for elimination (Fig. 11.1).

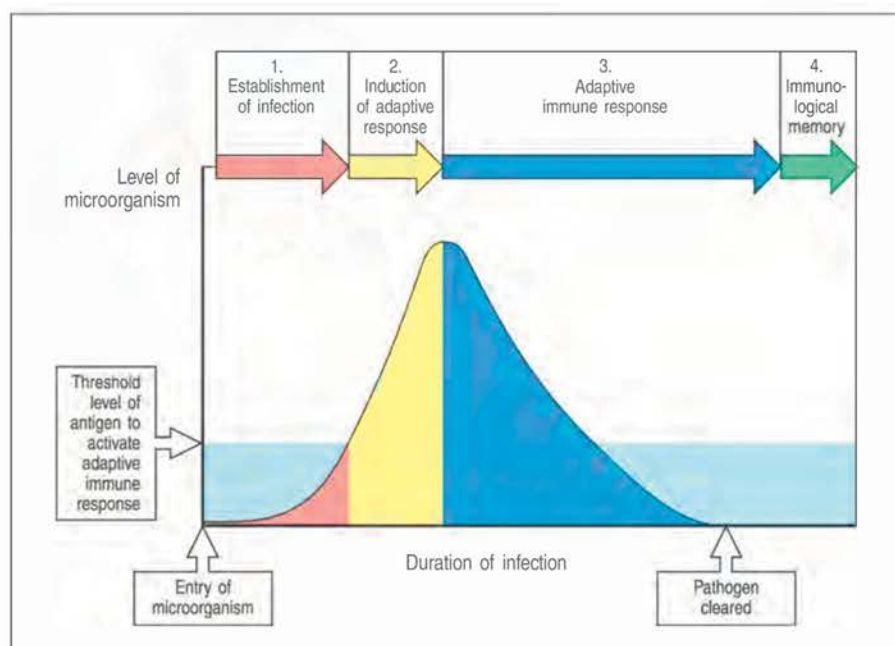


Fig. 11.1 The course of a typical acute infection that is cleared by an adaptive immune reaction. 1. The level of infectious agent increases as the pathogen replicates. 2. When numbers of the pathogen exceed the threshold dose of antigen required for an adaptive response, the response is initiated; the pathogen continues to grow, retarded only by responses of the innate immune system. At this stage, immunological memory also starts to be induced. 3. After 4–7 days, effector cells and molecules of the adaptive response start to clear the infection. 4. When the infection has been cleared and the dose of antigen has fallen below the response threshold, the response ceases, but antibody, residual effector cells, and immunological memory provide lasting protection against reinfection in most cases.

During this period, specific **immunological memory** is also established. This ensures a rapid reinduction of antigen-specific antibody and effector T cells on subsequent encounters with the same pathogen, thus providing long-lasting and often lifelong protection against it. Immunological memory is discussed in the last part of the chapter. Memory responses differ in several ways from primary responses. We discuss the reasons for this, and what is known about how immunological memory is maintained.

The course of the immune response to infection.

The immune response is a dynamic process, and both its nature and intensity change over time. It starts with the relatively nonspecific responses of innate immunity and becomes both more focused on the pathogen and more powerful as the adaptive immune response is initiated and rapidly develops. In this part of the chapter, we discuss how the different phases of an immune response are orchestrated in space and time, how the response develops in both strength and precision, how changes in specialized cell-surface molecules and chemokines guide effector lymphocytes to the appropriate site of action, and how these cells are regulated during the different stages.

An innate immune response is an essential prerequisite to a primary adaptive immune response, because the co-stimulatory molecules induced on cells of the innate immune system during their interaction with microorganisms are essential for the activation of the antigen-specific lymphocytes (see Chapter 9). Cells of the innate immune system hand on other important signals in the form of secreted cytokines that influence the characteristics of the adaptive response and tailor it to the type of pathogen encountered. For this to happen, cells from different locations must engage to coordinate the specific activation of naive T cells and B cells, and the migration of cells to precise locations within lymphoid tissues is thus critical for the coordination of an adaptive response.

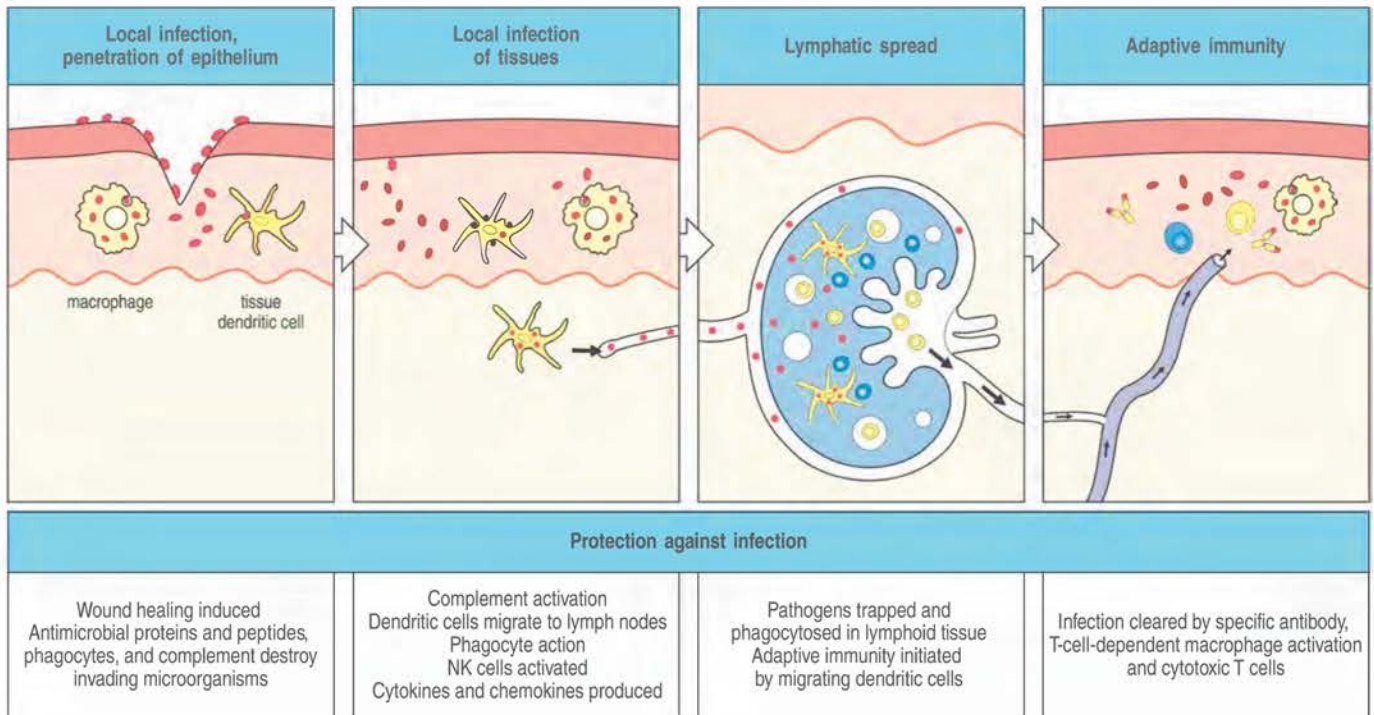
11-1 The course of an infection can be divided into several distinct phases.



An infection can be broken down into various stages (see Fig. 2.5), but in Chapters 2 and 3 we considered in detail only the responses of innate immunity. In this chapter, we return to the various stages of an infection but will now integrate the adaptive immune response into the picture.

In the first stage of infection with a pathogen, a new host is exposed to infectious particles either shed by an infected individual or present in the environment. The numbers, route, mode of transmission, and stability of an infectious agent outside the host determine its infectivity. Some pathogens, such as the anthrax bacterium, are spread by spores that are highly resistant to heat and drying, whereas others, such as the human immunodeficiency virus (HIV), are spread only by the exchange of bodily fluids or tissues because they are unable to survive as infectious agents outside the body.

The first contact with a new host occurs through an epithelial surface, such as the skin or the mucosal surfaces of the respiratory, gastrointestinal, or urogenital tracts. As most pathogens gain entry to the body through mucosal surfaces, the immune responses that occur in this specialized compartment of the immune system are of great importance and are considered in detail in Chapter 12. After making contact, an infectious agent must establish a focus of infection. It must either adhere to the epithelial surface and colonize it, or penetrate it to replicate in the tissues (Fig. 11.2, first two panels). Wounds



and insect and tick bites that breach the epidermal barrier help some microorganisms to get through the skin. Many microorganisms are repelled or kept in check at this stage by innate defenses, which include the induced innate immune response triggered by stimulation of the various germline-encoded receptors (such as TLRs and NK-cell receptors) that discriminate between foreign microbial and self host-cell surfaces or between infected and normal cells (see Chapters 2 and 3). These responses are not as effective as adaptive immune responses, which can afford to be more powerful because they are antigen specific and thus target the pathogen precisely. However, they can prevent an infection from being established or, failing that, can contain it and prevent the spread of a pathogen into the bloodstream while an adaptive immune response develops.

Only when a microorganism has successfully established a focus of infection in the host does disease occur. With the possible exception of lung infections such as tuberculosis, and diarrhea-inducing gut infections such as cholera, in which the primary infection can cause life-threatening disease, little damage will be caused unless the agent spreads from the original focus or secretes toxins that can spread to other parts of the body. Extracellular pathogens spread by direct extension of the infection through the lymphatics or the bloodstream. Usually, spreading into the bloodstream occurs only after the lymphatic system has been overwhelmed. Obligate intracellular pathogens spread from cell to cell; they do so either by direct transmission from one cell to the next or by release into the extracellular fluid and reinfection of both adjacent and distant cells. In contrast, some of the bacteria that cause gastroenteritis exert their effects without spreading into the tissues. They establish a site of infection on the luminal surface of the epithelium lining the gut and cause no direct pathology themselves, but secrete toxins that cause damage either *in situ* or after crossing the epithelial barrier and entering the circulation.

Most infectious agents show a significant degree of host specificity, causing disease in only one or a few related species. What determines host specificity for every agent is not known, but the requirement for attachment to

Fig. 11.2 Infections and the responses to them can be divided into a series of stages. These are illustrated here for a pathogenic microorganism (red) entering across a wound in an epithelium. The microorganism first adheres to epithelial cells and then invades beyond the epithelium into underlying tissues (first panel). A local innate immune response helps to contain the infection, and delivers antigen and antigen-loaded dendritic cells to lymphatics (second panel) and thence to local lymph nodes (third panel). This leads to an adaptive immune response in the lymph node that involves the activation and further differentiation of B cells and T cells with the eventual production of antibody and effector T cells, which clear the infection (fourth panel).

a particular cell-surface molecule is one critical factor. As other interactions with host cells are also commonly needed to support replication, most pathogens have a limited host range. The molecular mechanisms of host specificity comprise an area of research known as molecular pathogenesis, which is outside the scope of this book.

Adaptive immunity is triggered when an infection eludes or overwhelms the innate defense mechanisms and generates a threshold level of antigen (see Fig. 11.1). Adaptive immune responses are then initiated in the local lymphoid tissue, in response to antigens presented by dendritic cells activated during the course of the innate immune response (Fig. 11.2, second and third panels). Antigen-specific effector T cells and antibody-secreting B cells are generated by clonal expansion and differentiation over several days, as described in greater detail in Chapters 9 and 10. During this time, the induced responses of innate immunity, such as the acute-phase responses and interferon production (see Sections 3-7 and 3-18), continue to function. Eventually, antigen-specific T cells and then antibodies are released into the blood and from there can enter the site of infection (Fig. 11.2, fourth panel). Resolution of the infection involves the clearance of extracellular infectious particles by opsonizing antibodies and phagocytes (see Chapter 10) and the clearance of intracellular residues of infection through the actions of effector T cells (see Chapter 9).

After many types of infection, little or no residual pathology follows an effective primary adaptive response. In some cases, however, the infection or the response to it causes significant tissue damage. In yet other cases, such as infection with cytomegalovirus or *Mycobacterium tuberculosis*, the pathogen is contained but not eliminated, and can persist in a latent form. If the adaptive immune response is later weakened, as it is in acquired immune deficiency syndrome (AIDS), these pathogens may resurface to cause virulent systemic infections. We will focus on the strategies used by certain pathogens to evade or subvert adaptive immunity, and thereby establish a persistent, or chronic, infection, in the first part of Chapter 13. In addition to clearing the infectious agent, an effective adaptive immune response prevents reinfection. For some infectious agents, this protection is essentially absolute, whereas for others infection is only reduced or attenuated on reexposure to the pathogen.

It is not known how many infections are dealt with solely by the nonadaptive mechanisms of innate immunity, because such infections are eliminated early and produce little in the way of symptoms or pathology. Naturally occurring deficiencies in nonadaptive defenses are rare, so it has seldom been possible to study their consequences. Innate immunity does, however, seem to be essential for effective host defense, as shown by the progression of infection in mice that lack components of innate immunity but have an intact adaptive immune system (Fig. 11.3). In humans, for example, mutations in the Toll-like receptor TLR-3 have been associated with increased susceptibility to encephalitis due to the herpes simplex virus, which more usually causes self-limiting cold sores on the skin. Adaptive immunity is also essential, as shown by the immunodeficiency syndromes associated with defects in various components of the adaptive immune response (discussed in Chapter 13).

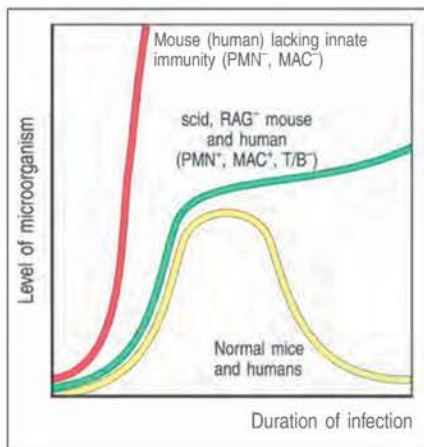


Fig. 11.3 The time course of infection in normal and immunodeficient mice and humans. The red curve shows the rapid growth of microorganisms in the absence of innate immunity, when macrophages (MAC) and polymorphonuclear leukocytes (PMN) are lacking. The green curve shows the course of infection in mice and humans that have innate immunity but have no T or B lymphocytes and so lack adaptive immunity. The yellow curve shows the normal course of an infection in immunocompetent mice or humans.

11-2 The nonspecific responses of innate immunity are necessary for an adaptive immune response to be initiated.

The establishment of a focus of infection in tissues and the response of the innate immune system produce changes in the immediate environment. Many of these changes have been described in earlier chapters, but we review them briefly here to provide a cohesive framework for the induction of adaptive immunity.

In a bacterial infection, the first thing that usually happens is that the infected tissue becomes inflamed. This is initially the result of the activation of the resident macrophages by bacterial components such as lipopolysaccharide (LPS) acting through Toll-like receptors (TLRs) on the macrophage. The cytokines and chemokines secreted by the activated macrophages, especially the cytokine tumor necrosis factor- α (TNF- α), induce numerous changes in the endothelial cells of nearby blood capillaries, a process known as endothelial cell activation. Inflammation also results from the activation of complement, resulting in the production of the anaphylatoxins C3a and C5a, which are able to activate vascular endothelium. In a primary infection, complement is activated mainly via the alternative and lectin pathways (see Fig. 2.12).

Activation of the vascular endothelium causes the release of the contents of Weibel–Palade bodies (the cell adhesion molecule P-selectin and von Willebrand factor) within the endothelial cells to the cell surface (see Section 3-15). Activation also induces the expression of E-selectin, which then also appears on the endothelial cell surface. These two selectins cause neutrophils, monocytes, and other leukocytes to adhere to and roll on the endothelial surface. Cytokine activation of the endothelium also induces the production of the adhesion molecule ICAM-1. By binding to adhesion molecules, such as LFA-1, on neutrophils and monocytes, ICAM-1 strengthens the interaction of these cells with the endothelium and aids their entry in large numbers into the infected tissue to form an inflammatory focus (see Fig. 3.26). As monocytes mature into tissue macrophages and become activated in their turn, additional inflammatory cells are attracted into the infected tissue, and the inflammatory response is maintained and reinforced. The inflammatory response can be thought of as putting up a flag on the endothelial cells to signal the presence of infection, but as yet the response is nonspecific for the pathogen.

A second crucial effect of infection is the activation of specialized antigen-presenting cells, the dendritic cells residing in most tissues, as described in Sections 9-4 to 9-6. Dendritic cells take up antigen in the infected tissues and, like macrophages, they are activated through innate immune receptors that respond to common constituents of pathogens, such as TLRs (Section 3-7) and NOD proteins (Section 3-8). Activated dendritic cells increase their synthesis of MHC class II molecules and, most importantly, begin to express the co-stimulatory molecules B7.1 and B7.2 on their surface. As described in Chapter 9, these antigen-presenting cells migrate away from the infected tissue through the lymphatics, along with their antigen cargo, to enter peripheral lymphoid tissues, where they initiate the adaptive immune response. They arrive in large numbers at the draining lymph nodes, or other nearby lymphoid tissue, attracted by the chemokines CCL19 and CCL21 produced by the lymph-node stroma (see Section 9-3).

When dendritic cells arrive in the lymphoid tissues, they seem to have reached their final destination. They activate antigen-specific naive T lymphocytes in these tissues, after which they die. Naive lymphocytes are continually passing through the lymph nodes, which they enter from the blood across the walls of high endothelial venules (see Fig. 9.4). Those naive T cells that recognize antigen on the surface of dendritic cells are activated, and divide and mature into effector cells that reenter the circulation. Where there is a local infection, the changes induced by inflammation in the walls of nearby venules induce these effector T cells to leave the blood vessel and migrate into the site of infection.

Thus, the local release of cytokines and chemokines at the site of infection has far-reaching consequences. In addition to recruiting neutrophils and macrophages, which are not specific for antigen, the changes induced in the blood vessel walls also enable newly activated effector T lymphocytes to enter infected tissue, as discussed in more detail later in this chapter.

11-3 Cytokines made during infection can direct differentiation of CD4 T cells toward the T_H17 subset.

