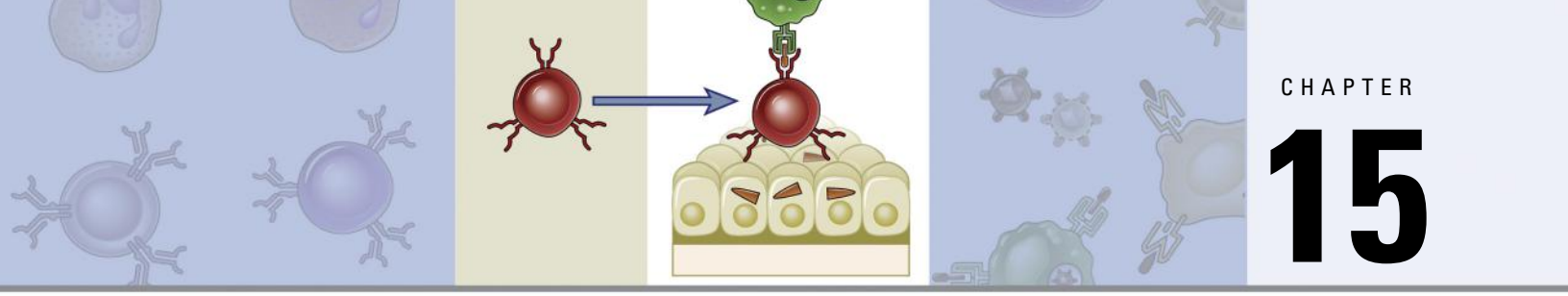

CHAPTER

15

Immunologic Tolerance and Autoimmunity

Immunologic tolerance is defined as unresponsiveness to an antigen that is induced by previous exposure to that antigen. The term arose from the experimental observation that animals that had encountered an antigen under particular conditions would not respond to, or would tolerate, subsequent exposures to the same antigen. When specific lymphocytes encounter antigens, the lymphocytes may be activated, leading to immune responses, or the cells may be inactivated or eliminated, leading to tolerance. Different forms of the same antigen may induce an immune response or tolerance. Antigens that induce tolerance are called tolerogens, or tolerogenic antigens, to distinguish them from immunogens, which generate immunity. A single antigen may be an immunogen or a tolerogen, depending on whether it is displayed to specific lymphocytes in the presence or absence, respectively, of inflammation and innate immune responses. Tolerance to self antigens, also called **self-tolerance**, is a fundamental property of the normal immune system, and failure of self-tolerance results in immune reactions against self (autologous) antigens. Such reactions are called **autoimmunity**, and the diseases they cause are called **autoimmune diseases**. The importance of self-tolerance for the health of individuals was appreciated from the early days of immunology. In Chapter 1, we introduced the concept of self–non-self discrimination, which is the ability of the immune system to recognize and respond to foreign antigens but not to self antigens. Macfarlane Burnet added to his clonal selection hypothesis the corollary that lymphocytes specific for self antigens are eliminated to prevent immune reactions against one's own tissues. Elucidating the mechanisms of self-tolerance is the key to understanding the pathogenesis of autoimmunity.

In this chapter, we will discuss immunologic tolerance mainly in the context of self-tolerance and how self-tolerance may fail, resulting in autoimmunity. We will also consider tolerance to foreign antigens and the potential of tolerance induction as a therapeutic strategy for allergic and autoimmune diseases and to prevent the rejection of cell and organ transplants.

OVERVIEW OF IMMUNOLOGIC TOLERANCE

There are several characteristics of tolerance in T and B lymphocyte populations. It is important to appreciate the general principles before we discuss the specific mechanisms of tolerance in these lymphocytes.

- ***Normal individuals are tolerant of their own (self) antigens because the lymphocytes that recognize self antigens are killed or inactivated or the specificity of these lymphocytes is changed.*** All individuals inherit essentially the same antigen receptor gene segments, and these recombine and are expressed in lymphocytes as the cells arise from precursor cells. The specificities of the receptors encoded by the recombined genes are random, and are not influenced by what is foreign or self for each individual (see Chapter 8). It is not surprising that during this process of generating a large and diverse repertoire, some developing T and B cells in every individual may express receptors capable of recognizing normal molecules in that individual (i.e., self antigens). Therefore, there is a risk

for lymphocytes to react against that individual's cells and tissues, causing disease. The mechanisms of immunologic tolerance have evolved to prevent such reactions.

- ***Tolerance results from the recognition of antigens by specific lymphocytes.*** In other words, tolerance, by definition, is antigen specific. This contrasts with therapeutic immunosuppression, which affects lymphocytes of many specificities. The key advance that allowed immunologists to study tolerance was the ability to induce this phenomenon in animals by exposure to defined antigens under various conditions and to then analyze the survival and functions of the lymphocytes that had encountered the antigens. Peter Medawar and colleagues showed in the 1950s that neonatal mice of one strain exposed to cells from other strains became unresponsive to subsequent skin grafts from the donor strain. Later studies showed that tolerance could be induced not only to foreign cells but also to proteins and other antigens.
- ***Self-tolerance may be induced in immature self-reactive lymphocytes in the generative lymphoid organs (central tolerance) or in mature lymphocytes in peripheral sites (peripheral tolerance)*** (Fig. 15-1). Central tolerance ensures that the repertoire of mature lymphocytes becomes incapable of responding to self antigens that are expressed in the generative lymphoid organs (the thymus for T cells and the bone marrow for B lymphocytes, also called central lymphoid organs). However, central tolerance is not perfect, and some self-reactive lymphocytes do complete their maturation. Therefore, the mechanisms of peripheral tolerance are needed to prevent activation of these potentially dangerous lymphocytes.
- ***Central tolerance occurs during a stage in the maturation of lymphocytes when encounter with antigen may lead to cell death or replacement of a self-reactive antigen receptor with one that is not self-reactive.*** The generative lymphoid organs contain mostly self antigens and not foreign antigens because foreign (e.g., microbial) antigens that enter from the external environment are typically captured and taken to peripheral lymphoid organs, such as the lymph nodes, spleen, and mucosal lymphoid tissues, and are not concentrated in the thymus or bone