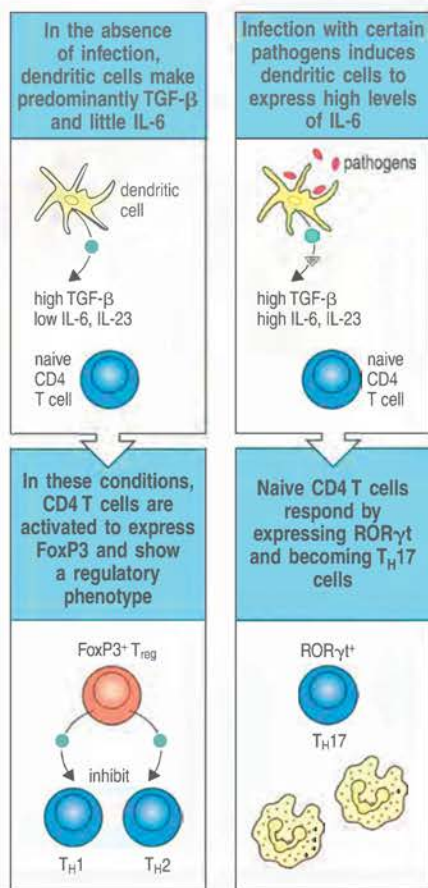


Fig. 11.4 Cytokines produced by dendritic cells regulate the balance of regulatory T-cell development and T_H17 differentiation. The balance between TGF- β and IL-6 production acts to induce either the transcription factor FoxP3, which is characteristic of regulatory T cells, or ROR γ t (an 'orphan' member of the nuclear receptor family), which is characteristic of T_H17 cells. In the absence of infection, IL-6 production by dendritic cells is low, and TGF- β production dominates. In these conditions, T cells that do encounter their cognate antigen will be induced to express FoxP3 and predominantly acquire a regulatory phenotype, whereas those that do not encounter antigen remain naive. Infection by certain bacteria and fungi induce dendritic cells to produce abundant IL-6 and IL-23, but less IL-12 (see Fig. 11.5); under these conditions, naive T cells will express ROR γ t and become T_H17 cells. The cytokines produced by this T-cell subset, IL-17 and IL-17F, induce cells such as epithelium to secrete chemokines that attract inflammatory cells such as neutrophils.

In Chapter 9 we described transcriptional mechanisms that control how specific cytokines direct the differentiation of naive CD4 T cells into distinct classes of CD4 effector T cells— T_H17 , T_H1 , or T_H2 , or regulatory subsets (see Fig. 9.29). The cytokines that are produced during the progression of an infection depend on how the microorganism influences the behavior of innate immune cells and antigen-presenting cells. The conditions produced by these interactions have a major impact on how T cells differentiate during their initial contact with antigen-presenting cells, thus determining the types of T cells that are generated (see Chapter 9). In turn, these T-cell subsets influence the nature of the effector responses that are recruited, such as the extent of macrophage activation, neutrophil and eosinophil recruitment to the site of infection, and which classes of antibody will predominate.

The subset of effector T cells generated in response to infection by extracellular bacteria and fungi is often T_H17 . At the beginning of an infection, dendritic cells are not yet fully activated; in this state they produce TGF- β , but little IL-6 or other cytokines that direct T-cell differentiation. Upon encountering such pathogens, dendritic cells become activated and are induced to synthesize IL-6 along with IL-23. As there is no source of IL-4 or IL-12 at this time, naive CD4 T cells will differentiate into T_H17 cells rather than T_H1 or T_H2 cells (Fig. 11.4). For example, activation of dendritic cells through Dectin-1, a receptor that recognizes carbohydrates common to yeast and other fungi, induces dendritic cells to produce abundant IL-23 but little IL-12. The effect is that dendritic cells activated by these organisms will tend to promote T_H17 differentiation.

When the T_H17 cells leave the lymph node and migrate to the sites of infection, they encounter pathogen antigens and are stimulated to synthesize and release cytokines, which include various members of the IL-17 family such as IL-17A and IL-17E (also known as IL-25). The receptor for IL-17 is expressed ubiquitously on cells such as fibroblasts, epithelial cells, and keratinocytes. IL-17 induces these cells to secrete various cytokines, including IL-6, the chemokines CXCL8 and CXCL2, and the hematopoietic factors granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF). These chemokines can act directly to recruit neutrophils, whereas G-CSF and GM-CSF act to augment neutrophil and macrophage production in the bone marrow or increase the differentiation of local monocytes into macrophages.

Thus, one important action of IL-17 at sites of infection is to induce local cells to secrete cytokines and chemokines that attract neutrophils. T_H17 cells also produce IL-22, a cytokine related to IL-10. IL-22 acts cooperatively with IL-17 to induce the expression of antimicrobial peptides, such as β -defensins, by the keratinocytes of the epidermis. In this way, the presence of pathogen-specific T_H17 cells serves as an efficient amplifier of an acute inflammatory response by the innate immune system at sites of early infection. CD4 T cells that acquire the T_H17 phenotype are not the only cells that can produce IL-17 in response to infections. CD8 T cells have also been shown to produce abundant IL-17.

The cytokine environment is also influential in preventing the immune system from making inappropriate responses to self antigens or those of commensal microorganisms, the microorganisms that normally inhabit the body. Even in the absence of infection, dendritic cells take up self and environmental antigens and eventually carry them to peripheral lymphoid tissues, where they may meet antigen-specific naive T cells. In such circumstances, the pro-inflammatory signals are not present; dendritic cells are not activated and so produce the cytokine TGF- β but not the other cytokines that can affect CD4 T-cell differentiation. In this setting, dendritic cells seem to actively

generate tolerance to the antigens that the naive T cells encounter (Fig. 11.4, left panels). TGF- β on its own inhibits the proliferation and differentiation of T_H17, T_H1, and T_H2 cells. When a naive CD4 T cell encounters its cognate peptide:MHC ligand in the presence of TGF- β alone, it acquires the phenotype of a regulatory T cell, in that it can inhibit the activation of other T cells. Regulatory T cells induced in this way outside the thymus are called adaptive regulatory T cells and some express the transcription factor FoxP3 (see Section 9-18). The regulatory T cells, in theory, should not be specific for pathogen antigens—which they have not yet encountered—but should instead be specific for either self antigens or peptides from commensal organisms. Other FoxP3-expressing regulatory CD4 T cells, the natural regulatory T cells, seem to acquire their regulatory phenotype in the thymus (see Section 8-18).

The reciprocal pathways for the development of T_H17 cells and regulatory T cells seem to be based on an evolutionarily ancient system of activation and inactivation, because proteins similar to TGF- β and IL-17 are present in invertebrates that possess primitive intestinal immune systems. This might suggest that the dichotomy between T_H17 cells and regulatory T cells is largely concerned with maintaining the lymphocyte balance in tissues exposed to large numbers of potential pathogens, such as the mucosae of the gut and the lungs, where a rapid response to infection is critical. For example, IL-17-producing T cells have an important role in mice in resistance to infections of the lung by Gram-negative bacteria such as *Klebsiella pneumoniae*. Mice lacking the receptor for IL-17 are significantly more susceptible than normal mice to lung infection by this pathogen, and they show decreased production of G-CSF and CXCL2 and poorer recruitment of neutrophils to infected lungs. T_H17 cells also promote resistance to the gut nematode *Nippostrongylus brasiliensis*. This effect seems to be due to the induction or recruitment by IL-17E of a population of non-T non-B leukocytes, perhaps similar to basophils, that secrete the T_H2 cytokines IL-4, IL-5, and IL-13. These cytokines, particularly IL-13, promote resistance to *N. brasiliensis* by, for example, inducing its expulsion from the gut by augmenting the production of mucus (see Chapter 12).

11-4 T_H1 and T_H2 cells are induced by cytokines generated in response to different pathogens.

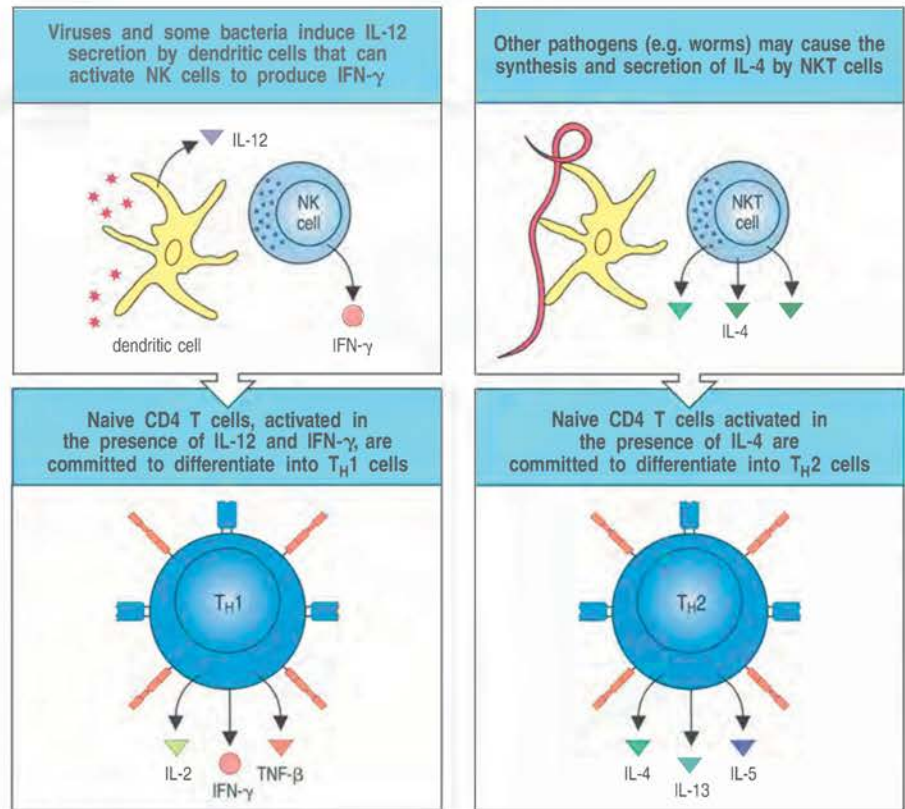
T_H1 responses tend to be induced by viruses and by bacterial and protozoan pathogens that can survive inside macrophage intracellular vesicles. In the case of viruses, the T_H1 response is generally involved in helping to activate the CD8 cytotoxic T cells that will recognize virus-infected cells and destroy them (see Chapter 9). T_H1 cells also induce the production of some subclasses of IgG antibodies, which neutralize virus particles in the blood and extracellular fluid. In the case of mycobacteria, and of protozoa such as *Leishmania* and *Toxoplasma*, which all take up residence inside macrophages, the role of T_H1 cells is to activate macrophages to a degree that will destroy the invaders.

Experiments *in vitro* have shown that naive CD4 T cells initially stimulated in the presence of IL-12 and IFN- γ tend to develop into T_H1 cells (Fig. 11.5, left panels). In part this is because these cytokines induce or activate the transcription factors leading to T_H1 development, and in part it is because IFN- γ inhibits the proliferation of T_H2 cells, as described in Chapter 9. NK cells and CD8 cells are also both activated in response to infections with viruses and some other intracellular pathogens, as discussed in Chapters 3 and 9, and both produce abundant IFN- γ . Dendritic cells and macrophages produce IL-12. Thus, CD4 T-cell responses in these infections tend eventually to be dominated by T_H1 cells.

Toll-like receptor (TLR) signaling is of major importance in driving dendritic cells to produce IL-12. This has been shown in mice lacking the adaptor

Fig. 11.5 The differentiation of naive CD4 T cells into subclasses of effector T cells is influenced by cytokines elicited by the pathogen. Left panels:

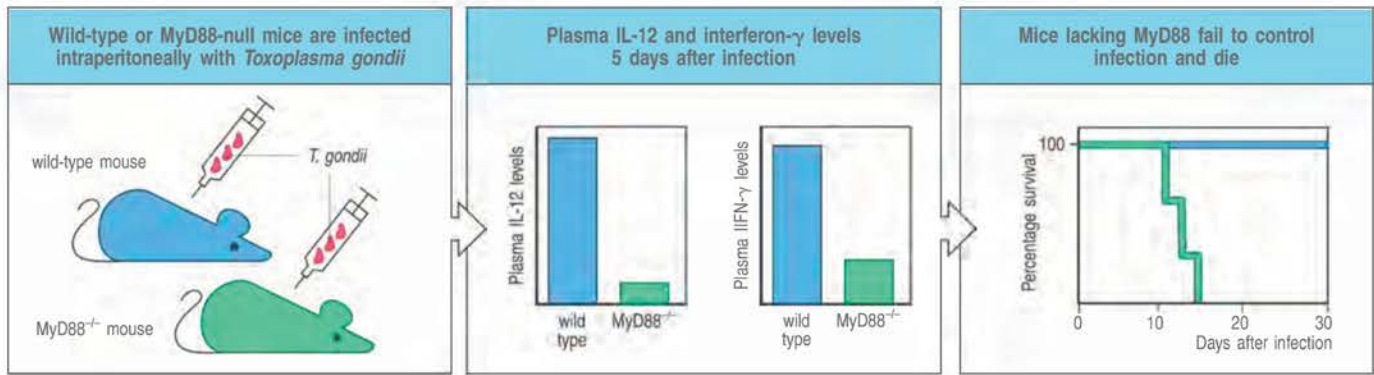
many pathogens, especially viruses and intracellular bacteria, activate dendritic cells to produce IL-12 and NK cells to produce IFN- γ . These cytokines cause proliferating CD4 T cells to differentiate into T_H1 cells. NK cells can be induced by certain stimuli and adjuvants to migrate into lymph nodes, where they could promote T_H1 responses. Right panels: IL-4, which can be produced by various cells, is made in response to parasitic worms and some other pathogens, and acts on proliferating CD4 T cells to cause them to become T_H2 cells. An iNKT cell is shown as a source of IL-4 here, but these cells are not the only source of IL-4 that can promote T_H2 responses (see the text). The mechanisms by which these cytokines induce the selective differentiation of CD4 T cells are discussed in Section 9-18 and Fig. 9.29. Selective induction of transcription factors induced by cytokine binding to cytokine receptors leads to the activation of these two different fates.



protein MyD88, a component of an intracellular signaling pathway activated by the stimulation of some TLRs (see Section 3-7). Mice deficient in MyD88 do not survive a challenge with *Toxoplasma gondii*, which normally elicits a strong T_H1 response. Dendritic and other cells from mice lacking MyD88 failed to produce IL-12 in response to parasite antigens, and the animals failed to mount a T_H1 response (Fig. 11.6). In contrast to dendritic cells stimulated through Dectin-1, dendritic cells stimulated through TLR-9 produce abundant IL-12 and support T_H1 differentiation. It is important to note that many immune responses to pathogens produce a mixed response, with both T_H17 and T_H1 cells being generated. The basis for such mixed responses, and for potential plasticity between these subsets, is an area of active research.

The killer lymphocytes of innate immunity, the NK cells, may contribute to T_H1 development (see Fig. 11.5). NK cells are not normally found within lymph nodes, but injection of mice with certain adjuvants, or with mature dendritic cells, can induce their recruitment to lymph nodes via expression of the chemokine receptor CXCR3 by the NK cell. As NK cells produce abundant IFN- γ , but little IL-4, they may act in lymph nodes during infections to direct the development of T_H1 cells.

For T_H2 responses, the mechanisms linking innate immunity to regulation of the adaptive T_H2 response are somewhat less clear (see Section 9-18). Naive CD4 T cells activated in the presence of IL-4, especially when IL-6 is also present, tend to differentiate into T_H2 cells (see Fig. 11.5, right panels). Some pathogens, such as helminths and other extracellular parasites, consistently induce development of T_H2 responses *in vivo*, and do so in a manner that requires IL-4 signaling by T cells. But it is still uncertain how these pathogens are initially sensed by the immune system and how this triggers commitment of the naive T cell to the T_H2 subset. But once some T cells have differentiated into effector T_H2 cells, their production of IL-4 can strongly reinforce the development of more T_H2 cells. Many cells have been proposed to act as



an initial and rapid source of IL-4, including invariant NKT cells (iNKT cells), mast cells, and basophils.

iNKT cells are a class of innate-like lymphocytes (see Section 3-24) that express the cell-surface marker NK1.1 normally associated with NK cells. Like other NKT cells (see Section 8-9), development of iNKT cells in the thymus does not involve selection by MHC class I or class II molecules, but instead depends on interactions with the MHC class IB molecules known as CD1 proteins (see Section 6-19), which bind self lipids. iNKT cells have a nearly invariant repertoire of $\alpha\beta$ T-cell receptor composed of gene segments $V_{\alpha}14$ – $J_{\alpha}28$ in mice, or the equivalent gene segments, $V_{\alpha}24$ – $J_{\alpha}18$, in humans. Expression of CD1 proteins can be induced at sites of infection, which may allow the presentation of microbial lipids to T cells. At least some iNKT cells recognize specific glycolipid antigens presented by CD1d. On activation, iNKT cells secrete very large amounts of IL-4 and IFN- γ , and may provide an initial source of cytokines that polarize a T-cell response, particularly in the direction of T_H2 cells. iNKT cells are not the only T cells that recognize antigens presented by CD1 molecules. CD1b presents the bacterial lipid mycolic acid to $\alpha\beta$ T cells, and other CD1 molecules are recognized by $\gamma\delta$ T cells. However, a particular role for iNKT cells in T_H2 responses has emerged from studies of mice in which the genes encoding $J_{\alpha}18$ or CD1d have been inactivated, and so iNKT cells fail to develop. These mice also fail to develop antigen-induced airway hypersensitivity that mimics a condition similar to asthma in humans and which is strongly associated with a T_H2 response.

Mast cells and basophils are also potent IL-4 producers and can migrate to peripheral lymphoid organs, making them possible candidates for an early source of IL-4 in response to infection. However, transgenic mice engineered to lack basophils still developed T_H2 responses and IgE production when infected by the helminth *Nippostrongylus brasiliensis*, but basophils were required for protection against secondary infection. This suggests that their role is mainly as an effector, not an initiator, of T_H2 -type responses. Some evidence suggests that certain TLR ligands can cause dendritic cells to produce cytokines favoring T_H2 development rather than T_H1 . For example, dendritic cells make more IL-10 and less IL-12 when stimulated by some ligands for TLR-2, including bacterial lipoproteins, peptidoglycans, and zymosan, a carbohydrate derivative of yeast cell walls, compared with other TLR ligands, but these can also stimulate production of IL-23, which favors T_H17 development.

11-5 CD4 T-cell subsets can cross-regulate each other's differentiation.

The various subsets of CD4 T cells—regulatory T cells (T_{reg}), T_H17 , T_H1 , and T_H2 —each have very different functions. T_{reg} cells maintain tolerance and limit immunopathology. T_H17 cells amplify acute inflammation at sites of infection. T_H1 cells are crucial for cell-mediated immunity due to phagocytes.

Fig. 11.6 Infection may trigger T_H1 polarization through Toll-like receptor signaling pathways. The adaptor protein MyD88 is a key component of Toll-like receptor signaling. Wild-type mice and mice deficient in MyD88 were infected intraperitoneally with the protozoan parasite *Toxoplasma gondii* (left panel). Five days after infection, mice lacking MyD88 showed a severe reduction in the amount of IL-12 in plasma compared with wild-type mice (center panel), and dendritic cells from the spleens of these animals failed to produce IL-12 on stimulation with antigens from *T. gondii*. The mice lacking MyD88 also failed to produce a large IFN- γ response (center panel) and a T_H1 response to the infection, and died about 2 weeks after infection (right panel, green line). In contrast, wild-type mice produced a strong IL-12, IFN- γ , and T_H1 response, controlled the infection, and survived (right panel, blue line). Sensing of *T. gondii* in mice may involve TLR-11 (see Section 3-5).

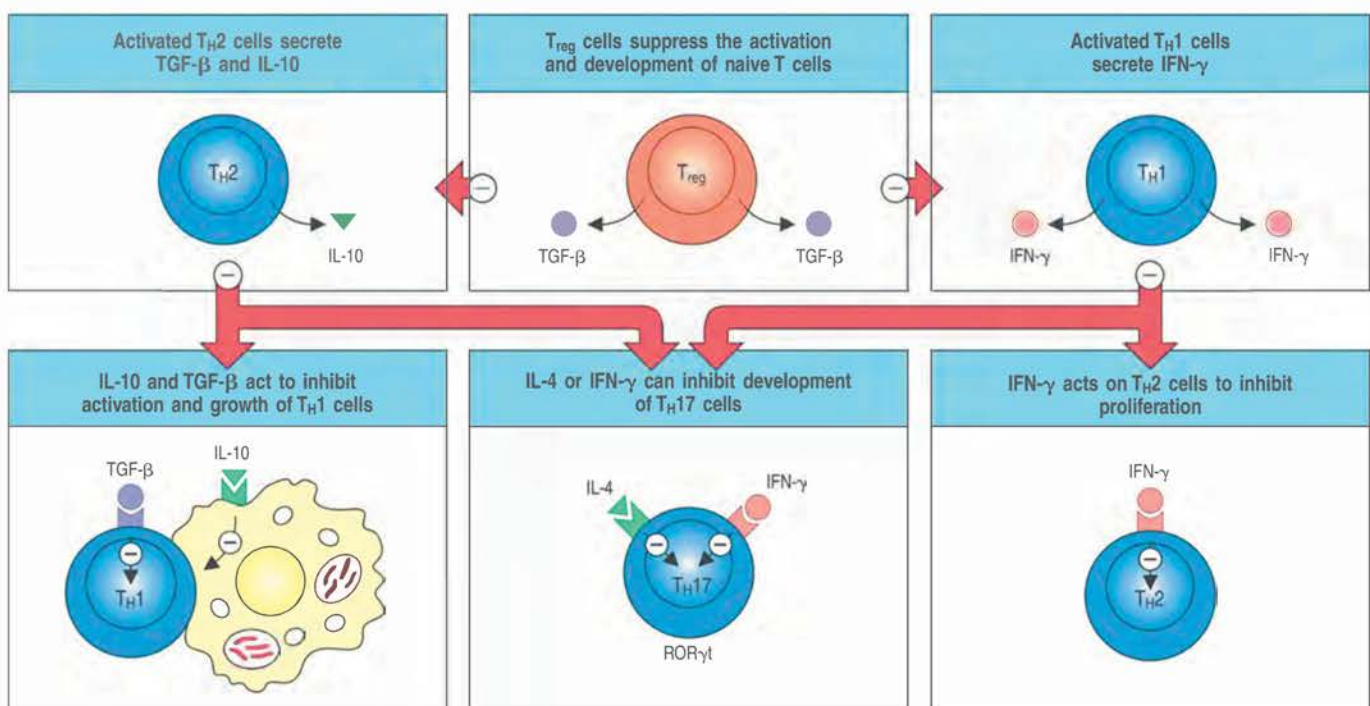
Fig. 11.7 The subsets of CD4 T cells each produce cytokines that can negatively regulate the development or effector activity of other subsets. In the absence of infection, in homeostatic conditions, the TGF- β produced by T_{reg} cells can inhibit the activation of naive T cells, thus preventing the development of a T_H17, T_H1, or T_H2 response (upper panels). During an infection, development of T_H17 cells occurs in response to IL-6 produced by dendritic cells. However, if signals are present to induce T_H1 or T_H2 cells, the cytokines IFN- γ or IL-4 produced by them can override the effect of IL-6 and inhibits T_H17 development (lower center panel). T_H2 cells can produce IL-10, which inhibits the production of IL-12 by macrophages, reducing T_H1 cells, and TGF- β , which inhibits T_H1 growth (left panels). T_H1 cells produce IFN- γ , which blocks the growth of T_H2 cells (right panels).

T_H2 cells promote allergic responses and protection against parasites by augmenting barrier immunity at epithelial surfaces. Cytokines mediate many of these distinct functions, but some can also influence the development of these T-cell subsets themselves. Thus, there is a complex pattern of cross-regulation during the development of CD4 T-cell subsets.

For example, T_H17 cells are induced by IL-6 and TGF- β , but IFN- γ (produced by T_H1 cells) or IL-4 (produced by T_H2 cells) can inhibit T_H17 development, promoting T_H1 or T_H2 development, respectively (Fig. 11.7). Furthermore, there is some degree of plasticity between these subsets, at least *in vitro*. Treatment with IFN- γ and IL-12 can convert established T_H17 cells into IFN- γ -producing T_H1 cells, and the combination of IFN- α , IFN- γ , and IL-12 can even induce IFN- γ production from T_H2 cells. There is also cross-regulation between T_H1 and T_H2 cells. IL-4 and IL-10, which are products of T_H2 cells, can inhibit the production of IL-12 by dendritic cells, and thereby inhibit T_H1 development. Conversely, IFN- γ , a product of T_H1 cells, can inhibit the proliferation of T_H2 cells (see Fig. 11.7). IL-10 is not made exclusively by T_H2 cells: it can also be made by T_H1 cells under conditions of strong stimulation of the T-cell receptor. In this way, antigen concentration can potentially modulate T_H1 and T_H2 differentiation by changing the overall balance of different cytokines such as IL-10.

It is sometimes possible to shift the balance between T_H1 and T_H2 cells by administering appropriate cytokines or antibodies, although studying their effects on human CD4 T-cells poses obvious difficulties. IL-2 and IFN- γ have been used to stimulate cell-mediated immunity in human diseases such as lepromatous leprosy and can cause both a local resolution of the lesion and a systemic change in T-cell responses. However, the links between cytokine action and disease have been explored mainly in mouse models. Such studies indicate that the appropriate choice of the CD4 T cell can sometimes be crucial for clearance of infection, and show that subtle differences in CD4 T-cell responses can have a significant impact on the outcome of infection.

One clear example of this is the murine model of infection by the protozoan parasite *Leishmania major*, which requires a T_H1 response and activation of



macrophages for clearance. C57BL/6 mice produce T_H1 cells that protect the animal by activating infected macrophages to kill *L. major*. In BALB/c mice infected with *L. major*, however, CD4 T cells fail to differentiate into T_H1 cells; instead, they become T_H2 cells, which are unable to activate macrophages to inhibit leishmanial growth. This difference seems to result from a population of memory T cells that are specific for gut-derived antigens but cross-react with an antigen, LACK (*Leishmania* analog of the receptors of activated C kinase), expressed by the *Leishmania* parasite. These memory cells are present in both strains of mice, but for unknown reasons they produce IL-4 in BALB/c mice but not in C57BL/6 mice. In BALB/c mice, the small amount of IL-4 secreted by these memory cells that are activated during *Leishmania* infection drives new *Leishmania*-specific CD4 T cells to become T_H2 cells, which leads eventually to a failure to eliminate the pathogen, and to death. The preferential development of T_H2 rather than T_H1 cells in BALB/c mice can be reversed if IL-4 is blocked in the first days of infection by injecting anti-IL-4 antibody, but this treatment is ineffective after a week or so of infection, demonstrating the crucial importance of the early exposure to cytokines for decisions by naive T cells (Fig. 11.8).

CD8 T cells are also able to regulate the immune response by producing cytokines. Effector CD8 T cells can, in addition to their familiar cytotoxic function, also respond to antigen by secreting cytokines typical of either T_H1 or T_H2 cells. Such CD8 T cells, called T_C1 or T_C2 by analogy with the T_H subsets, seem to be responsible for the development of leprosy in its lepromatous rather than its tuberculoid form. As we saw in Chapter 9, lepromatous leprosy is due to the predominance of a T_H2 cell response, which does not clear the bacteria. Patients with the less destructive tuberculoid leprosy make T_C1 cells whose cytokines induce T_H1 cells, which can activate macrophages to rid the body of its burden of leprosy bacilli. Patients with lepromatous leprosy have CD8 T cells that suppress the T_H1 response by making IL-10 and TGF- β . The expression of these cytokines could explain the suppression of CD4 T cells by CD8 T cells that has been observed in various situations.

Another factor that may possibly influence the differentiation of CD4 T cells into various subsets is the amount, or sequence, of the antigenic peptide that initiates the response. In some cases, large amounts of antigenic peptide, or peptides that interact strongly with the T-cell receptor, favor T_H1 responses, whereas a low density of peptide or peptides that bind weakly tend to elicit T_H2 responses. These effects do not seem to be due to differences in signaling through the T-cell receptor but may involve changes in the overall balance of different cytokines produced by the cells involved in activating naive T cells. It is not clear whether this effect is universal, but it could be important in certain circumstances. For instance, allergic reactions are caused by the production of IgE antibody, which requires high levels of IL-4 but does not occur in the presence of IFN- γ , a powerful inhibitor of IL-4-driven class switching to IgE. Antigens that elicit IgE-mediated allergy are generally delivered in minute doses and elicit T_H2 cells that make IL-4 and no IFN- γ . Also, it is possible that many allergens do not activate cytokine production from innate immune responses, which might override the effects of antigen dose. Finally, allergens are delivered to humans in minute doses across a thin mucosa, such as that of the lung, and something about this route of sensitization seems to favor T_H2 responses.

11-6 Effector T cells are guided to sites of infection by chemokines and newly expressed adhesion molecules.

The full activation of naive T cells takes 4–5 days and is accompanied by marked changes in their homing behavior. Effector cytotoxic CD8 T cells must travel from the peripheral lymphoid tissue in which they have been activated to attack and destroy infected cells. Effector CD4 T_H1 cells must also leave

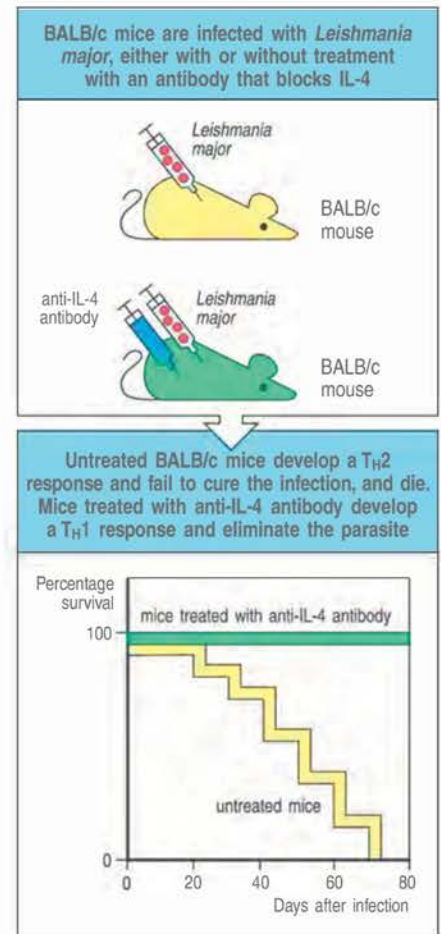


Fig. 11.8 The development of CD4 T-cell subsets can be manipulated by altering the cytokines acting during the early stages of infection. Elimination of infection with the intracellular protozoan parasite *Leishmania major* requires a T_H1 response, because IFN- γ is needed to activate the macrophages that provide protection. BALB/c mice are normally susceptible to *L. major* because they generate a T_H2 response to the pathogen. This is because they produce IL-4 early during infection and this induces naive T cells into the T_H2 lineage (see the text). Treatment of BALB/c mice with neutralizing anti-IL-4 antibodies at the beginning of infection inhibits this IL-4 and prevents the diversion of naive T cells toward the T_H2 lineage; these mice develop a protective T_H1 response.

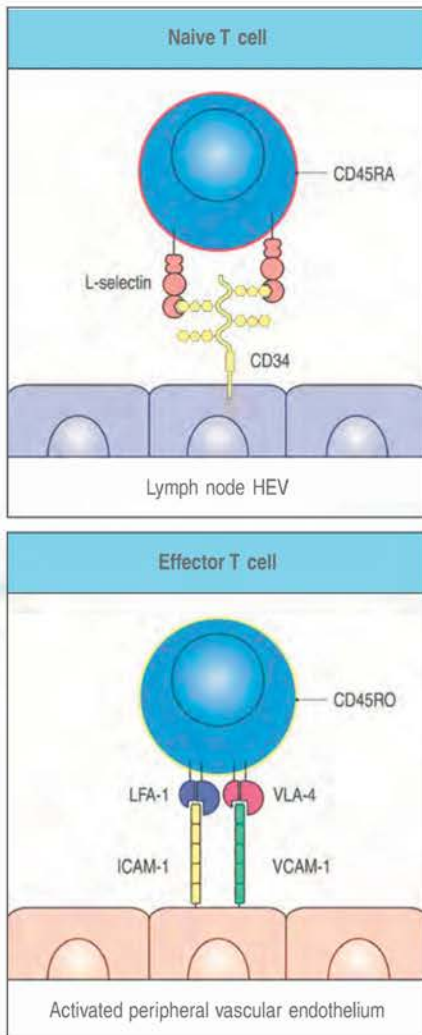


Fig. 11.9 Effector T cells change their surface molecules, allowing them to home to sites of infection. Naive T cells home to lymph nodes through the binding of L-selectin to sulfated carbohydrates displayed by various proteins, such as CD34 and GlyCAM-1, on the high endothelial venule (HEV, upper panel). After encounter with antigen, many of the differentiated effector T cells lose expression of L-selectin, leave the lymph node about 4–5 days later, and now express the integrin VLA-4 and increased levels of LFA-1. These bind to VCAM-1 and ICAM-1, respectively, on peripheral vascular endothelium at sites of inflammation (lower panel). On differentiating into effector cells, T cells also alter their splicing of the mRNA encoding the cell-surface protein CD45. The CD45RO isoform expressed by effector T cells lacks one or more exons that encode extracellular domains present in the CD45RA isoform expressed by naive T cells, and somehow makes effector T cells more sensitive to stimulation by specific antigen.

the lymphoid tissues to activate macrophages at the site of infection. Exit of effector T cells from lymphoid tissues is regulated by changes in their responsiveness to the chemotactic lipid sphingosine 1-phosphate, as described in Section 9-3. Most effector T cells cease production of L-selectin, which mediates homing to the lymph nodes, whereas the expression of other adhesion molecules is increased (Fig. 11.9). One important change is a marked increase in synthesis of the integrin $\alpha_4\beta_1$, also known as VLA-4. This binds to the adhesion molecule VCAM-1, a member of the immunoglobulin superfamily that is induced on activated endothelial cell surfaces and initiates the extravasation of the effector T cells. Thus, if the innate immune response has already activated the endothelium at the site of infection, as described in Section 11-2, effector T cells will be rapidly recruited.

In the early stage of an immune response, only a few of the effector T cells that enter the infected tissue will be expected to be specific for pathogen, because any effector T cell specific for any antigen will also be able to enter. Specificity of the reaction is maintained, however, because only those effector T cells that recognize pathogen antigens will carry out their function, destroying infected cells or specifically activating pathogen-loaded macrophages. By the peak of an adaptive immune response, after several days of clonal expansion and differentiation, most of the recruited T cells will be specific for the infecting pathogen.

Not all infections trigger innate immune responses that activate local endothelial cells, and it is not so clear how effector T cells are guided to the sites of infection in these cases. However, activated T cells seem to enter all tissues in very small numbers, perhaps via adhesive interactions such as the binding of P-selectin on the endothelial cells to its ligand **P-selectin glycoprotein ligand-1 (PSGL-1)**, which is expressed by activated T cells, and so effector T cells could encounter their antigens even in the absence of a previous inflammatory response.

By the means just described, one or a few specific effector T cells encountering antigen in a tissue can initiate or augment a potent local inflammatory response that recruits many more effector lymphocytes and nonspecific inflammatory cells to that site. Effector T cells that recognize pathogen antigens in the tissues produce cytokines such as $\text{TNF-}\alpha$, which activates endothelial cells to express E-selectin, VCAM-1, and ICAM-1, and chemokines such as CCL5, which acts on effector T cells to activate their adhesion molecules. VCAM-1 and ICAM-1 on endothelial cells bind VLA-4 and LFA-1, respectively, on effector T cells, recruiting more of these cells into tissues that contain the antigen. At the same time, monocytes and polymorphonuclear leukocytes are recruited by adhesion to E-selectin. $\text{TNF-}\alpha$ and $\text{IFN-}\gamma$ released by activated T cells also act synergistically to change the shape of endothelial cells, allowing increased blood flow, increased vascular permeability, and increased emigration of leukocytes, fluid, and protein into the infected tissue. Thus, at the later stages of an infection, the protective effects of macrophages secreting $\text{TNF-}\alpha$ and other pro-inflammatory cytokines at the infected site (see Section 3-17) are reinforced by the actions of effector T cells.

By contrast, effector T cells that enter tissues but do not recognize their antigen are rapidly lost. They either enter the lymph from the tissues and eventually return to the bloodstream, or undergo apoptosis. Most of the T cells in the afferent lymph that drains tissues are memory or effector T cells, which characteristically express the CD45RO isoform of the cell-surface molecule CD45 and lack L-selectin (see Fig. 11.9). Effector T cells and memory T cells have similar phenotypes, as we discuss later, and both seem to be committed to migration through potential sites of infection. In addition to allowing effector T cells to clear all sites of infection, this pattern of migration allows them to contribute, along with memory cells, to protecting the host against reinfection with the same pathogen.