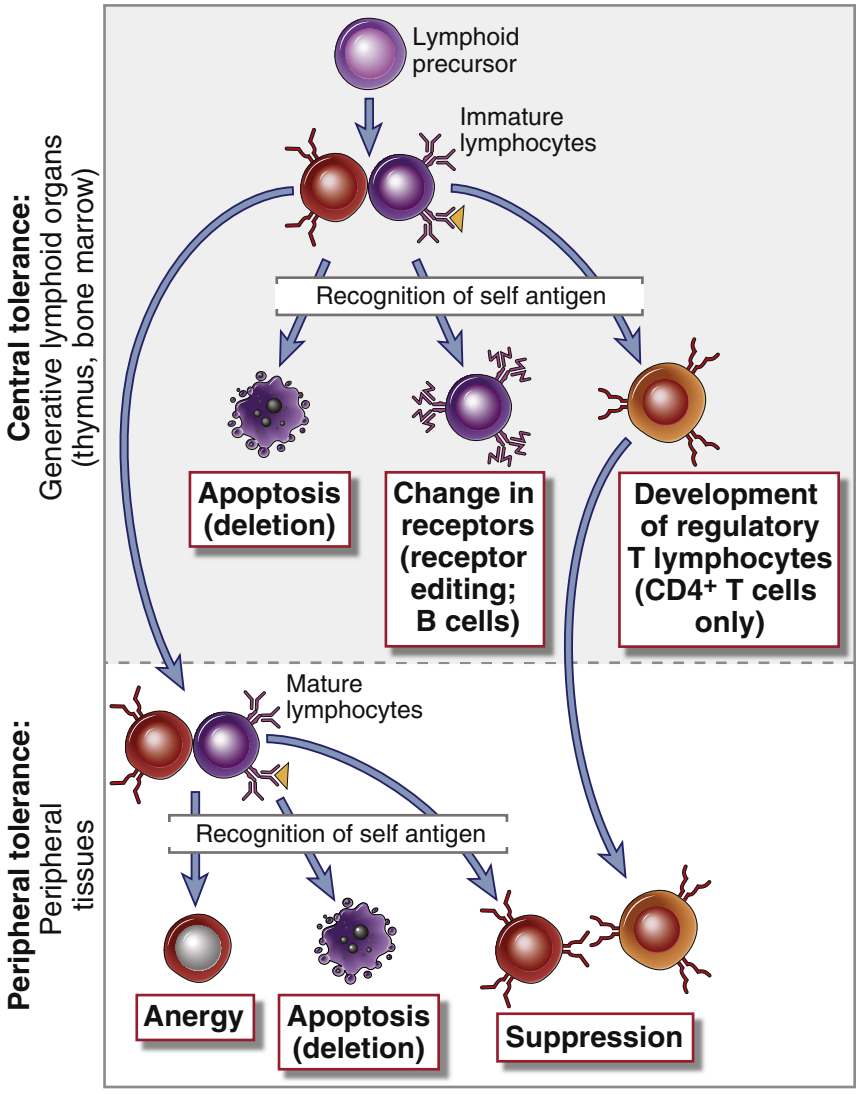

FIGURE 15-1 Central and peripheral tolerance to self antigens. Immature lymphocytes specific for self antigens may encounter these antigens in the generative (central) lymphoid organs and are deleted, change their specificity (B cells only), or (in the case of $CD4^+$ T cells) develop into regulatory lymphocytes (central tolerance). Some self-reactive lymphocytes may mature and enter peripheral tissues and may be inactivated or deleted by encounter with self antigens in these tissues or are suppressed by the regulatory T cells (peripheral tolerance). Note that T cells recognize antigens presented by antigen-presenting cells *(not shown)*.

marrow. The antigens normally present in the thymus and bone marrow include ubiquitous, or widely disseminated, self antigens including those bought in by the blood. In addition, many peripheral tissue–specific antigens are expressed in the thymus by a special mechanism that is described later. Therefore, in the generative lymphoid organs, the immature lymphocytes that specifically recognize antigens are typically cells specific for self, and not foreign, antigens. The fates of immature lymphocytes that recognize self antigens with high affinity are described later (see Fig. 15-1).

- ***Peripheral tolerance is induced when mature lymphocytes recognize self antigens and die by apoptosis, or become incapable of activation by re-exposure to that antigen.*** Peripheral tolerance is important for maintaining unresponsiveness to self antigens that are expressed in peripheral tissues and not in the generative lymphoid organs and for tolerance to self antigens that are expressed only in adult life, after many mature lymphocytes specific for these antigens may have already been generated. As mentioned earlier, peripheral mechanisms may also serve as a backup for the central mechanisms, which do not eliminate all self-reactive lymphocytes.
- ***Peripheral tolerance is also maintained by regulatory T cells (Treg) that actively suppress self antigen-specific lymphocytes.*** Treg suppression occurs in secondary lymphoid organs and in nonlymphoid tissues.
- ***Some self antigens are sequestered from the immune system, and other antigens are ignored.*** Antigens may be sequestered from the immune system by anatomic barriers, such as in the testes and eyes, and thus cannot engage antigen receptors (see Chapter 14). In experimental models, some self antigens are available for recognition by lymphocytes but, for unknown reasons, fail to elicit any response and are functionally ignored. The importance of this phenomenon of ignorance for the maintenance of self-tolerance is not established.
- ***Foreign antigens in the absence of costimulatory signals may inhibit immune responses by inducing tolerance in specific lymphocytes.*** Many of the mechanisms of tolerance to foreign antigens are similar to those of self-tolerance in mature lymphocytes (peripheral tolerance). Some microbes and tumors also evade immune attack by inducing unresponsiveness in specific lymphocytes.
- ***The induction of immunologic tolerance has been exploited as a therapeutic approach for preventing harmful immune responses.*** A great deal of effort is being devoted to the development of strategies for inducing tolerance to treat autoimmune and allergic diseases and to prevent the rejection of organ transplants. Tolerance induction may also be useful for preventing immune reactions to the products of newly expressed genes in gene therapy protocols, for preventing reactions to injected proteins in patients with deficiencies of these proteins (e.g., hemophiliacs treated with factor VIII), and for promoting acceptance of stem cell transplants.

Experimental approaches, especially the creation of genetically modified mice, have provided valuable models for analysis of self-tolerance, and many of our current concepts are based on studies with such models. Furthermore, by identifying genes that may be associated with autoimmunity in mice and humans, it has been possible to deduce some of the critical mechanisms of self-tolerance. However, we do not know which self antigens induce central or peripheral tolerance (or are ignored). More importantly, it is also not known which tolerance mechanisms might fail in common human autoimmune diseases, and this remains a major challenge in understanding autoimmunity.

In the sections that follow, we will discuss central and peripheral tolerance first in T cells and then in B lymphocytes, but many aspects of the processes are common to both lineages.

T LYMPHOCYTE TOLERANCE

Tolerance in $CD4^+$ helper T lymphocytes is an effective way of preventing both cell-mediated and humoral immune responses to protein antigens because helper T cells are necessary inducers of all such responses. This realization has been the impetus for a large amount of work on the mechanisms of tolerance in $CD4^+$ T cells. Immunologists have developed experimental models for studying tolerance in $CD4^+$ T cells that have proved to be informative. Also, many of the therapeutic strategies that are being developed to induce tolerance to transplants and autoantigens are aimed at inactivating or eliminating these T cells. Therefore, much of the following discussion, especially of peripheral tolerance, focuses on $CD4^+$ T cells. Less is known about peripheral tolerance in $CD8^+$ T cells, and this is summarized at the end of the section.

Central T Cell Tolerance

During their maturation in the thymus, many immature T cells that recognize antigens with high avidity are deleted, and some of the surviving cells in the $CD4^+$ lineage develop into regulatory T cells (Fig. 15-2). The process of **deletion**, or **negative selection**, of T lymphocytes in the thymus was described in Chapter 8, in the discussion of T cell maturation. This process affects both class I and class II MHC–restricted T cells and is therefore important for tolerance in both $CD8^+$ and $CD4^+$ lymphocyte populations. Negative selection of thymocytes is responsible for the fact that the repertoire of mature T cells that leave the thymus and populate peripheral lymphoid tissues is unresponsive to many self antigens that are present in the thymus. The two main factors that determine if a particular self antigen will induce negative selection of self-reactive thymocytes are the presence of that antigen in the thymus, either by local expression or delivery by the blood, and the affinity of the thymocyte T cell receptors (TCRs) that recognize the antigen. Thus, the important questions that are relevant to negative selection are what self antigens are present in the thymus and how immature T cells that recognize these antigens are deleted.