Expression of particular adhesion molecules can direct different subsets of effector T cells to specific sites. As we shall see in Chapter 12, the peripheral immune system is compartmentalized, such that different populations of lymphocytes migrate through different lymphoid compartments and—after activation—through the different tissues they serve. This is achieved by the selective expression of adhesion molecules that bind selectively to tissue-specific addressins. In this context, the adhesion molecules are often known as **homing receptors**. For example, some activated T cells specifically populate the skin. They are induced during activation to express the adhesion molecule **cutaneous lymphocyte antigen (CLA)** (Fig. 11.10). This is a specifically glycosylated isoform of PSGL-1 that binds to E-selectin on cutaneous vascular endothelium. CLA-expressing T lymphocytes also produce the chemokine receptor CCR4. This binds the chemokine CCL17 (TARC), which is present at high levels on the endothelium of cutaneous blood vessels. Interaction of CLA with E-selectin causes the T cell to roll against the wall of the vessel, and the signal delivered by endothelial CCL17 is thought to cause the arrest of lymphocytes and to bring about their adhesion to the wall, probably by inducing tight binding of integrin, as described for the action of CCL21 on naive T cells (see Section 9-3). The condition known as leukocyte adhesion deficiency is found in people who lack integrin β subunits, and such people suffer from recurrent infections with pyogenic bacteria and problems with wound healing. In addition to CCR4, skin-homing T cells carry the chemokine receptor CCR10 (GPR-2), which binds the chemokine CCL27 (CTACK) expressed by keratinocytes, the epithelial cells of the skin. Different combinations of chemokines and receptors are used by T cells that home to the mucosal immune system of the gut, and these are discussed in Chapter 12.



11-7 Differentiated effector T cells are not a static population but continue to respond to signals as they carry out their effector functions.

The commitment of CD4 T cells to become distinct lineages of effector cells begins in peripheral lymphoid tissues, such as lymph nodes, as described in Sections 11-3 and 11-4. However, the effector activities of these cells once they enter sites of infection are not defined simply by the signals received in the lymphoid tissues. Instead, evidence suggests that there is continuous regulation of the expansion and the effector activities of differentiated CD4 cells, in particular of T_H17 and T_H1 cells.

As noted in Chapter 9, commitment of naive T cells to become T_H17 cells is triggered by exposure to TGF- β and IL-6; commitment to T_H1 cells is initially triggered by IFN- γ . These initial conditions are not, however, sufficient to

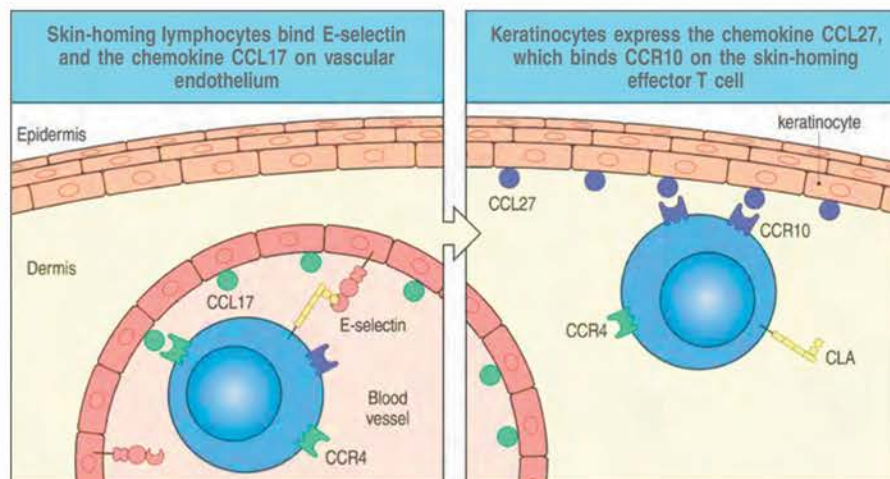


Fig. 11.10 Skin-homing T cells use specific combinations of integrins and chemokines to migrate specifically to the skin. Left panel: a skin-homing lymphocyte binds to the endothelium lining a cutaneous blood vessel by interactions between cutaneous lymphocyte antigen (CLA) and constitutively expressed E-selectin on the endothelial cells. The adhesion is strengthened by an interaction between lymphocyte chemokine receptor CCR4 and the endothelial chemokine CCL17. Right panel: once through the endothelium, effector T lymphocytes are attracted to keratinocytes of the epidermis by the chemokine CCL27, which binds to the receptor CCR10 on lymphocytes.

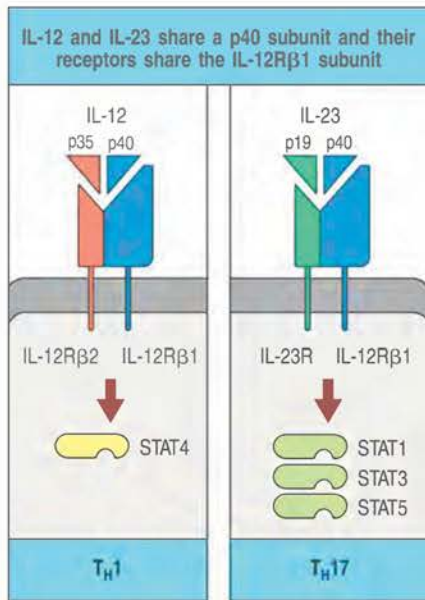


Fig. 11.11 The cytokines IL-12 and IL-23 share subunits, and their receptors have a common component. The dimeric cytokines IL-12 and IL-23 share the p40 subunit, and the receptors for IL-12 and IL-23 have the IL-12Rβ1 subunit in common. IL-12 signaling activates the transcriptional activators STAT1, STAT3, and STAT4, but its action in increasing IFN- γ production is due to STAT4. IL-23 activates several other STATs, but activates STAT4 only weakly. Both cytokines augment the activity and proliferation of the CD4 subsets that express receptors for them; T_H1 cells express IL-12R, and T_H17 cells express IL-23R. Mice deficient in p40 lack expression of both of these cytokines, and manifest immune defects as a result of deficiencies in both T_H1 and T_H17 activities.

generate complete or effective T_H17 or T_H1 responses. In addition, each T-cell subset also requires stimulation by another cytokine: IL-23 in the case of T_H17 cells, and IL-12 in the case of T_H1 cells. IL-23 and IL-12 are closely related in structure; each is a heterodimer and they share a subunit. IL-23 is composed of one p40 and one p19 subunit, whereas IL-12 has the p40 subunit and a unique p35 subunit. Committed T_H17 cells express a receptor for IL-23, whereas T_H1 cells express a receptor for IL-12. The receptors for IL-12 and IL-23 are also related, having a common subunit (Fig. 11.11).

IL-23 and IL-12 amplify the activities of T_H17 and T_H1 cells, respectively. Like many other cytokines, they both act through the JAK–STAT intracellular signaling pathway (see Fig. 7.29). IL-23 signaling activates the intracellular transcriptional activators STAT1, STAT3, and STAT5, but activates STAT4 very weakly. In contrast, IL-12 activates STAT1 and STAT3 and also strongly activates STAT4. IL-23 does not initiate the commitment of naive CD4 T cells to T_H17 cells, but it does stimulate their expansion. Many *in vivo* responses that depend on IL-17 are diminished in the absence of IL-23. For example, mice lacking the IL-23-specific subunit p19 show decreased production of IL-17 and IL-17F in the lung after infection by *Klebsiella pneumoniae*.

Mice lacking the p40 subunit, which is shared by IL-12 and IL-23, are deficient in both IL-23 and IL-12. This was the source of some confusion before the separate role of IL-23 in T_H17 activity was understood. It had been assumed that inflammation in the brains of mice with experimental autoimmune encephalomyelitis (EAE) was due to T_H1 cells and IFN- γ , because EAE could not be induced in p40-deficient mice. But later it was found that EAE could be induced in p35-deficient mice, which lack IL-12 but maintain IL-23, and that EAE cannot be induced in p19-deficient mice. It turns out that the brain inflammation in EAE is largely a result of the activity of T_H17 cells.

IL-12 regulates the effector activity of committed T_H1 cells at sites of infection, but other cytokines such as IL-18 may also be involved. Studies of two different pathogens have shown that the initial differentiation of T_H1 cells is not sufficient for protection, and that continuous signals are required. Mice deficient in p40 can resist initial infection by *T. gondii* as long as IL-12 is administered continuously. If IL-12 is administered during the first 2 weeks of infection, p40-deficient mice survive the initial infection and establish a latent chronic infection characterized by cysts containing the pathogen. When IL-12 administration is stopped, however, these mice gradually reactivate the latent cysts and the animals eventually die of toxoplasmic encephalitis. IFN- γ production by pathogen-specific T cells decreases in the absence of IL-12 but could be restored by IL-12 administration. Similarly, the adoptive transfer of differentiated T_H1 cells from mice cured of *L. major* protects RAG-deficient mice infected by *L. major*, but cannot protect p40-deficient mice (Fig. 11.12). Together, these experiments suggest that T_H1 cells continue to respond to signals during an infection, and that continuous IL-12 is needed to sustain the effectiveness of differentiated T_H1 cells against at least some pathogens.

11-8 Primary CD8 T-cell responses to pathogens can occur in the absence of CD4 T-cell help.

Many CD8 T-cell responses require help from CD4 T cells (see Section 9-16). This is typically the case where the antigen recognized by the CD8 T cells is derived from an agent that does not cause inflammation on initial infection. In such circumstances, CD4 T-cell help is required to activate dendritic cells to become able to stimulate a complete CD8 T-cell response, an activity that has been described as licensing of the antigen-presenting cell (see Section 9-6). Licensing involves the induction of co-stimulatory molecules such as B7, CD40, and 4-1BBL on the dendritic cell, which can then deliver signals that

Fig. 11.12 Continuous IL-12 is required for resistance to pathogens requiring T_H1 responses. Mice that have eliminated an infection with *Leishmania major* and have generated T_H1 cells specific to the pathogen were used as a source of T cells that were adoptively transferred into either RAG2-deficient mice, which lack T cells and B cells and cannot control *L. major* infection but can produce IL-12, or into mice lacking p40, which cannot produce IL-12. On subsequent infection of the RAG2-deficient mice, lesions did not enlarge because the transferred T_H1 cells conferred immunity. But despite the fact that the transferred cells were already differentiated T_H1 cells, they did not confer resistance to IL-12 p40-deficient mice in which a continuous source of IL-12 was not present.

fully activate naive CD8 T cells (see Fig. 9.27). Licensing enforces a requirement for dual recognition of an antigen by the immune system by both CD4 and CD8 T cells, which provides a useful safeguard against autoimmunity. Dual recognition is also seen in the cooperation between T cells and B cells for antibody generation (see Chapter 10). However, not all CD8 T-cell responses require such help.

Some infectious agents, such as the intracellular Gram-positive bacterium *Listeria monocytogenes* and the Gram-negative bacterium *Burkholderia pseudomallei*, provide the inflammatory environment required to license dendritic cells and thus can induce primary CD8 T-cell responses without help from CD4 T cells. These pathogens carry a number of immunostimulatory signals, such as ligands for TLRs, and so can directly activate antigen-presenting cells to express the co-stimulatory molecules B7 and CD40. Thus, fully activated dendritic cells presenting *Listeria* or *Burkholderia* antigens can activate naive antigen-specific CD8 T cells without the help of CD4 T cells, and can induce them to undergo clonal expansion (Fig. 11.13). The activated dendritic cell also secretes cytokines such as IL-12 and IL-18, which act on naive CD8 T cells in a so-called 'bystander' effect to induce them to produce IFN- γ , which in turn induces other protective effects (see Fig. 11.13).

Primary CD8 T-cell responses to *L. monocytogenes* were examined in mice that were genetically deficient in MHC class II molecules and thus lacked CD4 T cells (see Section 8-18). The numbers of CD8 T cells specific for a particular antigen expressed by the pathogen were measured by using MHC tetramers (see Appendix I, Section A-28). On day 7 after infection, wild-type mice and mice lacking CD4 T cells show equivalent expansion, and equivalent cytotoxic capacity, of pathogen-specific CD8 T cells. Mice lacking CD4 T cells cleared the initial infection by *L. monocytogenes* as effectively as wild-type mice. These experiments clearly show that protective responses can be generated by pathogen-specific CD8 T cells without CD4 T-cell help. However, as we will see later, the nature of the CD8 memory response is different and is diminished in the absence of CD4 T-cell help.

Naive CD8 T cells can also undergo 'bystander' activation by IL-12 and IL-18 to produce IFN- γ very early during infection (see Fig. 11.13). Mice infected with *L. monocytogenes* or *B. pseudomallei* rapidly produce a strong IFN- γ response, which is essential for their survival. The source of this IFN- γ seems to be both the NK cells of innate immunity and naive CD8 T cells, which begin to secrete it within the first few hours after infection. This is believed to be too soon for any significant expansion of pathogen-specific CD8 T cells, which would initially be too rare to contribute in an antigen-specific manner. The production of IFN- γ by both NK and CD8 T cells at this early time can be blocked experimentally by antibodies against IL-12 and IL-18, suggesting that these cytokines are responsible. The source of the IL-12 and IL-18 was not identified in this experiment, but they are produced by macrophages and dendritic cells in response to activation via TLRs. These experiments indicate that naive CD8 T cells can contribute nonspecifically in a kind of innate defense, not requiring CD4 T cells, in response to early signals of infection.

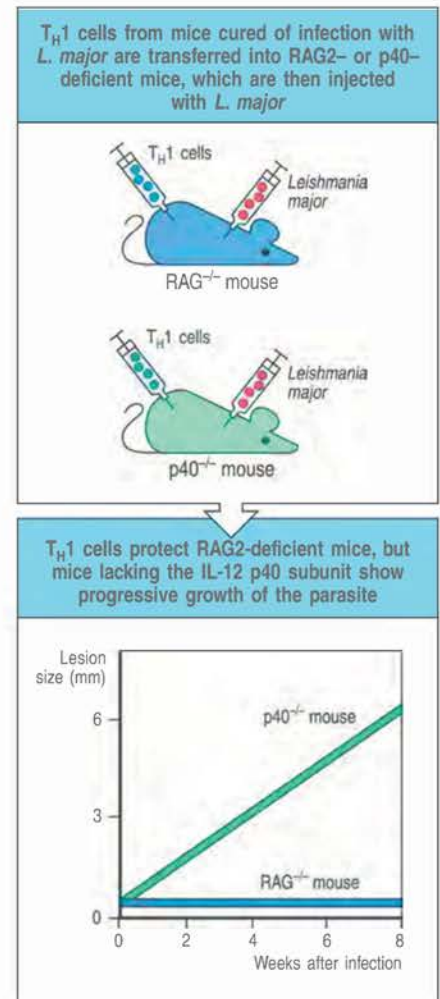
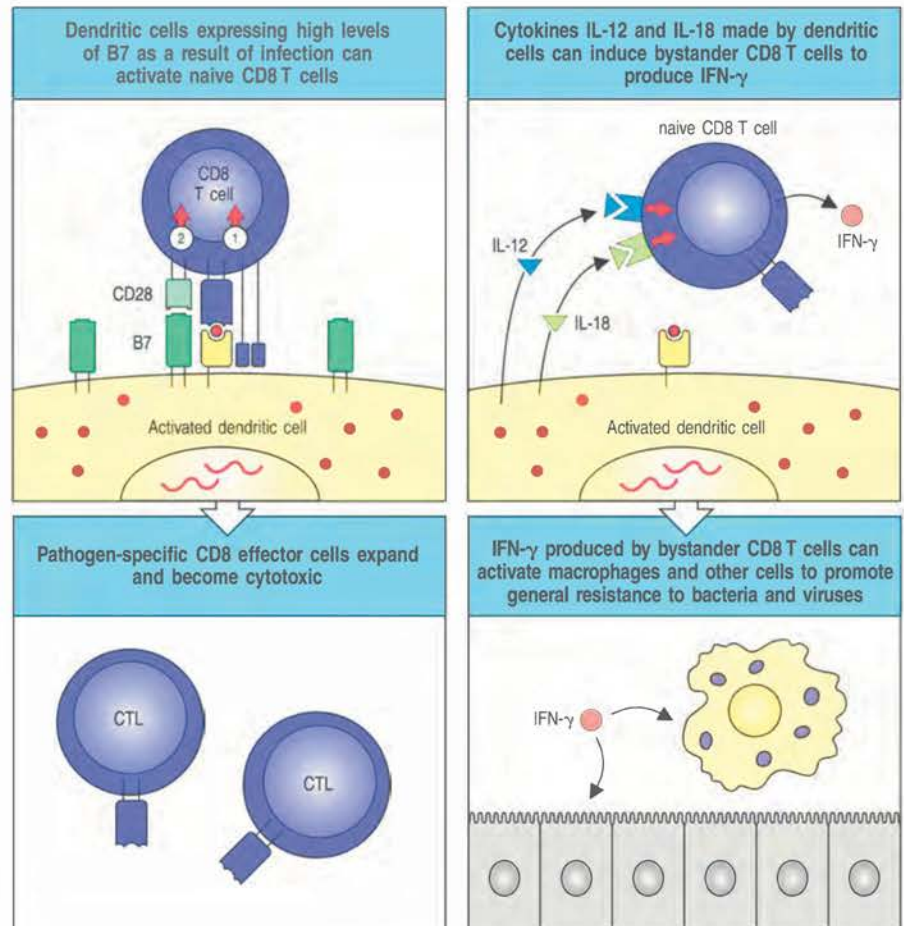


Fig. 11.13 Naive CD8 T cells can be activated directly by potent antigen-presenting cells through their T-cell receptor or through the action of cytokines. Left panels: naive CD8 T cells that encounter peptide:MHC class I complexes on the surface of dendritic cells expressing high levels of co-stimulatory molecules as a result of the inflammatory environment produced by some pathogens (upper left panel) are activated to proliferate in response, eventually differentiating into cytotoxic CD8 T cells (lower left panel). Right panels: activated dendritic cells also produce the cytokines IL-12 and IL-18, whose combined effect on CD8 T cells rapidly induces the production of IFN- γ (upper right panel). This activates macrophages for the destruction of intracellular bacteria and can promote antiviral responses in other cells (lower right panel).