Negative selection occurs in double-positive T cells in the thymic cortex and newly generated single-positive T cells in the medulla. In both locations, immature thymocytes with

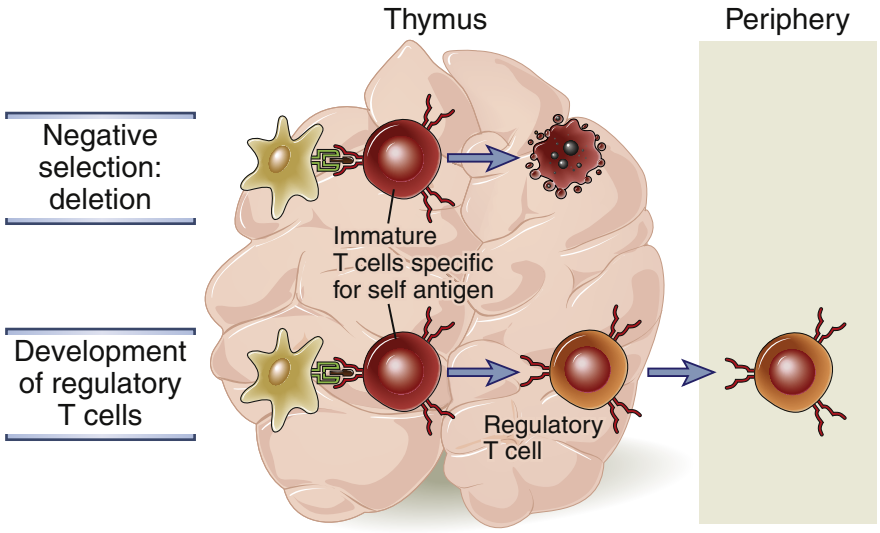

FIGURE 15-2 Central T cell tolerance. Recognition of self antigens by immature T cells in the thymus leads to the death of the cells (negative selection, or deletion) or to the development of regulatory T cells that enter peripheral tissues.

high-affinity receptors for self antigens that encounter these antigens die by apoptosis. T cell receptor (TCR) signaling in immature T cells triggers the mitochondrial pathway of apoptosis. The mechanisms of apoptosis are described later in this chapter, when we discuss deletion as a mechanism of peripheral T cell tolerance. Clearly, immature and mature lymphocytes interpret antigen receptor signals differently—the former die, and the latter are activated. The biochemical basis of this difference is not known.

The antigens that are present in the thymus include many circulating and cell-associated proteins that are widely distributed in tissues. The thymus also has a special mechanism for expressing many protein antigens that are typically present only in certain peripheral tissues, so that immature T cells specific for these antigens can be deleted from the developing T cell repertoire. These peripheral tissue antigens are expressed in thymic medullary epithelial cells under the control of the **autoimmune regulator (AIRE)** protein. Mutations in the *AIRE* gene are the cause of a multiorgan autoimmune disease called **autoimmune polyendocrine syndrome type 1 (APS1).** This group of diseases is characterized by antibody- and lymphocyte-mediated injury to multiple endocrine organs, including the parathyroids, adrenals, and pancreatic islets. A mouse model of APS1 has been developed by knockout of the *AIRE* gene, and it recapitulates many of the features of the human disease. Studies with mice have shown that several proteins that are produced in peripheral organs (such as pancreatic insulin) are also expressed at low levels in medullary thymic epithelial cells, and immature T cells that recognize these antigens are deleted in the thymus. In the absence of functional AIRE (as in APS1 patients and knockout mice) these antigens are not displayed in the thymus, and T cells specific for the antigens escape deletion, mature, and enter the periphery, where they attack the target tissues in which the antigens are expressed independent of AIRE (Fig. 15-3). The AIRE protein may function as a transcriptional regulator to promote the expression of selected tissue-restricted antigens in the thymus. It is a component of a multiprotein complex that is involved in transcriptional elongation and chromatin unwinding and remodeling. How AIRE drives expression of a wide range of tissue antigens in one cell population in the thymus is still not known.

Some self-reactive CD4⁺ T cells that see self antigens in the thymus are not deleted but instead differentiate into regulatory T cells specific for these antigens (see Fig. 15-2). The regulatory cells leave the thymus and inhibit responses against self antigens in the periphery. What determines the choice between deletion and development of regulatory T cells is not known. Possible factors include the affinity of antigen recognition, the types of antigen-presenting cells (APCs) presenting the antigen, and the availability of certain cytokines locally in the thymus. We will describe the characteristics and functions of regulatory T cells later in the context of peripheral tolerance because these cells suppress immune responses in the periphery.

Peripheral T Cell Tolerance

The mechanisms of peripheral tolerance are anergy (functional unresponsiveness), suppression by regulatory T cells, and deletion (cell death) (Fig. 15-4). These mechanisms may be responsible for T cell tolerance to tissue-specific self antigens, especially those that are not abundant in the thymus. We do not know if tolerance to different self antigens is maintained by one or another mechanism or if all of these mechanisms function cooperatively to prevent autoimmunity. The same mechanisms may induce unresponsiveness to tolerogenic forms of foreign antigens.

Anergy (Functional Unresponsiveness)

Exposure of mature CD4⁺ T cells to an antigen in the absence of costimulation or innate immunity may make the cells incapable of responding to that antigen. In this process, which is called anergy, the self-reactive cells do not die but become unresponsive to the antigen. We previously introduced the concept that full activation of T cells requires the recognition of antigen by the TCR (which provides signal 1) and recognition of costimulators, mainly B7-1 and B7-2, by CD28 (signal 2) (see Chapter 9). Prolonged signal 1

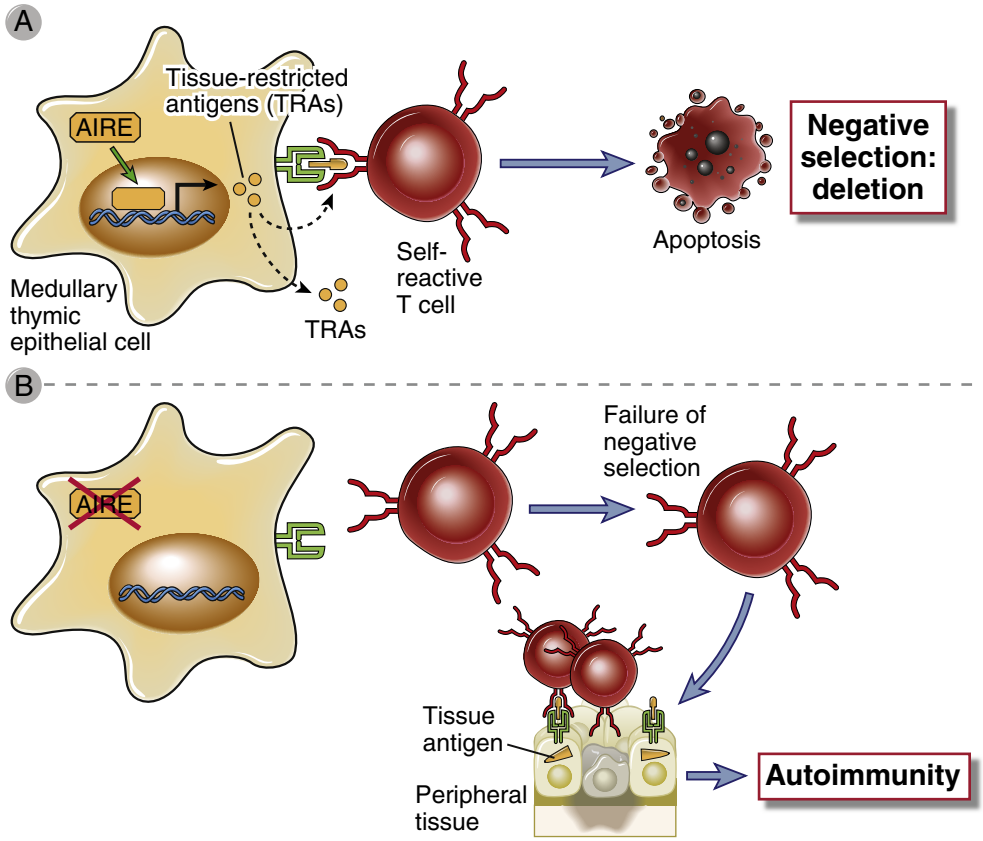

FIGURE 15-3 The function of AIRE in deletion of T cells in the thymus. A, The AIRE protein is part of a complex that regulates the expression of tissue-restricted antigens (TRAs) in medullary thymic epithelial cells (MTEC). Peptides derived from these antigens are displayed on the MTEC and recognized by immature antigen-specific T cells, leading to the deletion of many self-reactive T cells. **B,** In the absence of functional AIRE, these self-reactive T cells are not eliminated; they can enter tissues where the antigens continue to be produced and cause injury.