11-9 Antibody responses develop in lymphoid tissues under the direction of T_{FH} cells.

As described in Chapter 10, B cells specific for a protein antigen cannot be activated to proliferate, form germinal centers, or differentiate into plasma cells until they encounter a helper T cell that is specific for one of the peptides derived from that antigen. Recall that naive antigen-binding B cells first enter the follicle, but when they encounter antigen, CCR7 is expressed and they move toward the boundary of the B-cell and T-cell zones (see Section 10-5). Similarly, in the T-cell zones, some naive T cells that are activated by antigen-bearing dendritic cells become follicular T helper (T_{FH}) cells that express CXCR5; these move toward the boundary of the B-cell and T-cell zones, where they can interact with recently activated B cells (see Fig. 10.5). Through linked recognition (see Section 10-3), this initial T cell–B cell interaction gives rise to the primary focus after about 5 days, and later can generate a germinal center reaction in the follicle that facilitates B cells to undergo isotype switching and affinity maturation (see Sections 5-18 and 10-10). Some B cells activated in the primary focus migrate to the medullary cords of the lymph node, or to those parts of the red pulp that are next to the T-cell zones of the spleen, where they become plasma cells and secrete specific antibody for a few days (see Fig. 10.7). The final products of the germinal center response are high-affinity memory B cells and long-lived plasma cells, which are important for the maintenance of serum antibody levels and long-lasting immunity.

Antigen is retained for very long periods in lymphoid follicles in the form of antigen:antibody complexes on the surface of the local follicular dendritic

cells. The antigen:antibody complexes, which become coated with fragments of C3, are held on the cell by receptors for the complement fragments (CR1, CR2, and CR3) as well as by a nonphagocytic Fc receptor (see Fig. 10.16). The function of this long-lived antigen is unclear, because there is evidence that it is not absolutely required for the stimulation of B cells in the germinal center (see Section 10-10), but it may regulate the long-term antibody response.

11-10 Antibody responses are sustained in medullary cords and bone marrow.

The B cells activated in primary foci migrate either to adjacent follicles or to local extrafollicular sites of proliferation. B cells grow exponentially in these sites for 2–3 days and undergo six or seven cell divisions before the progeny come out of the cell cycle and form antibody-producing plasma cells *in situ* (Fig. 11.14, upper panel). Most of these plasma cells have a life-span of 2–4 days, after which they undergo apoptosis. About 10% of plasma cells in these extrafollicular sites live longer; their origin and ultimate fate are unknown. Surviving B cells that have undergone class switching and affinity maturation in the germinal center will either become memory cells or can leave the germinal center as plasmablasts (pre-plasma cells) to become relatively long-lived antibody-producing cells in other locations (see Sections 10-7 to 10-9).

Plasmablasts originating in the follicles of Peyer's patches and mesenteric lymph nodes migrate via lymph to the blood and then enter the lamina propria of the gut and other epithelial surfaces. Those originating in peripheral lymph node or splenic follicles migrate to the bone marrow (Fig. 11.14, lower panel). In these distant sites of antibody production, the plasmablasts differentiate into plasma cells that have a life-span of months to years. These cells are thought to provide the antibody that can be present in the blood for years after the initial immune response. Whether this supply of plasma cells is replenished by the continual but occasional differentiation of memory cells is not yet known. Studies of responses to nonreplicating antigens show that germinal centers are present for only 3–4 weeks after initial exposure to antigen. However, small numbers of B cells continue to proliferate in the follicles for months and may be the precursors of antigen-specific plasma cells in the mucosa and bone marrow throughout the subsequent months and years.

11-11 The effector mechanisms used to clear an infection depend on the infectious agent.

Most infections engage both the cell-mediated and the humoral aspects of immunity, and in many cases both are helpful in clearing or containing the pathogen and setting up protective immunity, as shown in Fig. 11.15, although the relative importance of the different effector mechanisms, and the effective classes of antibody involved, vary with different pathogens. As we learned in Chapter 9, cytotoxic T cells are important in destroying virus-infected cells, and in some viral diseases they are the predominant class of lymphocytes present in the blood during a primary infection. Nevertheless, the role of antibodies in clearing viruses from the body and preventing them from getting a hold should not be forgotten. Ebola virus causes a hemorrhagic fever and is one of the most lethal viruses known, but some patients do survive and some people even become infected but remain asymptomatic. In both cases, a strong antiviral IgG response early in infection seems to be essential for survival. The antibody response seems to clear the virus from the bloodstream and gives the patient time to activate cytotoxic T cells. In contrast, this antibody response did not occur in infections that proved fatal; the virus continued to replicate, and even though there was some activation of T cells, the disease progressed.

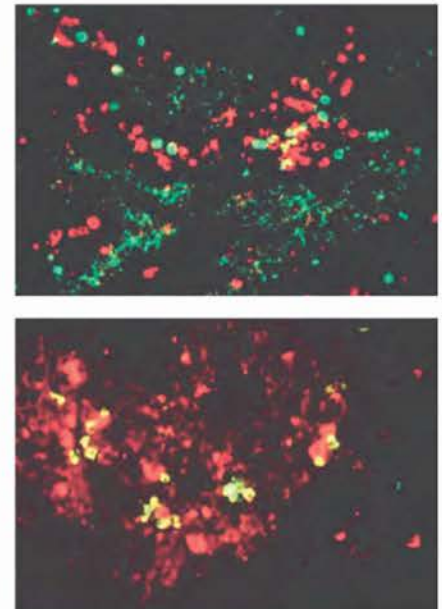


Fig. 11.14 Plasma cells are dispersed in medullary cords and bone marrow. In these sites they secrete antibody at high rates directly into the blood for distribution to the rest of the body. In the upper micrograph, plasma cells in lymph node medullary cords are stained green (with fluorescein anti-IgA) if they are secreting IgA, and red (with rhodamine anti-IgG) if they are secreting IgG. The plasma cells in these local extrafollicular sites are short lived (2–4 days). The lymphatic sinuses are outlined by green granular staining selective for IgA. In the lower micrograph, longer-lived plasma cells (3 weeks to 3 months or more) in the bone marrow are revealed with antibodies specific for light chains (fluorescein anti- λ and rhodamine anti- κ stain). Plasma cells secreting immunoglobulins containing λ light chains show on this micrograph as yellow. Those secreting immunoglobulins containing κ light chains stain red. Photographs courtesy of P. Brandtzaeg.

	Pathological agent	Disease	Humoral immunity				Cell-mediated immunity	
			IgM	IgG	IgE	IgA	CD4 T cells (macrophages)	CD8 killer T cells
Viruses	Herpes zoster	Chickenpox						
	Epstein-Barr virus	Mononucleosis						
	Influenza virus	Influenza						
	Polio virus	Poliomyelitis						
Intra-cellular bacteria	<i>Rickettsia prowazekii</i>	Typhus						
	Mycobacteria	Tuberculosis, leprosy						
Extra-cellular bacteria	<i>Staphylococcus aureus</i>	Boils						
	<i>Streptococcus pneumoniae</i>	Pneumonia						
	<i>Neisseria meningitidis</i>	Meningitis						
	<i>Corynebacterium diphtheriae</i>	Diphtheria						
	<i>Vibrio cholerae</i>	Cholera						
Fungi	<i>Candida albicans</i>	Candidiasis						
Protozoa	<i>Plasmodium</i> spp.	Malaria						
	<i>Trypanosoma</i> spp.	Trypanosomiasis						
Worms	Schistosome	Schistosomiasis						
Toxins	<i>Corynebacterium diphtheriae</i>	Diphtheria						
	<i>Clostridium tetani</i>	Tetanus						

Fig. 11.15 Different effector mechanisms are used to clear primary infections with different classes of pathogens and to protect against reinfection. The defense mechanisms used to clear a primary infection are identified by red shaded boxes. Yellow shading indicates a role in protective immunity. Paler shades indicate less well established mechanisms. It is clear that classes of pathogens elicit similar protective immune responses, reflecting similarities in their lifestyles. The CD4 responses indicated on this diagram refer only to those involved in macrophage activation. In addition, in virtually all diseases, helper CD4 T-cell responses will be involved in stimulating antibody production, class switching, and the production of memory cells.

Cytotoxic T cells are also required for the destruction of cells infected with some intracellular bacterial pathogens, such as *Rickettsia*, the causative agent of typhus. In contrast, mycobacteria, which live inside macrophage vesicles, are mainly kept in check by CD4 T_H1 cells, which activate infected macrophage to kill the bacteria. Antibodies are the principal immune reactants that clear primary infections with common extracellular bacteria such as *Staphylococcus aureus* and *Streptococcus pneumoniae*. The IgM and IgG antibodies produced against components of the bacterial surface coat opsonize the bacteria and make them more susceptible to phagocytosis.

Figure 11.15 also indicates the mechanisms involved in immunity to reinfection, or protective immunity, against the pathogens listed. Inducing protective immunity is the goal of vaccine development, which we discuss more detail in Chapter 16. To achieve protective immunity requires a vaccine to induce an adaptive immune response that has both the antigen specificity and the appropriate functional elements to combat the particular pathogen concerned. Pathogens carry multiple epitopes for both B cells and T cells, and thus generate diverse antibody and T-cell responses, but not all of these will be equally effective in clearing the disease. Protective immunity consists of two components—immune reactants, such as antibody or effector T cells generated in the initial infection or by vaccination, and long-lived immunological memory (Fig. 11.16).

The type of antibody or effector T cell that offers protection depends on the infectious strategy and lifestyle of the pathogen. Thus, when opsonizing

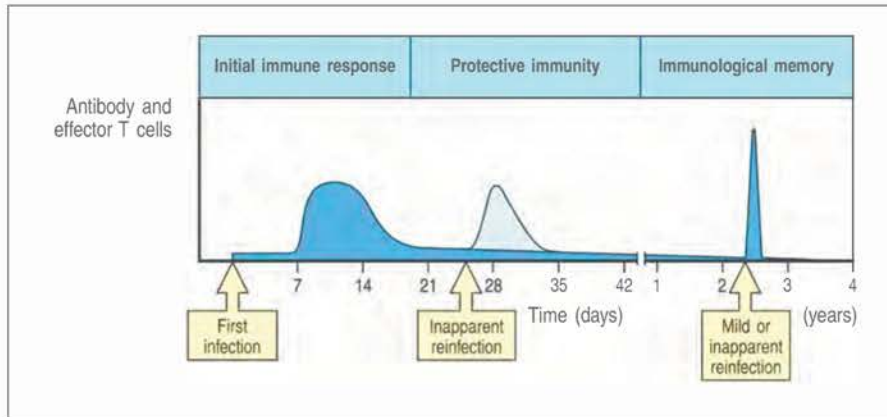


Fig. 11.16 Protective immunity consists of preformed immune reactants and immunological memory. The first time a particular pathogen is encountered, pathogen-specific antibody and effector T cells are produced. After the infection has been cleared, their levels gradually decline. An early reinfection with the same pathogen is rapidly cleared by these preformed immune reactants. There are few symptoms, but levels of immune reactants are found to increase temporarily (light blue peak). Reinfection years afterward leads to an immediate and rapid increase in pathogen-specific antibody and effector T cells as a result of immunological memory, and disease symptoms are mild or inapparent.

antibodies such as IgG1 are present (see Section 10-14), opsonization and phagocytosis of extracellular pathogens will be more efficient. If specific IgE is present, pathogens will trigger activation of mast cells, rapidly initiating an inflammatory response through the release of histamine and leukotrienes. In many cases the most efficient protective immunity is mediated by neutralizing antibody that can prevent pathogens from establishing an infection, and most of the established vaccines against acute childhood viral infections work primarily by inducing protective antibodies. Effective immunity against polio virus, for example, requires preexisting antibody (see Fig. 11.15), because the virus rapidly infects motor neurons and destroys them unless it is immediately neutralized by antibody and prevented from spreading within the body. In polio, specific IgA on mucosal epithelial surfaces also neutralizes the virus before it enters the tissues. Thus, protective immunity can involve effector mechanisms (IgA in this case) that do not operate in the elimination of the primary infection.

When a primary adaptive immune response is successful in halting an infection, it will often clear the primary infection from the body by the effector mechanisms discussed in Chapters 9 and 10. However, as we discuss in Chapter 13, many pathogens evade complete clearance and persist for the life of the host. The virus herpes zoster, which causes chickenpox on primary infection, then lies latent in the body for years without causing disease, but can later in life, or if the body is stressed, become reactivated and cause shingles.

11-12 Resolution of an infection is accompanied by the death of most of the effector cells and the generation of memory cells.

When an infection is effectively repelled by the adaptive immune system, two things occur. The actions of effector cells remove the specific stimulus that originally recruited them. In the absence of this stimulus, the cells then undergo 'death by neglect,' removing themselves by apoptosis. The dying cells are rapidly cleared by phagocytes and other cells, which recognize the membrane lipid phosphatidylserine. This lipid is normally found only on the inner surface of the plasma membrane, but in apoptotic cells it rapidly redistributes to the outer surface, where it can be recognized by specific receptors on many cells. Thus, not only does the ending of infection lead to the removal of the pathogen, it also leads to the loss of most of the pathogen-specific effector cells.

However, some of the effector cells are retained, and these provide the raw material for memory T-cell and B-cell responses. These are crucially important to the operation of the adaptive immune system. Memory T cells, in particular, are retained virtually forever. The mechanisms underlying the decision to induce apoptosis in most effector cells and to retain only a few are only now being discovered and are still not well understood. It seems likely that

the answers will lie in the cytokines produced by the environment and by the T cells themselves, and in the affinity of the T-cell receptors for their antigens.

Summary.

The adaptive immune response is required for effective protection of the host against pathogenic microorganisms. The response of the innate immune system to pathogens helps to initiate the adaptive immune response. Pathogens cause the activation of dendritic cells to full antigen-presenting cell status, and interactions with other cells of the innate immune system lead to the production of cytokines that direct the quality of the CD4 T-cell response. Pathogen antigens are transported to local lymphoid organs by the migrating dendritic cells and are presented to antigen-specific naive T cells that continuously recirculate through the lymphoid organs. T-cell priming and the differentiation of effector T cells occur here, and the effector T cells either leave the lymphoid organ to provide cell-mediated immunity in sites of infection in the tissues or remain in the lymphoid organ to participate in humoral immunity by activating antigen-binding B cells. Distinct types of CD4 T cells develop in response to infection by different types of pathogens. T_H17 responses provide for the generation of acute inflammation at sites of infection by robust recruitment of neutrophils. T_H1 responses activate the phagocytic pathways to protect against intracellular pathogens. T_H2 responses are directed against infections by parasites such as helminths by promoting barrier and mucosal immunity, IgE production, and recruitment of eosinophils to sites of infection. CD8 T cells have an important role in protective immunity, especially in protecting the host against infection by viruses and intracellular infections by *Listeria* and other microbial pathogens that have special means for entering the host cell's cytoplasm. Primary CD8 T-cell responses to pathogens usually require CD4 T-cell help, but can occur in response to some pathogens without such help. CD4-independent responses can lead either to the generation and expansion of antigen-specific cytotoxic T cells or to the nonspecific activation of naive CD8 T cells to secrete IFN- γ , which in turn contributes to host protection. Ideally, the adaptive immune response eliminates the infectious agent and provides the host with a state of protective immunity against reinfection with the same pathogen.

Immunological memory.

Having considered how an appropriate primary immune response is mounted, we now turn to how long-lasting protective immunity is generated. The establishment of immunological memory is perhaps the most important consequence of an adaptive immune response, because it enables the immune system to respond more rapidly and effectively to pathogens that have been encountered previously, and prevents them from causing disease. Memory responses, which are called **secondary immune responses**, **tertiary immune responses**, and so on, depending on the number of exposures to antigen, also differ qualitatively from primary responses. This is particularly clear in the antibody response, in which the characteristics of antibodies produced in secondary and subsequent responses are distinct from those produced in the primary response to the same antigen. Memory T-cell responses can also be distinguished qualitatively from the responses of naive or effector T cells. The principal focus of this part of the chapter is the altered character of memory responses, although we also discuss emerging explanations of how immunological memory persists after exposure to antigen.

11-13 Immunological memory is long-lived after infection or vaccination.

Most children in developed countries are now vaccinated against measles virus; before vaccination was widespread, most were naturally exposed to this virus and developed an acute, unpleasant, and potentially dangerous illness. Whether through vaccination or infection, children exposed to the virus acquire long-term protection from measles, lasting for most people for the whole of their life. The same is true of many other acute infectious diseases (see Chapter 16): this state of protection is a consequence of immunological memory.

The basis of immunological memory has been hard to explore experimentally. Although the phenomenon was first recorded by the ancient Greeks and has been exploited routinely in vaccination programs for more than 200 years, it is only now becoming clear that this memory reflects a small population of specialized **memory cells** formed during the adaptive immune response that can persist in the absence of the antigen that originally induced them. This mechanism of maintaining memory is consistent with the finding that only individuals who were themselves previously exposed to a given infectious agent are immune, and that memory is not dependent on repeated exposure to infection as a result of contacts with other infected individuals. This was established by observations made of populations on remote islands, where a virus such as measles can cause an epidemic, infecting all people living on the island at that time, after which the virus disappears for many years. On reintroduction from outside the island, the virus does not affect the original population but causes disease in those people born since the first epidemic.

Studies have attempted to determine the duration of immunological memory by evaluating responses in people who received vaccinia, the virus used to immunize against smallpox (Fig. 11.17). Because smallpox was eradicated in 1978, it is presumed that their responses represent true immunological memory and are not due to restimulation from time to time by the smallpox virus. The study found strong vaccinia-specific CD4 and CD8 T-cell memory responses as long as 75 years after the original immunization, and from the strength of these responses it was estimated that the memory response had an approximate half-life of between 8 and 15 years. Half-life represents the time that a response takes to reduce to 50% of its original strength. Titers of antiviral antibody remained stable, without measurable decline.

These findings show that immunological memory need not be maintained by repeated exposure to infectious virus. Instead, it is likely that memory is sustained by long-lived antigen-specific lymphocytes that were induced by the original exposure and that persist until a second encounter with the pathogen. Although most of the memory cells are in a resting state, careful studies have shown that a small percentage of these cells are dividing at any one time. What stimulates this infrequent cell division is unclear, but it is likely that cytokines produced either constitutively or during antigen-specific immune responses directed at other, non-cross-reactive, antigens are responsible. The number of memory cells for a given antigen is highly regulated, remaining practically constant during the memory phase, which reflects a control mechanism that maintains a balance between cell proliferation and cell death.