(i.e., antigen recognition) alone may lead to anergy. It is likely that self antigens are continuously displayed to specific T cells in the absence of innate immunity and strong costimulation. Antigen-induced anergy has been demonstrated in a variety of experimental models, including studies with T cell clones exposed to antigens in vitro (which were the basis for the original definition of anergy), experiments in which antigens are administered to mice without adjuvants, and studies with transgenic mice in which particular protein antigens are expressed throughout life and are recognized by T cells in the absence of the inflammation and innate immune responses that normally accompany exposure to microbes. In many of these situations, the T cells that recognize the antigens become functionally unresponsive and survive for days or weeks in a quiescent state.

Anergy results from biochemical alterations that reduce the ability of lymphocytes to respond to signals from their antigen receptors (Fig. 15-5). It is believed that several biochemical pathways cooperate to maintain this unresponsive state.

- ***TCR-induced signal transduction is blocked in anergic cells.*** The mechanisms of this signaling block are not fully known. In different experimental models, it is attributable to decreased TCR expression (perhaps because of increased degradation; see later) and recruitment to the TCR complex of inhibitory molecules such as tyrosine phosphatases.
- ***Self antigen recognition may activate cellular ubiquitin ligases, which ubiquitinate TCR-associated proteins and target them for proteolytic degradation in proteasomes or lysosomes.*** The net result is loss of these signaling molecules and defective T cell activation (see Chapter 7, Fig. 7-22). One ubiquitin ligase that is important in T cells is called Cbl-b. Mice in which Cbl-b is knocked out show spontaneous T cell proliferation and manifestations of autoimmunity, suggesting that this enzyme is involved in maintaining T cell unresponsiveness to self antigens. It is not known why self antigen recognition, which occurs typically without strong costimulation, activates these ubiquitin ligases, whereas foreign antigens that are recognized with costimulation do so much less or not at all.
- ***When T cells recognize self antigens, they may engage inhibitory receptors of the CD28 family, whose function is to terminate T cell responses.*** The functions of the best known inhibitory receptors of T cells are described in the section that follows.

Regulation of T Cell Responses by Inhibitory Receptors

In Chapter 9, we introduced the general concept that the outcome of antigen recognition by T cells, particularly $CD4^+$ cells, is determined by a balance between engagement of

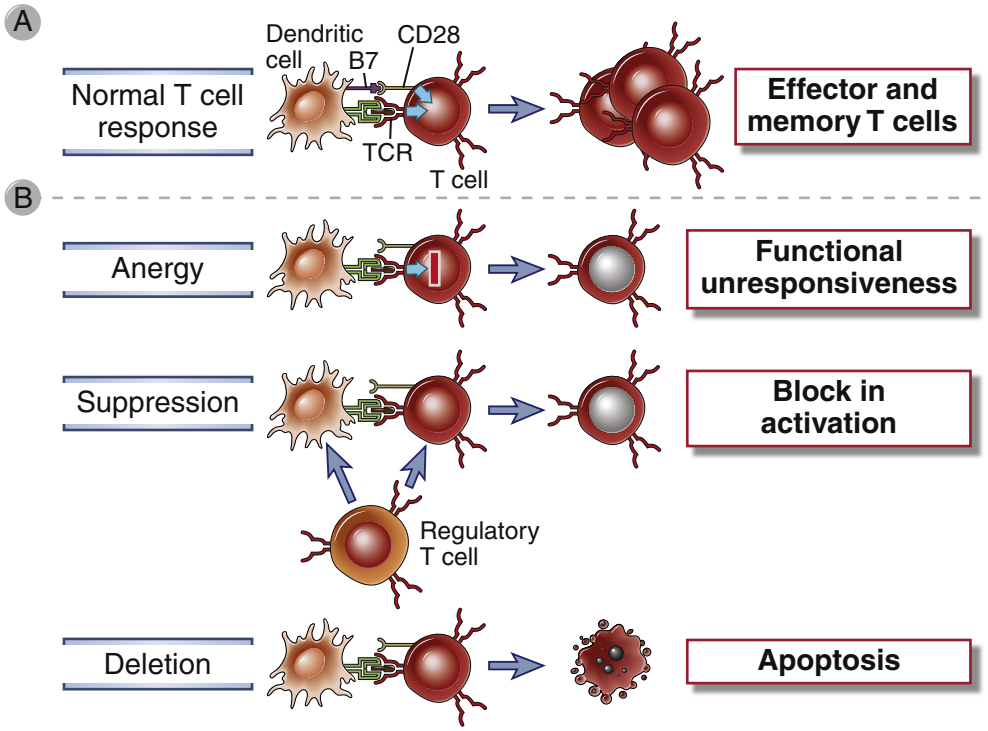

FIGURE 15-4 Mechanisms of peripheral T cell tolerance. The signals involved in a normal immune response **(A)** and the three major mechanisms of peripheral T cell tolerance **(B)** are illustrated.

activating and inhibitory receptors. Although many inhibitory receptors have been described, the two whose physiologic role in self-tolerance is best established are CTLA-4 and PD-1. Studies of these inhibitory receptors have increased our understanding of tolerance mechanisms and led to new therapeutic approaches for manipulating immune responses. The functions and mechanisms of action of these receptors are discussed next.

CTLA-4. CTLA-4 is a member of the CD28 receptor family (see Fig. 9-5) and, like the activating receptor CD28, it binds to B7 molecules. The importance of CTLA-4 in tolerance induction is illustrated by the finding that knockout mice lacking CTLA-4 develop uncontrolled lymphocyte activation with massively enlarged lymph nodes and spleen and fatal multiorgan lymphocytic infiltrates suggestive of systemic autoimmunity. In other words, elimination of this one control mechanism results in failure of peripheral tolerance and a severe T cell–mediated disease. Blocking of CTLA-4 with antibodies also enhances autoimmune diseases in animal models, such as encephalomyelitis induced by immunization with myelin antigens and diabetes induced by T cells reactive with antigens in the β cells of pancreatic islets. Polymorphisms in the *CTLA4* gene are associated with several autoimmune diseases in humans, including type 1 diabetes and Graves' disease. All of these findings, as well as results of clinical trials discussed below, indicate that CTLA-4 functions continuously to keep self-reactive T cells in check.

CTLA-4 has two important actions:

- CTLA-4 expression is low on most T cells until the cells are activated by antigen, and once expressed CTLA-4 terminates continuing activation of these responding T cells.
- CTLA-4 is expressed on regulatory T cells, described later, and mediates the suppressive function of these cells by inhibiting the activation of naive T cells.

CTLA-4 is thought to mediate its inhibitory activity by two main mechanisms (Fig. 15-6):

- ***Signaling block.*** Engagement of CTLA-4 by B7 activates a phosphatase, which removes phosphates from TCR- and CD28-associated signaling molecules and thus terminates responses.
- ***Reducing the availability of B7.*** CTLA-4, especially on regulatory T cells, binds to B7 molecules on APCs and blocks them from binding to CD28. It also captures B7 molecules and endocytoses them, thus reducing their expression on APCs. The net result is that the level of B7 on APCs available to bind CD28 is reduced, and the deficiency of costimulation results in a reduced T cell response.

It is still not clear what determines if CD28 will engage B7 molecules to activate T cells (as in infections or immunization with adjuvants) or if CTLA-4 will bind to B7 to block T cell responses (e.g., when self antigens are being presented). In Chapter 9, we discussed the hypothesis that CTLA-4, having a higher affinity for B7 than CD28, is preferentially engaged when APCs are presenting self antigens and expressing little B7. In contrast, microbes increase B7 expression and tilt the balance towards CD28 engagement and T cell activation. Other possibilities are that CD28, which is expressed on naive cells, binds B7 at the initiation of a T cell response, whereas CTLA-4, which is expressed after T cells are activated, functions to terminate these responses.

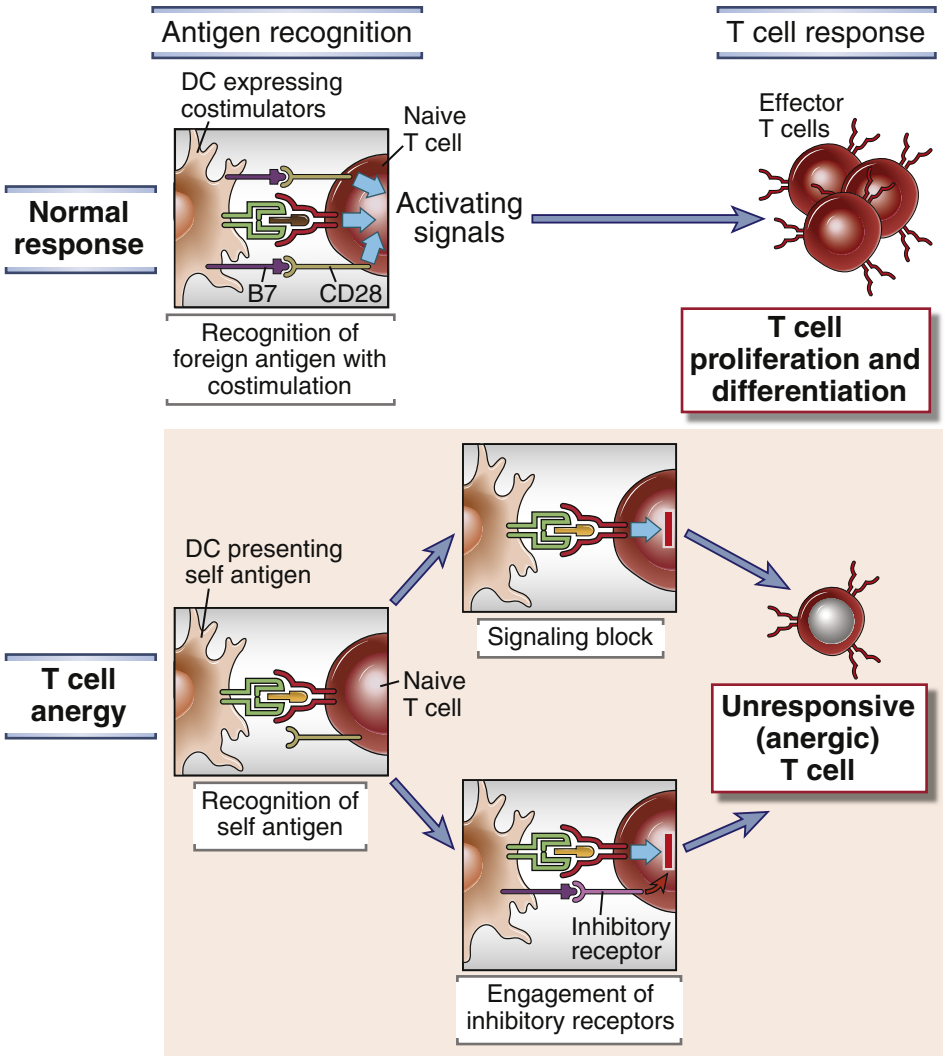

FIGURE 15-5 Mechanisms of T cell anergy. T cell responses are induced when the cells recognize an antigen presented by a professional antigen-presenting cell (APC) and activating receptors on the T cells (such as CD28) recognize costimulators on the APCs (such as B7). If the T cell recognizes a self antigen without costimulation, the T cell becomes unresponsive to the antigen because of a block in signaling from the TCR complex or engagement of inhibitory receptors (such as CTLA-4 and PD-1). The signaling block may be the result of recruitment of phosphatases to the TCR complex or the activation of ubiquitin ligases that degrade signaling proteins. The T cell remains viable but is unable to respond to the self antigen. *DC,* dendritic cell.

The realization that CTLA-4 sets checkpoints in immune responses has led to the idea that lymphocyte activation can be promoted by reducing inhibition, a process known as checkpoint blockade. Blocking CTLA-4 with antibodies results in increased immune responses to tumors (see Chapter 18). Anti-CTLA-4 antibody is now approved for the treatment of advanced melanomas, and it is effective in other cancers as well. Predictably, some of the treated patients develop manifestations of autoimmunity with inflammation in various organs.

PD-1. Another inhibitory receptor of the CD28 family is PD-1 (programmed cell death 1, so called because it was originally thought to be involved in programmed cell death but now is known not to have a role in T cell apoptosis). PD-1 recognizes two ligands, called PD-L1 and PD-L2; PD-L1 is expressed on APCs and many other tissue cells, and PD-L2 is expressed mainly on APCs. Engagement of PD-1 by either ligand leads to inactivation of the T cells. Mice in which PD-1 is knocked out develop autoimmune diseases, including a lupus-like kidney disease and arthritis in different inbred strains. The autoimmune disorders in PD-1 knockout mice are less severe than in CTLA-4 knockouts. PD-1 inhibits T cell responses to antigen stimulation, presumably by inducing inhibitory signals in the T cells. Checkpoint blockade with anti-PD-1 and anti-PD-L1 antibodies is showing even more efficacy and less toxicity than with anti-CTLA-4 in several cancers (see Chapter 18).

Although CTLA-4 and PD-1 are both inhibitory receptors of the same family, their functions may not overlap. CTLA-4 may be more important for controlling the initial activation of $CD4^+$ T cells in lymphoid organs and is a mediator of the suppressive function of regulatory T cells, whereas PD-1 is clearly important in terminating the peripheral responses of effector T cells, especially $CD8^+$ cells, and may not be required for the function of regulatory T cells. Also, several other inhibitory receptors have been identified, including some belonging to the TNF-receptor family and others to the TIM family. There is great interest in defining the role of these receptors in self-tolerance and the regulation of immune responses and the potential of targeting these molecules therapeutically.

Suppression by Regulatory T Cells

The concept that some lymphocytes could control the responses of other lymphocytes was proposed many years

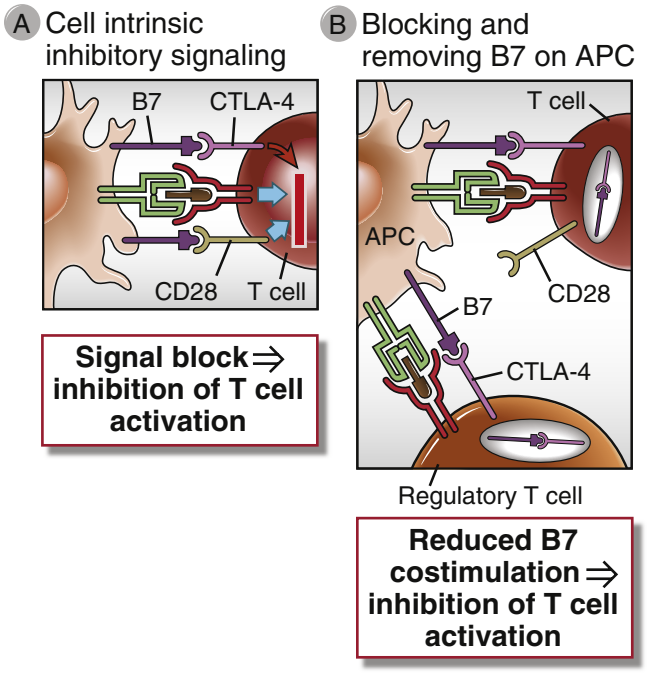

FIGURE 15-6 Mechanisms of action of CTLA-4. A, Engagement of CTLA-4 on a T cell may deliver inhibitory signals that terminate further activation of that cell (cell-intrinsic function of CTLA-4). **B,** CTLA-4 on regulatory or responding T cells binds to B7 molecules on APCs or removes these molecules from the surface of the APCs, making the B7 costimulators unavailable to CD28 and blocking T cell activation. CTLA-4–mediated inhibition by regulatory T cells is a cell-extrinsic action of this inhibitory receptor (since the responding T cells are suppressed by another cell).

ago and was soon followed by experimental demonstrations of populations of T lymphocytes that suppressed immune responses. These initial findings led to enormous interest in the topic, and *suppressor T cells* became one of the dominant topics of immunology research in the 1970s. However, this field has had a somewhat checkered history, mainly because initial attempts to define populations of suppressor cells and their mechanisms of action were largely unsuccessful. More than 20 years later, the idea had an impressive rebirth, with the application of better approaches to define, purify, and analyze populations of T lymphocytes that inhibit immune responses. These cells are called *regulatory T lymphocytes*; their properties and functions are described next.