Immunological memory can be measured experimentally in various ways. Adoptive transfer assays (see Appendix I, Section A-36) of lymphocytes from animals immunized with simple, nonliving antigens have been favored for such studies, because the antigen cannot proliferate. In these experiments, the existence of memory cells is measured purely in terms of the transfer of specific responsiveness from an immunized, or 'primed,' animal to a nonimmunized recipient, as tested by a subsequent immunization with the antigen. Animals that received memory cells have a faster and more robust response to

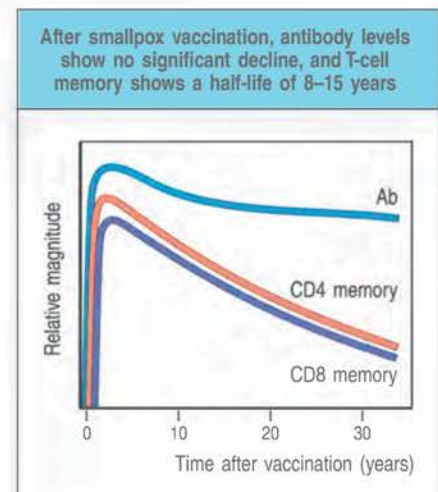


Fig. 11.17 Antiviral immunity after smallpox vaccination is long lived.

Because smallpox has been eradicated, recall responses measured in people who were vaccinated for smallpox can be taken to represent true memory in the absence of reinfection. After smallpox vaccination, antibody levels show an early peak with a period of rapid decay, which is followed by long-term maintenance that shows no significant decay. CD4 and CD8 T-cell memory is long-lived but gradually decays, with a half-life in the range 8–15 years.

antigen challenge than do controls that did not receive cells, or that received cells from nonimmune donors.

Experiments like these have shown that when an animal is first immunized with a protein antigen, functional helper T-cell memory against that antigen appears abruptly and reaches a maximum after 5 days or so. Functional antigen-specific B-cell memory appears some days later, then enters a phase of proliferation and selection in lymphoid tissue. By 1 month after immunization, memory B cells are present at their maximum level. These levels of memory cells are then maintained, with little alteration, for the lifetime of the animal. It is important to recognize that the functional memory elicited in these experiments can be due to the precursors of memory cells as well as the memory cells themselves. These precursors are probably activated T cells and B cells, some of whose progeny will later differentiate into memory cells. Thus, precursors to memory can appear very shortly after immunization, even though resting memory-type lymphocytes may not yet have developed.

In the following sections we look in more detail at the changes that occur in lymphocytes after antigen priming that lead to the development of resting memory lymphocytes, and discuss the mechanisms that might account for these changes.

11-14 Memory B-cell responses differ in several ways from those of naive B cells.

Immunological memory in B cells can be examined quite conveniently *in vitro* by isolating B cells from immunized or unimmunized mice and restimulating them with antigen in the presence of helper T cells specific for the same antigen (Fig. 11.18). B cells from immunized mice produce responses that differ both quantitatively and qualitatively compared with naive B cells from unimmunized mice. B cells that respond to the antigen increase in frequency by up to 100-fold after their initial priming in the primary immune response, and, as a result of the process of affinity maturation (see Section 10-8), produce antibody of higher affinity than unprimed B lymphocytes. The response observed from immunized mice is due to **memory B cells**, which we introduced in Section 11-9 as B cells that arise from the germinal center reaction. These cells populate the spleen and lymph nodes as well as circulating through the blood, and express some markers that distinguish them from naive B cells and plasma cells. In the human, a marker of memory B cells is CD27, a member of the TNF receptor family that is also expressed by naive T cells and binds the TNF-family ligand CD70, which is expressed by dendritic cells (see Section 9-13).

Fig. 11.18 The generation of secondary antibody responses from memory B cells is distinct from the generation of the primary antibody response.

These responses can be studied and compared by isolating B cells from immunized and unimmunized donor mice, and stimulating them in culture in the presence of antigen-specific effector T cells. The primary response usually consists of antibody molecules made by plasma cells derived from a quite diverse population of precursor B cells specific for different epitopes of the antigen and with receptors with a range of affinities for the antigen. The antibodies are of relatively low affinity overall, with few somatic mutations. The secondary response derives from a far more limited population of high-affinity B cells, which have, however, undergone significant clonal expansion. Their receptors and antibodies are of high affinity for the antigen and show extensive somatic mutation. The overall effect is that although there is usually only a 10–100-fold increase in the frequency of activatable B cells after priming, the quality of the antibody response is radically altered, in that these precursors induce a far more intense and effective response.

	Source of B cells	
	Unimmunized donor Primary response	Immunized donor Secondary response
Frequency of antigen-specific B cells	$1:10^4 - 1:10^5$	$1:10^2 - 1:10^3$
Isotype of antibody produced	IgM > IgG	IgG, IgA
Affinity of antibody	Low	High
Somatic hypermutation	Low	High

A primary antibody response is characterized by the initial rapid production of IgM, accompanied by an IgG response, due to class switching, which lags slightly behind it (Fig. 11.19). The secondary antibody response is characterized in its first few days by the production of relatively small amounts of IgM antibody and much larger amounts of IgG antibody, with some IgA and IgE. At the beginning of the secondary response, the source of these antibodies is memory B cells that were generated in the primary response and that have already switched from IgM to another isotypes, and express either IgG, IgA, or IgE on their surface, as well as a somewhat higher level of MHC class II molecules and B7.1 than is typical of naive B cells. The average affinity of IgG antibodies increases throughout the primary response and continues to increase during the ongoing secondary and subsequent responses (see Fig. 11.19). The higher affinity of memory B cells for antigen and their higher levels of cell-surface MHC class II molecules facilitate antigen uptake and presentation, which together with an increased expression of co-stimulatory molecules allows memory B cells to initiate their crucial interactions with helper T cells at lower doses of antigen than do naive B cells. This means that B-cell differentiation and antibody production start earlier after antigen stimulation than in the primary response. The secondary response is characterized by a more vigorous and earlier generation of plasma cells than in the primary response, thus accounting for the almost immediate abundant production of IgG (see Fig. 11.19).

The antibodies made in primary and secondary responses can be clearly distinguished in cases in which the primary response is dominated by antibodies that are closely related to each other and show little somatic hypermutation. This occurs in some inbred mouse strains in which certain haptens are recognized by a limited set of naive B cells. For example, in C57BL/6 mice, antibodies against the hapten nitrophenol (NP) are encoded by the same V_H (VH186.2) and V_L ($\lambda 1$) genes in all animals of the strain. As a result of this uniformity in the primary response, changes in the antibody molecules produced in secondary responses to the same antigens are easy to observe. These differences include not only numerous somatic hypermutations in antibodies containing the dominant V regions (see Fig. 5.24) but also the addition of antibodies containing V_H and V_L gene segments not detected in the primary response. These are thought to derive from B cells that were activated at low frequency during the primary response, and thus were not detected, and which differentiated into memory B cells.

11-15 Repeated immunization leads to increasing affinity of antibody due to somatic hypermutation and selection by antigen in germinal centers.

In secondary and subsequent immune responses, any antibodies persisting from previous responses are immediately available to bind the newly introduced pathogen. These antibodies divert the antigen to phagocytes for degradation and disposal (see Section 10-22), and if there is sufficient antibody to clear or inactivate the pathogen completely, it is possible that no secondary immune response will ensue. If the levels of pathogen overwhelm the amount of circulating antibody, excess antigens will bind to receptors on B cells and initiate a secondary B-cell response in the peripheral lymphoid organs. B cells with the highest avidity for antigen are the first to be recruited into this secondary response, and so memory B cells, which have already been selected for their avidity to antigen, make up a substantial part of the cells that contribute to the secondary response.

Secondary B-cell responses begin with the proliferation of B cells and T cells at the interface between the T-cell and B-cell zones, as in primary responses. Memory T cells reside in lymphoid tissues, but can also enter nonlymphoid tissues (see Section 11-6). Memory B cells, in contrast, continue to recirculate

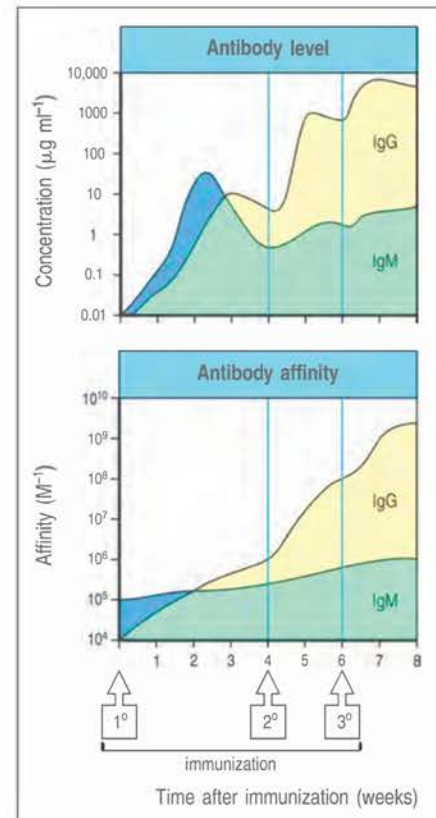


Fig. 11.19 Both the affinity and the amount of antibody increase with repeated immunization. The upper panel shows the increase in antibody concentration with time after a primary (1°), followed by a secondary (2°) and a tertiary (3°), immunization; the lower panel shows the increase in the affinity of the antibodies (affinity maturation). Affinity maturation is seen largely in IgG antibody (as well as in IgA and IgE, which are not shown) coming from mature B cells that have undergone isotype switching and somatic hypermutation to yield higher-affinity antibodies. The blue shading represents IgM on its own; the yellow shading IgG, and the green shading the presence of both IgG and IgM. Although some affinity maturation occurs in the primary antibody response, most arises in later responses to repeated antigen injections. Note that these graphs are on a logarithmic scale; it would otherwise be impossible to represent the overall increase of around a million-fold in the concentration of specific IgG antibody from its initial level.

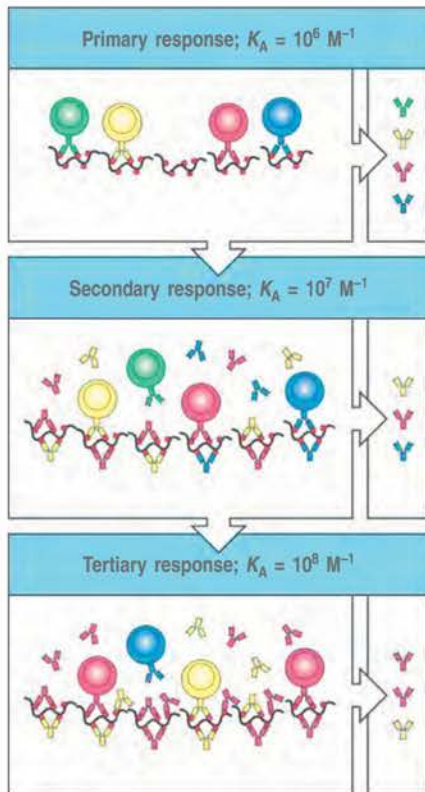


Fig. 11.20 The mechanism of affinity maturation in an antibody response. At the beginning of a primary response, B cells with receptors of a wide variety of affinities (K_A), most of which will bind antigen with low affinity, take up antigen, present it to helper T cells, and become activated to produce antibody of varying and relatively low affinity (top panel). These antibodies then bind and clear antigen, so that only those B cells with receptors of the highest affinity can continue to capture antigen and interact effectively with helper T cells. Such B cells will therefore be selected to undergo further expansion and clonal differentiation, and the antibodies they produce will dominate a secondary response (middle panel). These higher-affinity antibodies will in turn compete for antigen and select for the activation of B cells bearing receptors of still higher affinity in the tertiary response (bottom panel).

through the same secondary lymphoid compartments as naive B cells, principally the follicles of the spleen, lymph nodes, and the Peyer's patches of the gut mucosa. Memory B cells that have picked up antigen are able to present peptide:MHC class II complexes to their cognate helper T cells surrounding the follicle. Contact between the antigen-presenting B cells and helper T cells leads to the rapid proliferation of both the B cells and T cells. As the higher-affinity memory B cells compete most effectively for antigen, these B cells are most efficiently stimulated in the secondary immune response. Reactivated B cells that have not yet undergone differentiation into plasma cells migrate into the follicle and become germinal center B cells. There, they enter a second round of proliferation, during which the DNA encoding their immunoglobulin V domains undergoes somatic hypermutation, before differentiating into antibody-secreting plasma cells (see Section 10-8). The affinity of the antibodies produced rises progressively and rapidly, because B cells with the highest-affinity antigen receptors produced by somatic hypermutation bind antigen most efficiently, and will be selected to proliferate by their interactions with antigen-specific helper T cells in the germinal center (Fig. 11.20).

Memory B cells may not produce all antibodies in the secondary response. On secondary exposure to antigen, preexisting antibody can sometimes permit the formation of immune complexes, which do not form immediately in the primary response. Recent studies have shown that such immune complexes can bind to and activate signaling by Fc receptors on antigen-specific naive B cells, which acts to accelerate their kinetics of response. The formation of immune complexes, however, requires equivalent levels of antigen and antibody, which may not always occur, for example if antibody is present in great excess. Still, the shorter lag phase in antibody production in the secondary response may arise not only from the faster intrinsic responses of memory B cells, but also from accelerated naive B-cell responses if antigen:antibody complexes can form.

11-16 Memory T cells are increased in frequency compared with naive T cells specific for the same antigen, and have distinct activation requirements and cell-surface proteins that distinguish them from effector T cells.

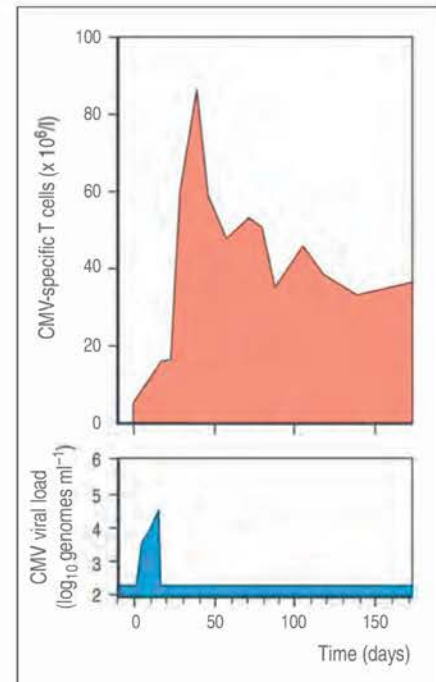
Because the T-cell receptor does not undergo class switching or somatic hypermutation, it is not as easy to identify a memory T cell unequivocally as it is to identify a memory B cell. After immunization, the number of T cells reactive to a given antigen increases markedly as effector T cells are produced, and then falls back to persist at a level 100–1000-fold above the initial frequency for the rest of the life of the animal or person (Fig. 11.21). These persisting cells are designated **memory T cells**. They are long-lived cells with a particular set of cell-surface proteins, responses to stimuli, and expression of genes that control cell survival. Overall, their cell-surface proteins are similar to those of effector cells, but there are some distinctive differences (Fig. 11.22). In B cells, there is an obvious distinction between effector and memory cells, because effector B cells are terminally differentiated plasma cells that have already been activated to secrete antibody until they die.

A major problem in experiments aimed at establishing the existence of memory T cells is that many assays for T-cell effector function take several days, during which the putative memory T cells are reinduced to effector cell status. Thus, assays requiring several days do not distinguish preexisting effector cells from memory T cells, because memory cells can acquire effector activity during the period of the assay. This problem does not apply to cytotoxic T cells, however, because cytotoxic effector T cells can program a target cell for lysis in 5 minutes, whereas memory CD8 T cells need more time

Fig. 11.21 Generation of memory T cells after a virus infection. After an infection, in this case a reactivation of latent cytomegalovirus (CMV), the number of T cells specific for viral antigen increases dramatically and then falls back to give a sustained low level of memory T cells. The upper panel shows the numbers of T cells (orange); the lower panel shows the course of the virus infection (blue), as estimated by the amount of viral DNA in the blood. Data courtesy of G. Aubert.

than this to be reactivated to become cytotoxic. Thus, their cytotoxic actions will appear later than those of any preexisting effector cells, even though they can become activated without undergoing DNA synthesis, as shown by studies conducted in the presence of mitotic inhibitors.