Regulatory T lymphocytes are a subset of CD4⁺ T cells whose function is to suppress immune responses and maintain self-tolerance (Fig. 15-7). The majority of these $CD4^+$ regulatory T lymphocytes express high levels of the interleukin-2 (IL-2) receptor α chain (CD25). A transcription factor called FoxP3, a member of the forkhead family of transcription factors, is critical for the development and function of the majority of regulatory T cells. Mice with spontaneous or experimentally induced mutations in the *foxp3* gene develop a multisystem autoimmune disease associated with an absence of $CD25^+$ regulatory T cells. A rare autoimmune disease in humans called **IPEX** syndrome (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) is caused by mutations in the *FOXP3* gene and is associated with deficiency of regulatory T cells. These observations have established

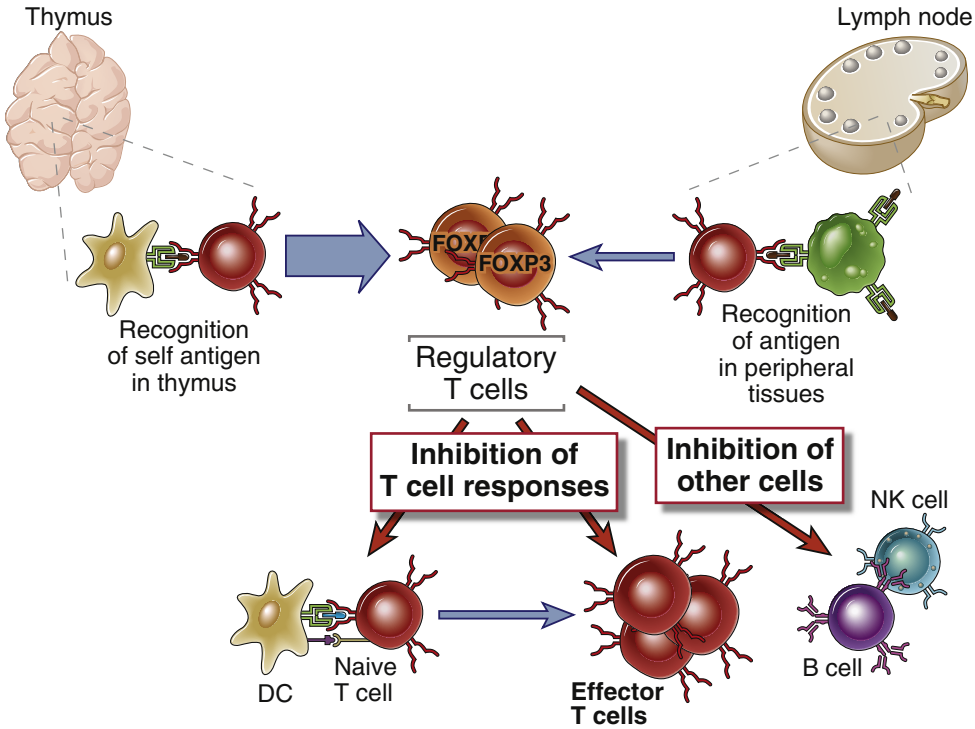

FIGURE 15-7 Regulatory T cells. Regulatory T cells are generated by self antigen recognition in the thymus (sometimes called natural regulatory cells) and (probably to a lesser extent) by antigen recognition in peripheral lymphoid organs (called inducible or adaptive regulatory cells). The development and survival of these regulatory T cells require IL-2 and the transcription factor FoxP3. In peripheral tissues, regulatory T cells suppress the activation and effector functions of other self-reactive and potentially pathogenic lymphocytes.

the importance of regulatory T cells for maintaining self-tolerance. The recent surge of interest in regulatory T cells is because of an increasing appreciation of their physiologic roles, as well as the possibility that defects in these cells may result in various autoimmune diseases and, conversely, that regulatory T cells can be used to treat inflammatory diseases.

Phenotypic Markers and Heterogeneity of Regulatory T Cells

Although numerous T cell populations have been described as possessing suppressive activity, the cell type whose regulatory role is best established is $CD4^+$ $FoxP3^+$ $CD25^{high}$. Both FoxP3 and CD25 are essential for the generation, maintenance, and function of these cells. These cells typically express low levels of receptors for IL-7 (CD127), and as predicted from this pattern of receptor expression, they use IL-2 but not IL-7 as their growth and survival factor. $FoxP3^+$ regulatory T cells typically express high levels of CTLA-4, which is also required for their function (discussed earlier). Demethylation of the *FOXP3* gene locus as well as of other loci containing genes that are expressed in these cells serves to maintain a stable regulatory T cell phenotype, and these epigenetic changes are now used to identify regulatory T cells in basic and clinical research.

Generation and Maintenance of Regulatory T Cells

Regulatory T cells are generated mainly by self antigen recognition in the thymus and by recognition of self and foreign antigens in peripheral lymphoid organs. In the thymus, development of regulatory T cells is one of the fates of T cells committed to the CD4 lineage that recognize self antigens; these thymic regulatory T cells (tTreg) have also been called natural regulatory T cells. In peripheral lymphoid organs, antigen recognition in the absence of strong innate immune responses favors the generation of regulatory cells from naive $CD4^+$ T lymphocytes; regulatory T cells can also develop after inflammatory reactions. These peripheral regulatory T cells (pTreg) have been called adaptive or inducible because they may be induced to develop from naive $CD4^+$ T cells in the peripheral lymphoid tissues as an adaptation of the immune system in response to certain types of antigen exposure. Predictably, thymic regulatory cells are specific for self antigens because these are the antigens mainly encountered in the thymus. Peripheral regulatory cells may be specific for self or foreign antigens.

The generation of some regulatory T cells requires the cytokine TGF-β. Culture of naive T cells with activating anti-TCR antibodies together with TGF-β (and IL-2, discussed next) can induce the development of regulatory cells in vitro. In mice, elimination of TGF-β or blocking of TGF-β signals in T cells leads to a systemic inflammatory disease because of uncontrolled leukocyte activation and deficiency of functional regulatory T cells. TGF-β stimulates expression of FoxP3, the transcription factor that is required for the development and function of regulatory T cells.

The survival and functional competence of regulatory T cells are dependent on the cytokine IL-2. Mice in which the gene for IL-2 or for the α or β chain of the IL-2 receptor is knocked out develop autoimmunity, manifested by inflammatory bowel disease, autoimmune hemolytic anemia, and multiple autoantibodies (including anti-erythrocyte and anti-DNA). These mice lack a full complement of $CD25^+$ $FoxP3^+$ regulatory T cells, and their disease can be corrected by restoring these cells. IL-2 promotes differentiation of T cells into the regulatory subset and is also required for the maintenance of this cell population. IL-2 activates the transcription factor STAT5, which may enhance expression of FoxP3 as well as other genes that are involved in the function of regulatory T cells. These results are the basis for ongoing clinical trials testing the ability of IL-2 to promote regulatory T cells in humans, for the control of graft-versus-host disease, autoimmune inflammation, and graft rejection.

Particular populations or subsets of dendritic cells may be especially important for stimulating the development of regulatory T cells in peripheral tissues. There is some evidence that dendritic cells exposed to retinoic acid, the vitamin A analogue, are inducers of regulatory T cells, especially in mucosal lymphoid tissues (see Chapter 14).

Mechanisms of Action of Regulatory T Cells

Regulatory T cells appear to suppress immune responses at multiple steps—at the induction of T cell activation in lymphoid organs as well as the effector phase of these responses in tissues. They may also directly suppress B cell activation and inhibit the proliferation and differentiation of natural killer (NK) cells. Although several mechanisms of suppression have been proposed, the following are the best supported by available data.

- ***Production of the immunosuppressive cytokines IL-10 and TGF-β***. The biology of these cytokines is described in more detail later.
- ***Reduced ability of APCs to stimulate T cells.*** One proposed mechanism of this action is dependent on binding of CTLA-4 on regulatory cells to B7 molecules on APCs, described earlier (see Fig. 15-6).
- ***Consumption of IL-2.*** Because of the high level of expression of the IL-2 receptor, these cells may absorb IL-2 and deprive other cell populations of this growth factor, resulting in reduced proliferation and differentiation of other IL-2–dependent cells.

It is not established if all regulatory cells work by all of these mechanisms or if there are subpopulations that use different mechanisms to control immune responses. In fact, there is some evidence in humans that two different populations of regulatory T cells can be distinguished by the expression of FoxP3 or production of IL-10, but this separation may not be absolute.

Inhibitory Cytokines Produced by Regulatory T Cells

TGF-β and IL-10 are involved in both the generation and the functions of regulatory T cells. These cytokines are produced by and act on many other cell types besides regulatory cells. Here we describe the properties and actions of these cytokines.

Transforming Growth Factor-β. TGF-β was discovered as a tumor product that promoted the survival of tumor cells in vitro. It is actually a family of closely related

molecules encoded by distinct genes, commonly designated TGF-β1, TGF-β2, and TGF-β3. Cells of the immune system synthesize mainly TGF-β1. TGF-β1 is produced by $CD4^+$ regulatory T cells, activated macrophages, and many other cell types. It is synthesized as an inactive precursor that is proteolytically cleaved in the Golgi complex and forms a homodimer. Mature TGF-β1 is secreted in a latent form in association with other polypeptides, which must be removed extracellularly by enzymatic digestion before the cytokine can bind to receptors and exert biologic effects. The TGF-β1 receptor consists of two different proteins, TGF-βRI and TGF-βRII, both of which phosphorylate transcription factors called SMADs. On cytokine binding, a serine/threonine kinase domain of TGF-βRI phosphorylates SMAD2 and SMAD3, which in complex with SMAD4 translocate to the nucleus, bind to promoters of target genes, and regulate their transcription.

TGF-β has many important and quite diverse roles in the immune system.

- ***TGF-β inhibits the proliferation and effector functions of T cells and the activation of macrophages.*** TGF-β inhibits classical macrophage activation but is one of the cytokines secreted by alternatively activated macrophages (see Chapter 10). TGF-β also suppresses the activation of other cells, such as neutrophils and endothelial cells. By these inhibitory actions, TGF-β functions to control immune and inflammatory responses.
- ***TGF-β regulates the differentiation of functionally distinct subsets of T cells.*** As described earlier, TGF-β stimulates the development of peripheral $FoxP3^+$ regulatory T cells. In combination with cytokines produced during innate immune responses, such as IL-1 and IL-6, TGF-β promotes the development of the T_H17 subset of $CD4^+$ T cells by virtue of its ability to induce the transcription factor RORγt (see Chapter 10). The ability of TGF-β to suppress immune and inflammatory responses, in part by generating regulatory T cells, and also to promote the development of proinflammatory T_H17 cells in the presence of other cytokines, is an interesting example of how a single cytokine can have diverse and sometimes opposing actions depending on the context in which it is produced. TGF-β can also inhibit development of T_H1 and T_H2 subsets.
- ***TGF-β stimulates production of IgA antibodies by inducing B cells to switch to this isotype.*** IgA is the major antibody isotype required for mucosal immunity (see Chapter 14).
- ***TGF-β promotes tissue repair after local immune and inflammatory reactions subside.*** This function is mediated mainly by the ability of TGF-β to stimulate collagen synthesis and matrix-modifying enzyme production by macrophages and fibroblasts and by promotion of angiogenesis. This cytokine may play a pathologic role in diseases in which fibrosis is an important component, such as pulmonary fibrosis and systemic sclerosis.

Interleukin-10. IL-10 is an inhibitor of activated macrophages and dendritic cells and is thus involved in the control of innate immune reactions and cell-mediated immunity. It is a member of a family of heterodimeric cytokines that includes IL-22, IL-27, and others. The IL-10 receptor belongs to the type II cytokine receptor family (similar to the receptor for interferons) and consists of two chains, which associate with JAK1 and TYK2 Janus family kinases and activate STAT3. IL-10 is produced by many immune cell populations, including activated macrophages and dendritic cells, regulatory T cells, and T_H1 and T_H2 cells. Because it is both produced by and inhibits macrophage and dendritic cell functions, it functions as a negative feedback regulator. IL-10 is also produced by some B lymphocytes, which have been shown to have immune suppressive functions and have been called **regulatory B cells**.

The biologic effects of IL-10 result from its ability to inhibit many of the functions of activated macrophages and dendritic cells.

- ***IL-10 inhibits the production of IL-12 by activated dendritic cells and macrophages.*** Because IL-12 is a critical stimulus for IFN-γ secretion, which plays an important role in innate and adaptive cell-mediated immune reactions against intracellular microbes, IL-10 functions to suppress all such reactions. In fact, IL-10 was first identified as a protein that inhibited IFN-γ production.
- ***IL-10 inhibits the expression of costimulators and class II MHC molecules on dendritic cells and macrophages.*** Because of these actions, IL-10 serves to inhibit T cell activation and terminate cell-mediated immune reactions.

A rare inherited autoimmune disease has been described in which mutations in the IL-10 receptor cause severe colitis that develops early in life, before 1 year of age. Knockout mice lacking IL-10 either in all cells or only in regulatory T cells also develop colitis, probably as a result of uncontrolled activation of lymphocytes and macrophages reacting to enteric microbes. Because of these findings, it is believed that this cytokine is especially important for controlling inflammatory reactions in mucosal tissues, particularly in the gastrointestinal tract (see Chapter 14).

The Epstein-Barr virus contains a gene homologous to human IL-10, and viral IL-10 has the same activities as the natural cytokine. This raises the intriguing possibility that acquisition of the IL-10–like gene during the evolution of the virus has given it the ability to inhibit host immunity and thus a survival advantage in the infected host.

Roles of Regulatory T Cells in Self-Tolerance and Autoimmunity

The elucidation of the genetic basis of IPEX syndrome and the similar disease in mice caused by mutations in the *Foxp3* gene, described earlier, is convincing proof of the importance of regulatory T cells in maintaining self-tolerance and homeostasis in the immune system. Numerous attempts are being made to identify defects in the development or function of regulatory T cells in more common autoimmune diseases in humans, such as inflammatory bowel disease, type 1 diabetes, and multiple sclerosis, as well as in allergic disorders. It appears

likely that defects in regulatory T cells or resistance of effector cells to suppression contribute to the pathogenesis of autoimmune and allergic diseases. There is also potential for expanding regulatory cells in culture and injecting them back into patients to control pathologic immune responses. Clinical trials of regulatory T cell transfer are ongoing in attempts to treat transplant rejection, graft-vs-host disease, and autoimmune and other inflammatory disorders. Attempts are also under way to induce these cells in patients by administering self peptides that are the targets of autoimmunity or low doses of the cytokine IL-2, either separately or in combination.