Recently it has become possible to track particular clones of antigen-specific CD8 T cells by staining them with tetrameric peptide:MHC complexes (see Appendix I, Section A-28). It has been found that the number of antigen-specific CD8 T cells increases markedly during an infection, and then decreases by up to 100-fold; nevertheless, this final level is distinctly higher than before priming. These cells continue to express some markers characteristic of activated cells, such as CD44, but stop expressing other activation markers, such



Protein	Naive	Effector	Memory	Comments
CD44	+	+++	+++	Cell-adhesion molecule
CD45RO	+	+++	+++	Modulates T-cell receptor signaling
CD45RA	+++	+	+++	Modulates T-cell receptor signaling
CD62L	+++	-	Some +++	Receptor for homing to lymph node
CCR7	+++	+/-	Some +++	Chemokine receptor for homing to lymph node
CD69	-	+++	-	Early activation antigen
Bcl-2	++	+/-	+++	Promotes cell survival
Interferon- γ	-	+++	+++	Effector cytokine; mRNA present and protein made on activation
Granzyme B	-	+++	+/-	Effector molecule in cell killing
FasL	-	+++	+	Effector molecule in cell killing
CD122	+/-	++	++	Part of receptor for IL-15 and IL-2
CD25	-	++	-	Part of receptor for IL-2
CD127	++	-	+++	Part of receptor for IL-7
Ly6C	+	+++	+++	GPI-linked protein
CXCR4	+	+	++	Receptor for chemokine CXCL12; controls tissue migration
CCR5	+/-	++	Some +++	Receptor for chemokines CCL3 and CCL4; tissue migration

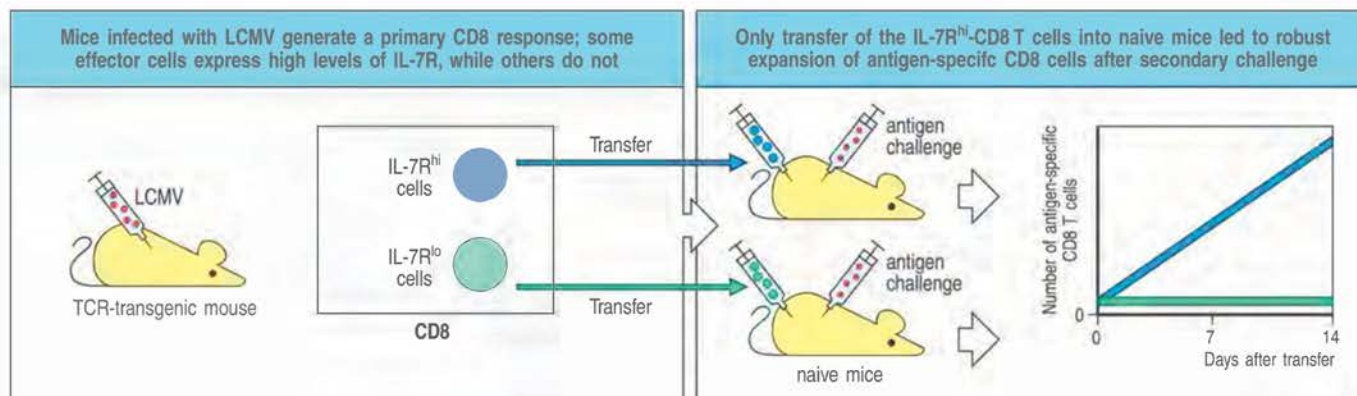
Fig. 11.22 Expression of many proteins alters when naive T cells become memory T cells. Proteins that are differently expressed in naive T cells, effector T cells, and memory T cells include adhesion molecules, which govern interactions with antigen-presenting cells and endothelial cells; chemokine receptors, which affect migration to lymphoid tissues and sites of inflammation; proteins and receptors that promote the survival of memory cells; and proteins that are involved in effector functions, such as granzyme B. Some changes also increase the sensitivity of the memory T cell to antigen stimulation. Many of the changes that occur in memory T cells are also seen in effector cells, but some, such as expression of the cell-surface proteins CD25 and CD69, are specific to effector T cells; others, such as expression of the survival factor Bcl-2, are limited to long-lived memory T cells. This list represents a general picture that applies to both CD4 and CD8 T cells in mice and humans, but some details that may differ between these sets of cells have been omitted for simplicity.

Fig. 11.23 Expression of the IL-7 receptor (IL-7R) indicates which CD8 effector T cells can generate robust memory responses. Mice expressing a T-cell receptor (TCR) transgene specific for a viral antigen from lymphocytic choriomeningitis virus (LCMV) were infected with the virus and effector cells were collected on day 11. Effector CD8 T cells expressing high levels of IL-7R (IL-7R^{hi}, blue) were separated and transferred into one group of naive mice, and effector CD8 T cells expressing low IL-7R (IL-7R^{lo}, green) were transferred into another group. Three weeks after transfer, the mice were challenged with a bacterium engineered to express the original viral antigen, and the numbers of responding transferred T cells (detected by their expression of the transgenic TCR) were measured at various times after challenge. Only the transferred IL-7R^{hi} effector cells could generate a robust expansion of CD8 T cells after the secondary challenge.

as CD69. In addition they express more Bcl-2, a protein that promotes cell survival and may be responsible for the long half-life of memory CD8 cells.

The α subunit of the IL-7 receptor (IL-7R α or CD127) may be a good marker for activated T cells that will become long-lived memory cells (see Fig. 11.22). Naive T cells express IL-7R α , but it is rapidly lost upon activation and is not expressed by most effector T cells. For example, during the peak of the effector response against lymphocytic choriomeningitis virus (LCMV) in mice, around day 7 of the infection, a small population of approximately 5% of CD8 effector T cells expressed high levels of IL-7R α . Adoptive transfer of these cells, but not the effector T cells expressing low levels of IL-7R α , could provide functional CD8 T-cell memory to uninfected mice (Fig. 11.23). This experiment suggests that the early maintenance, or the reexpression, of IL-7R α identifies effector CD8 T cells that generate memory T cells, although it is still not known whether, and how, this process is regulated. Memory T cells are more sensitive to restimulation by antigen than are naive T cells, and more quickly and more vigorously produce several cytokines such as IFN- γ , TNF- α , and IL-2 in response to such stimulation. A similar progression occurs for T cells in humans after immunization with a vaccine against yellow fever virus.

CD4 T-cell memory has been more difficult to study, in part because their responses are smaller than those of CD8 T cells and also because, until recently, there were no peptide:MHC class II reagents similar to the peptide:MHC class I tetramers. Nevertheless, the transfer and priming of naive T cells carrying T-cell receptor transgenes that give the T cells a known peptide:MHC specificity has made it possible to visualize memory CD4 T cells. They appear as a long-lived population of cells that share some surface characteristics of activated effector T cells but are distinct from effector T cells in that they require additional restimulation before acting on target cells. Changes in three cell-surface proteins—L-selectin, CD44, and CD45—that occur on the putative memory CD4 T cells after exposure to antigen are particularly significant. L-selectin is lost on most memory CD4 T cells, whereas CD44 levels are increased on all memory T cells; these changes contribute to directing the migration of memory T cells from the blood into the tissues rather than directly into lymphoid tissue. The isoform of CD45 changes because of alternative splicing of exons that encode the extracellular domain of CD45, leading to isoforms, such as CD45RO, that are smaller and more readily associated with the T-cell receptor and facilitate antigen recognition (see Fig. 11.22). These changes are characteristic of cells that have been activated to become effector T cells, yet some of the cells on which these changes have occurred have many characteristics of resting CD4 T cells, suggesting that they represent memory CD4 T cells. Only after reexposure to antigen on an antigen-presenting cell do they achieve effector T-cell status and acquire all the characteristics of T_H2 or T_H1 cells, secreting IL-4 and IL-5, or IFN- γ , respectively.



It therefore seems reasonable to designate these cells as memory CD4 T cells and to surmise that naive CD4 T cells differentiate into effector T cells, some of which then become memory cells. As with memory CD8 T cells, direct staining of CD4 T cells with peptide:MHC class II tetramers can identify antigen-specific CD4 T cells, and analysis using intracellular cytokine staining (see Appendix I, Section A-27) can determine whether they are T_H1 , T_H17 , or T_H2 cells. These improvements in the identification and phenotyping of CD4 T cells will rapidly increase our knowledge in this area and could contribute valuable comparative information on naive, memory, and effector CD4 T cells.

The homeostatic mechanisms governing the survival of memory T cells differ from those for naive T cells. Memory T cells divide more frequently than naive T cells, and their expansion is controlled by a balance between proliferation and cell death. As with naive cells, the survival of memory T cells requires stimulation by the cytokines IL-7 and IL-15. IL-7 is required for the survival of both CD4 and CD8 memory T cells, but in addition, IL-15 is critical for the long-term survival and proliferation of CD8 memory T cells under normal conditions. For memory CD4 T cells, the role of IL-15 is still under investigation.

In addition to cytokine stimulation, naive T cells also require contact with self-peptide:self-MHC complexes for their long-term survival in the periphery (see Section 8-29), but it seems that memory T cells do not have this requirement. It has been found, however, that memory T cells surviving after transfer to MHC-deficient hosts have some defects in typical T-cell memory functions, indicating that stimulation by self-peptide:self-MHC complexes may be required for their continued proliferation and optimal function (Fig. 11.24).

11-17 Memory T cells are heterogeneous and include central memory and effector memory subsets.

CD4 and CD8 T cells can differentiate into two types of memory cells with distinct activation characteristics (Fig. 11.25). One type is called an **effector memory cell** because it can rapidly mature into an effector T cell and secrete large amounts of IFN- γ , IL-4, and IL-5 early after restimulation. These cells lack the chemokine receptor CCR7 but express high levels of β_1 and β_2 integrins, as well as receptors for inflammatory chemokines. This profile suggests that these effector memory cells are specialized for rapidly entering inflamed tissues. The other type is called a **central memory cell**. It expresses CCR7 and would therefore be expected to recirculate more easily to the T-cell zones of peripheral lymphoid tissues, as do naive T cells. Central memory cells are very sensitive to cross-linking of their T-cell receptors and rapidly express CD40 ligand in response; however, they take longer than effector memory cells to differentiate into effector T cells and thus do not secrete such large amounts of cytokines early after restimulation.

The distinction between central memory cells and effector memory cells has been made both in humans and in the mouse. However, this general

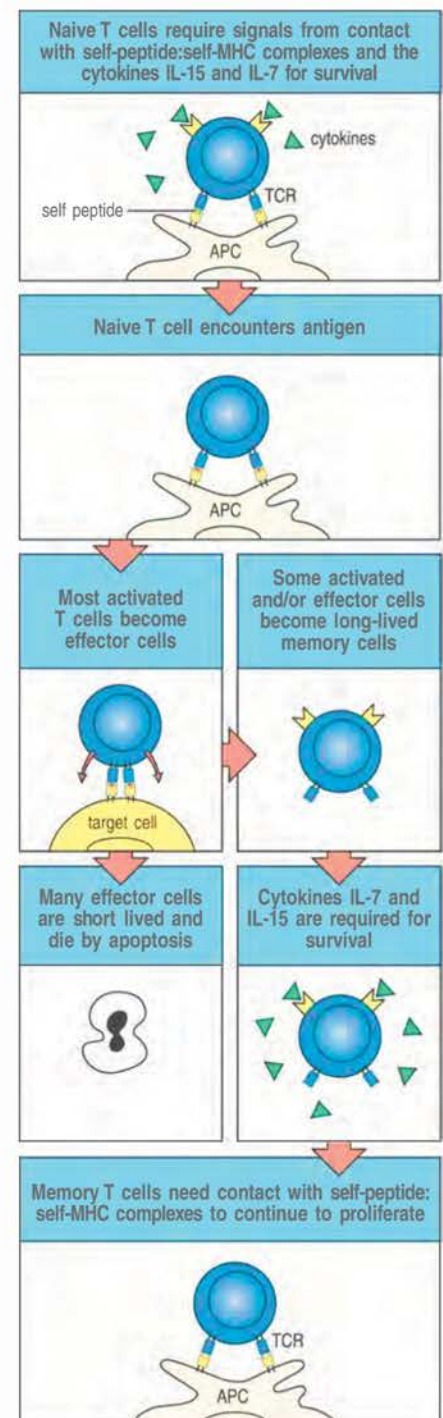
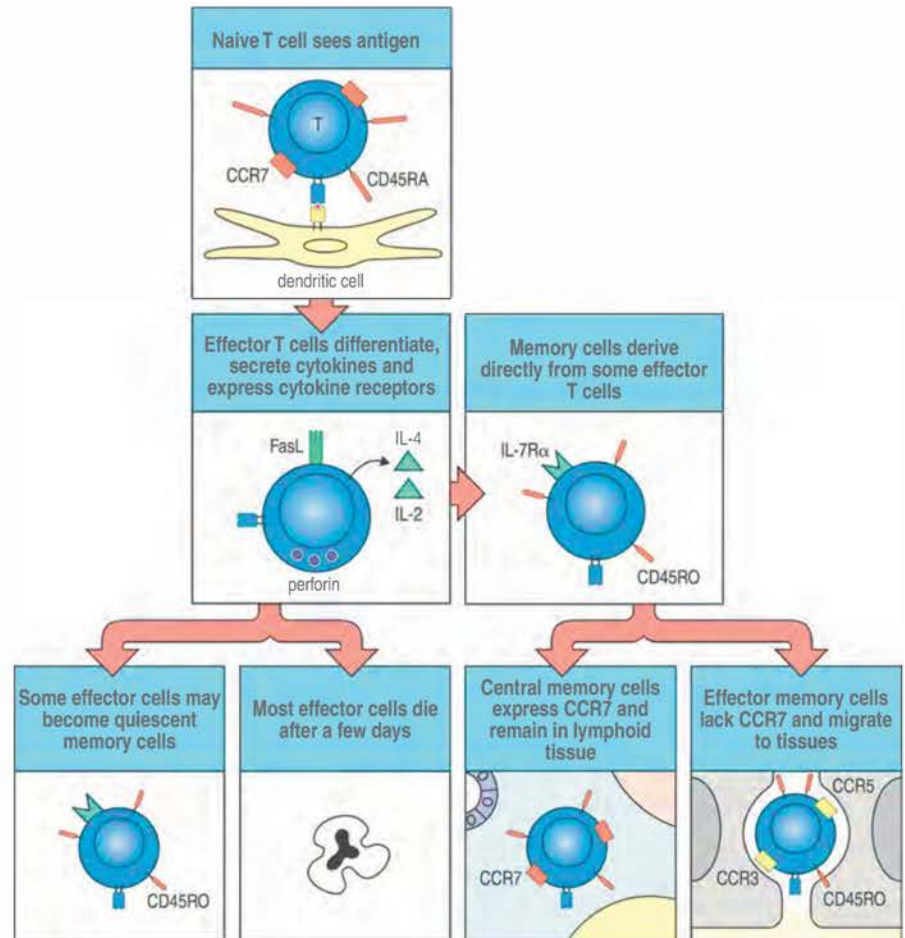


Fig. 11.24 Naive T cells and memory T cells have different requirements for survival.

For their survival in the periphery, naive T cells require periodic stimulation with the cytokines IL-7 and IL-15 and with self antigens presented by MHC molecules. On priming with its specific antigen, a naive T cell divides and differentiates. Most of the progeny differentiate into relatively short-lived effector cells, but some effector cells become long-lived memory T cells, which need to be sustained by cytokines but do not require contact with self-peptide:self-MHC complexes purely for survival. However, contact with self antigens does seem necessary for memory T cells to continue to proliferate and thus keep up their numbers in the memory pool.

Fig. 11.25 T cells differentiate into central memory and effector memory subsets distinguished by expression of the chemokine receptor CCR7.

Quiescent memory cells bearing the characteristic CD45RO surface protein can arise from activated effector cells (right half of diagram) or directly from activated naive T cells (left half of diagram). Two types of quiescent memory T cells can derive from the primary T-cell response. Central memory cells express CCR7 and remain in peripheral lymphoid tissues after restimulation. Memory cells of the other type—effector memory cells—mature rapidly into effector T cells after restimulation, and secrete large amounts of IFN- γ , IL-4, and IL-5. They do not express the receptor CCR7, but express receptors (CCR3 and CCR5) for inflammatory chemokines.



distinction does not imply that each subset is a uniform population. Within the CCR7-expressing central memory subset there are extensive differences in the expression of other markers, particularly receptors for other chemokines. For example, a subset of the CCR7-positive central memory cells also expresses CXCR5, similarly to T_H cells, although it is not yet clear whether these memory cells can provide help to B cells in the germinal center.

On stimulation by antigen, central memory cells rapidly lose expression of CCR7 and differentiate into effector memory cells. Effector memory cells are also heterogeneous in the chemokine receptors they express, and have been classified according to chemokine receptors typical of T_H1 cells, such as CCR5, and of T_H2 cells, such as CCR4. Central memory cells are not yet committed to particular effector lineages, and even effector memory cells are not fully committed to the T_H1 or the T_H2 lineage, although there is some correlation between their eventual output of T_H1 or T_H2 cells and the chemokine receptors expressed. Further stimulation with antigen seems to drive the differentiation of effector memory cells gradually into the distinct effector T-cell lineages.

11-18 CD4 T-cell help is required for CD8 T-cell memory and involves CD40 and IL-2 signaling.

In Section 11-8 we saw how primary CD8 T-cell responses to *Listeria monocytogenes* can occur in mice that lack CD4 T cells. After 7 days of infection, wild-type mice and mice lacking CD4 T cells both showed equivalent expansion and activity of pathogen-specific CD8 effector T cells. However, they are not equally able to generate memory CD8 T cells. Mice that lack CD4 T cells as

the result of a deficiency in MHC class II were found to generate much weaker secondary responses, characterized by many fewer expanding memory CD8 T cells specific for the pathogen. In this experiment, the *Listeria* carried a gene for the protein ovalbumin, and it was the response to this protein that was measured as a marker for CD8 T-cell memory (Fig. 11.26). CD4 T cells in these mice are lacking both during the primary response and in any secondary challenge, so the requirement for CD4 T cells could be either in the initial programming of CD8 T cells during their primary activation to enable memory development, or, alternatively, in providing help only during the secondary memory response.

Other experiments indicate that this CD4 T-cell help is necessary for programming naive CD8 T cells to be able to generate memory cells capable of robust secondary expansion. Memory CD8 T cells that developed in the absence of CD4 help were transferred into wild-type mice. After transfer, the recipient mice were challenged again. In mice that received CD8 memory T cells that developed without CD4 T-cell help, the CD8 T cells showed a reduced ability to proliferate even though the recipient mice expressed MHC class II. This result indicates that CD4 T-cell help is required during the priming of CD8 T cells and not simply at the time of secondary responses. This requirement for CD4 help in CD8 memory generation has also been demonstrated by experiments in which CD4 T cells were depleted by treatment with antibody or in which mice were deficient in the CD4 gene.

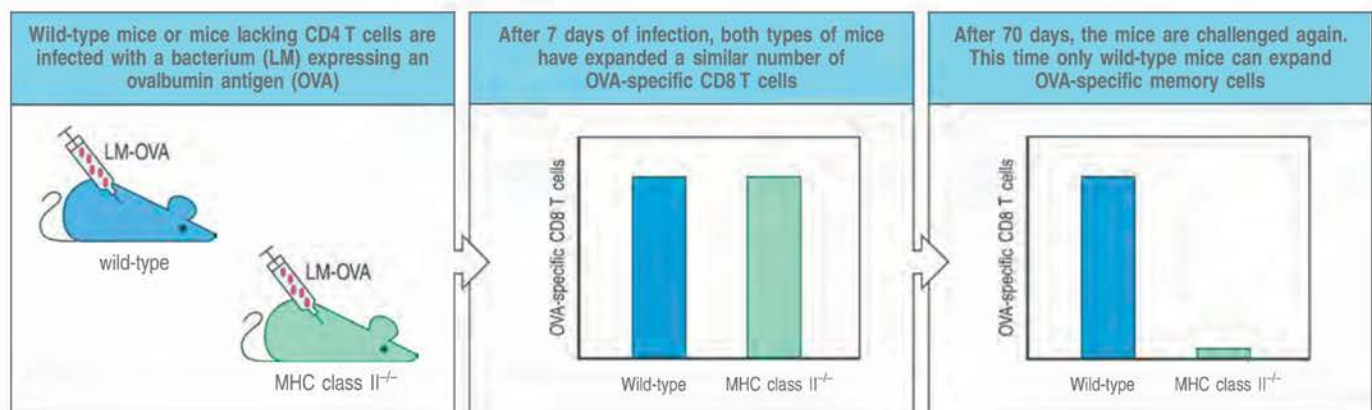
The mechanism underlying this requirement for CD4 T cells is not completely understood. It may involve two types of signals received by the CD8 T cell—those received through CD40 and those received through the IL-2 receptor. CD8 T cells that do not express CD40 are unable to generate memory T cells. Although many cells could potentially express the CD40 ligand needed to stimulate CD40, it is most likely that CD4 T cells are the source of this signal.

The requirement for IL-2 signaling in programming CD8 memory was discovered by using CD8 T cells with a genetic deficiency in the IL-2R α subunit, which were therefore unable to respond to IL-2. Because IL-2R α signaling is required for the development of T_{reg} cells, mice lacking IL-2R α develop a lymphoproliferative disorder. However, this disorder does not develop in mice that are mixed bone marrow chimeras harboring both wild-type and IL-2R α -deficient cells, and these chimeras can be used to study the behavior of IL-2R α -deficient cells. When these chimeric mice were infected with LCMV and their responses were tested, memory CD8 responses were found to be defective specifically in the T cells lacking IL-2R α .

CD4 T cells also provide help in maintaining the numbers of CD8 memory T cells that is distinct from their effect in programming naive CD8 T cells to become memory cells (Fig. 11.27). When CD8 memory T cells are transferred

Fig. 11.26 CD4 T cells are required for the development of functional CD8 memory T cells.

Mice that do not express MHC class II molecules (MHC II^{-/-}) fail to develop CD4 T cells. Wild-type and MHC II^{-/-} mice were infected with *Listeria monocytogenes* expressing the model antigen ovalbumin (LM-OVA). After 7 days, the numbers of OVA-specific CD8 T cells can be measured by using specific MHC tetramers that contain an OVA peptide, and therefore bind to T-cell receptors that react with this antigen. After 7 days of infection, mice lacking CD4 T cells have the same number of OVA-specific CD8 T cells as wild-type mice do. However, when mice are allowed to recover for 60 days, during which time memory T cells develop, and are then rechallenged with LM-OVA, the mice lacking CD4 T cells fail to expand CD8 memory cells specific to OVA, whereas there is a strong CD8 memory response in the wild-type mice.



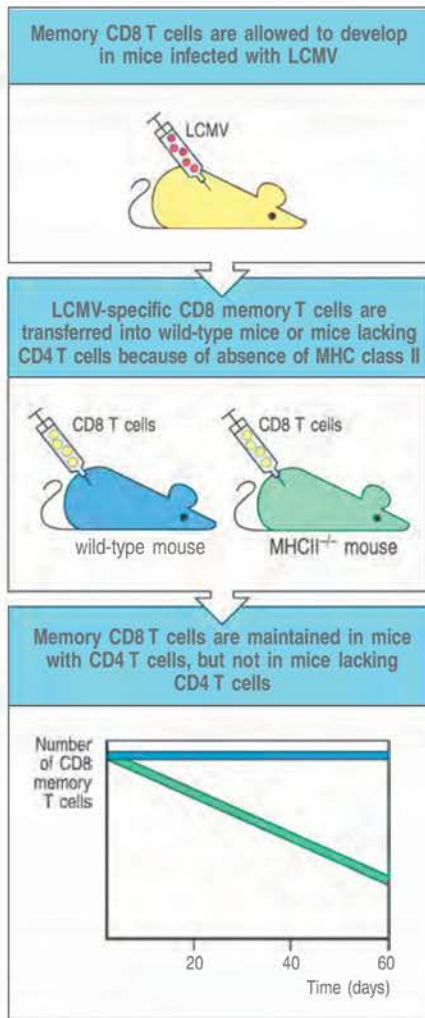


Fig. 11.27 CD4 T cells promote the maintenance of CD8 memory cells.

The dependence of memory CD8 T cells on CD4 T cells is shown by the different lifetimes after transfer into host mice that either have normal CD4 T cells (wild-type), or lack CD4 T cells (MHC II^{-/-}). In the absence of MHC class II proteins, CD4 T cells fail to develop in the thymus. When CD8 memory T cells specific for LCMV were isolated from donor mice 35 days after infection with the virus and transferred into these hosts, memory cells were maintained only in mice that had CD4 T cells. The basis for this action of CD4 T cells is not yet clear, but has implications for conditions such as HIV-AIDS in which numbers of CD4 T cells are diminished.

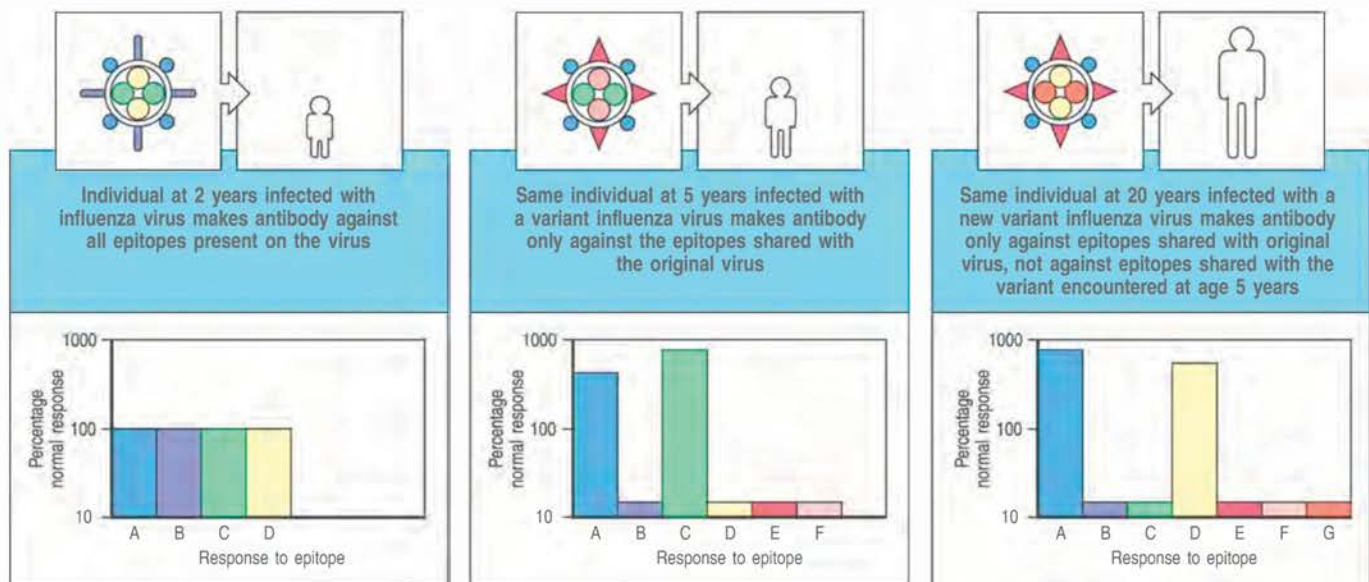
into immunologically naive mice, the presence or absence of CD4 T cells in the recipient influences the maintenance of the CD8 memory cells. Transfer of CD8 memory cells into mice lacking CD4 T cells is followed by a gradual decrease in the number of memory cells in comparison with a similar transfer into wild-type mice. In addition, CD8 effector cells transferred into mice lacking CD4 T cells had a relative impairment of CD8 effector functions. These experiments show that the CD4 T cells activated during an immune response have a significant impact on the quantity and quality of the CD8 T-cell response, even when they are not needed for the initial CD8 T-cell activation. CD4 T cells help to program naive CD8 T cells to be able to generate memory T cells, help to promote efficient effector activity, and help to maintain memory T-cell numbers.

11-19 In immune individuals, secondary and subsequent responses are mainly attributable to memory lymphocytes.

In the normal course of an infection, a pathogen proliferates to a level sufficient to elicit an adaptive immune response and then stimulates the production of antibodies and effector T cells that eliminate the pathogen from the body. Most of the effector T cells then die, and antibody levels gradually decline, because the antigens that elicited the response are no longer present at the level needed to sustain it. We can think of this as feedback inhibition of the response. Memory T and B cells remain, however, and maintain a heightened ability to mount a response to a recurrence of infection with the same pathogen.

Antibody and memory lymphocytes remaining in an immunized individual can have the effect of reducing the activation of naive B and T cells on a subsequent encounter with the same antigen. In fact, passively transferring antibody to a naive recipient can be used to inhibit naive B cell responses to that same antigen. This phenomenon has been put to practical use to prevent Rh⁻ mothers from making an immune response to a Rh⁺ fetus, which can result in hemolytic disease of the newborn (see Appendix I, Section A-11). If anti-Rh antibody is given to the mother before she is first exposed to her child's Rh⁺ red blood cells, her response will be inhibited. The mechanism of this suppression is likely to involve the antibody-mediated clearance and destruction of fetal red blood cells that have entered the mother, thus preventing naive B cells and T cells from mounting an immune response. Presumably, anti-Rh antibody is in excess over antigen, so that not only is antigen eliminated, but immune complexes are not formed to stimulate naive B cells through Fc receptors. Memory B-cell responses are, however, not inhibited by antibody, so the Rh⁻ mothers at risk must be identified and treated before a primary response has occurred. Because of their high affinity for antigen and alterations in their B-cell receptor signaling requirements, memory B cells are much more sensitive to the small amounts of antigen that cannot be efficiently cleared by the passive anti-Rh antibody. The ability of memory B cells to be activated to produce antibody, even when exposed to preexisting antibody, also allows secondary antibody responses to occur in individuals who are already immune.

These suppressive mechanisms might also explain the phenomenon known as **original antigenic sin**. This term was coined to describe the tendency of people to make antibodies only against epitopes expressed on the first influenza virus variant to which they are exposed, even in subsequent infections with variants that bear additional, highly immunogenic, epitopes (Fig. 11.28). Antibodies against the original virus will tend to suppress responses of naive B cells specific for the new epitopes. This might benefit the host by using only those B cells that can respond most rapidly and effectively to the virus. This pattern is broken only if the person is exposed to an influenza virus that lacks



all epitopes seen in the original infection, because now no preexisting antibodies bind the virus, and naive B cells are able to respond.

A similar suppressive effect of antigen-specific memory T cells on naive T-cell responses has been observed in some settings, such as infection by lymphocytic choriomeningitis virus (LCMV) in the mouse or dengue virus in humans. For example, mice that were primed with one strain of LCMV responded to a subsequent infection with a variant LCMV by using CD8 T cells directed against antigens specific for the first, rather than the new, variant. However, this effect was not observed when responses to variable ovalbumin antigenic epitopes were examined in the setting of recurrent infections using the bacterial pathogen *Listeria monocytogenes*, suggesting that original antigenic sin may not be a universal occurrence in all immune responses.

Summary.

Protective immunity against reinfection is one of the most important consequences of adaptive immunity. Protective immunity depends not only on preformed antibody and effector T cells but most importantly on the establishment of a population of lymphocytes that mediate long-lived immunological memory. The capacity of memory cells to respond rapidly to restimulation with the same antigen can be transferred to naive recipients by primed B and T cells. The precise changes that distinguish naive, effector, and memory lymphocytes include the regulation of expression of receptors for cytokines, such as IL-7 and IL-15, that help to maintain these cells, and the regulation of chemokine receptors, such as CCR7, that distinguish between functional subsets of memory cells. The advent of receptor-specific reagents—MHC tetramers—has allowed an analysis of the relative contributions of clonal expansion and differentiation to the memory phenotype. Memory B cells can be distinguished by changes in their immunoglobulin genes because of isotype switching and somatic hypermutation, and secondary and subsequent immune responses are characterized by antibodies with increasing affinity for the antigen. As for T-cell memory, there is a complex interplay between CD4 and CD8 T cells that is only partly understood. Although CD8 T cells can generate effective primary responses in the absence of help from CD4 T cells, it is becoming clear that CD4 T cells have an integral role in regulating CD8 T-cell memory. These issues will be critical in understanding, for example, how to design effective vaccines for diseases such as HIV/AIDS.

Fig. 11.28 When individuals who have been infected with one variant of influenza virus are infected with a second or third variant, they make antibodies only against epitopes that were present on the initial virus. A child infected for the first time with an influenza virus at 2 years of age makes a response to all epitopes (left panel). At age 5 years, the same child exposed to a different influenza virus responds preferentially to those epitopes shared with the original virus, and makes a smaller than normal response to new epitopes on the virus (center panel). Even at age 20 years, this commitment to respond to epitopes shared with the original virus, and the subnormal response to new epitopes, is retained (right panel). This phenomenon is called 'original antigenic sin.'

Summary to Chapter 11.

Vertebrates resist infection by pathogenic microorganisms in several ways. The innate defenses can act immediately and may succeed in repelling the infection, but if not they are followed by a series of induced early responses that help to contain the infection as adaptive immunity develops. These first two phases of the immune response rely on recognizing the presence of infection by using the nonclonotypic receptors of the innate immune system. They are summarized in Fig. 11.29 and covered in detail in Chapter 3. Specialized subsets of lymphocytes, which can be viewed as intermediates between innate and adaptive immunity, include iNKT cells, which can help to bias the CD4 T-cell response toward a T_H1 or a T_H2 phenotype, and NK cells, which can be recruited to lymph nodes and secrete IFN- γ , and thus promote a T_H1 response. The third phase of an immune response is the adaptive immune response (see Fig. 11.29), which is mounted in the peripheral lymphoid tissue that serves the particular site of infection and takes several days to develop, because T and B lymphocytes must encounter their specific antigen, proliferate, and differentiate into effector cells. T-cell dependent B-cell responses cannot be initiated until antigen-specific T_{FH} cells have had a chance to proliferate and differentiate. Once an adaptive immune response has occurred, the antibodies and effector T cells are dispersed via the circulation and recruited into the infected tissues; the infection is usually controlled and the pathogen is contained or eliminated. The final effector mechanisms used to clear an infection depend on the type of infectious agent, and in most cases they are the same as those employed in the early phases of immune defense; only the recognition mechanism changes and is more selective (see Fig. 11.29).

An effective adaptive immune response leads to a state of protective immunity. This state consists of the presence of effector cells and molecules produced in the initial response, and of immunological memory. Immunological memory

Fig. 11.29 The components of the three phases of the immune response against different classes of microorganisms. The mechanisms of innate immunity that operate in the first two phases of the immune response are described in Chapters 2 and 3, and thymus-independent (T-independent) B-cell responses are covered in Chapter 10. The early phases contribute to the initiation of adaptive immunity, and they influence the functional character of the antigen-specific effector T cells and antibodies that appear on the scene in the late phase of the response. There are striking similarities in the effector mechanisms at each phase of the response; the main change is in the recognition structure used.

	Phases of the immune response		
	Immediate (0–4 hours)	Early (4–96 hours)	Late (96–100 hours)
	Nonspecific Innate No memory No specific T cells	Nonspecific + specific Inducible No memory No specific T cells	Specific Inducible Memory Specific T cells
Barrier functions	Skin, epithelia, mucins, acid	Local inflammation (C5a) Local TNF- α	IgA antibody in luminal spaces IgE antibody on mast cells Local inflammation
Response to extracellular pathogens	Phagocytes Alternative and MBL complement pathway Lysozyme Lactoferrin Peroxidase Defensins	Mannan-binding lectin C-reactive protein T-independent B-cell antibody Complement	IgG antibody and Fc receptor-bearing cells IgG, IgM antibody + classical complement pathway
Response to intracellular bacteria	Macrophages	Activated NK-dependent macrophage activation IL-1, IL-6, TNF- α , IL-12	T-cell activation of macrophages by IFN- γ
Response to virus-infected cells	Natural killer (NK) cells	IFN- α and IFN- β IL-12-activated NK cells	Cytotoxic T cells IFN- γ

is manifested as a heightened ability to respond to pathogens that have previously been encountered and successfully eliminated. Memory T and B lymphocytes have the property of being able to transfer immune memory to naive recipients. The mechanisms that maintain immunological memory include certain cytokines, such as IL-7 and IL-15, as well as homeostatic interactions between the T cell receptors on memory cells with self-peptide:self-MHC complexes. The artificial induction of protective immunity, which includes immunological memory, by vaccination is the most outstanding accomplishment of immunology in the field of medicine. The understanding of how this is accomplished is now catching up with its practical success. However, as we will see in Chapter 13, many pathogens do not induce protective immunity that completely eliminates the pathogen, so we will need to learn what prevents this before we can prepare effective vaccines against these pathogens.

Questions.

- 11.1 *Communication is critical in any large enterprise. (a) How is the body alerted to an invasion by microbes, and (b) how does it ensure that its responses reach the site of infection?*
- 11.2 *The immune system responds to particular classes of pathogens in different ways. What properties of viruses and bacteria are used to induce T_H1 responses to them, and what host cells provide the information about the type of pathogen present?*
- 11.3 *Differentiated T cells require continued signals to maintain their function. (a) What signals do T_H1 cells need? (b) What advantages might the requirement for continued signals have? What disadvantages?*
- 11.4 *One could question the need for immunological memory. Invertebrates get by without adaptive immunity or memory. After all, if you survive the first infection without memory, you should be able to survive it the second time without memory. And if you fail to survive the first infection, memory is of no help. (a) What are the advantages of immunological memory that counter this argument? What features of pathogens might have driven the evolution of immunological memory? (b) Innate immune responses seem to lack memory, but may be augmented for a time after infection. What features of immunological memory provided by adaptive immunity are of greater value than simply an augmented innate response? In what way could these features be a disadvantage? Give an example.*
- 11.5 *Memory responses differ from primary immune responses in several important properties. Name three ways in which they differ, and describe the underlying mechanism(s) involved in each case.*
- 11.6 *(a) Discuss the relative roles of cytokine signals and signals received through the T-cell receptor in the survival and function of memory T cells. (b) Compare and contrast their requirements and responses to such signals with those of naive T cells.*
- 11.7 *You are swimming in freshwater and are infected by a parasite that entered your body through your skin. Outline how your body would generate an immune response against this pathogen. Would the response be T_H1 or T_H2 polarized? Include all relevant cell types and molecules that would be involved.*

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