Deletion of T Cells by Apoptotic Cell Death

T lymphocytes that recognize self antigens with high affinity or are repeatedly stimulated by antigens may die by apoptosis. There are two major pathways of apoptosis in various cell types (Fig. 15-8), both of which have been implicated in peripheral deletion of mature T cells.

- The **mitochondrial** (or **intrinsic**) **pathway** is regulated by the Bcl-2 family of proteins, named after the founding member, Bcl-2, which was discovered as an oncogene in a B cell lymphoma and shown to inhibit apoptosis. Some members of this family are pro-apoptotic and others are anti-apoptotic. The pathway is initiated when cytoplasmic proteins of the Bcl-2 family that belong to the BH3-only subfamily (so called because they contain one domain that is homologous to the third conserved domain of Bcl-2) are induced or activated as a result of growth factor deprivation, noxious stimuli, DNA damage, or certain types of receptor-mediated signaling (such as strong signals delivered by self antigens in immature lymphocytes). BH3-only proteins are sensors of cell stress that bind to and influence death effectors and regulators. In lymphocytes, the most important of these sensors is a protein called Bim. Activated Bim binds to two pro-apoptotic effector proteins of the Bcl-2 family called Bax and Bak, which oligomerize and insert into the outer mitochondrial membrane, leading to increased mitochondrial permeability. Growth factors and other survival signals induce the expression of anti-apoptotic members of the Bcl-2 family, such as Bcl-2 and Bcl-X_L, which function as inhibitors of apoptosis by blocking Bax and Bak and thus maintaining intact mitochondria. BH3-only proteins also antagonize Bcl-2 and Bcl-X_L. When cells are deprived of survival signals, the

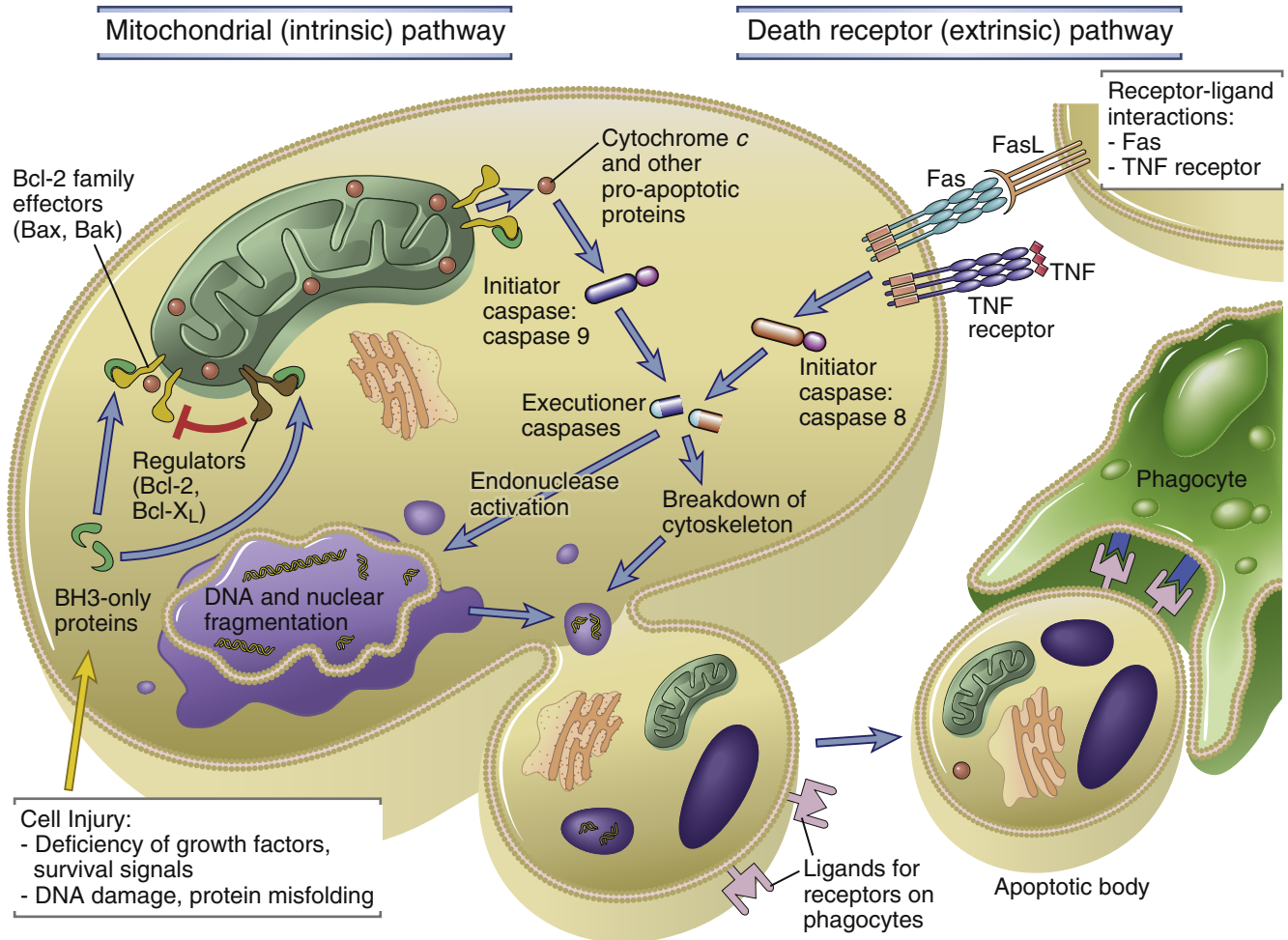

FIGURE 15-8 Pathways of apoptosis. Apoptosis is induced by the mitochondrial and death receptor pathways, described in the text, which culminate in fragmentation of the dead cell and phagocytosis of apoptotic bodies.

mitochondria become leaky because of the actions of the BH3-only protein sensors and Bax and Bak effectors and the relative deficiency of anti-apoptotic proteins such as Bcl-2 and Bcl-X_L. The result is that many mitochondrial components, including cytochrome *c*, leak out into the cytosol. These proteins activate cytosolic enzymes called **caspases**, initially caspase-9, which in turn cleaves downstream caspases that lead to nuclear DNA fragmentation and other changes that culminate in apoptotic death.

- In the **death receptor** (or **extrinsic**) **pathway**, cell surface receptors homologous to tumor necrosis factor (TNF) receptors are engaged by their ligands, which are homologous to the cytokine TNF. The receptors oligomerize and activate cytoplasmic adaptor proteins, which assemble procaspase-8, which cleaves itself when oligomerized to yield active caspase-8. The active caspase-8 then cleaves downstream caspases, again resulting in apoptosis. In many cell types, caspase-8 cleaves and activates a BH3-only protein called Bid that binds to Bax and Bak and induces apoptosis via the mitochondrial pathway. Thus, the mitochondrial pathway may serve to amplify death receptor signaling.

Cells undergoing apoptosis develop membrane blebs, and fragments of the nucleus and cytoplasm break off in membrane-bound structures called apoptotic bodies. There are also biochemical changes in the plasma membrane, including the exposure of lipids such as phosphatidylserine, which is normally on the inner face of the plasma membrane. These alterations are recognized by receptors on phagocytes, and apoptotic bodies and cells are rapidly engulfed and eliminated, without ever having elicited a host inflammatory response.

The best evidence for the involvement of the two apoptotic pathways in the elimination of mature self-reactive lymphocytes is that genetic ablation of both in mice results in systemic autoimmunity. These two death pathways may function in different ways to maintain self-tolerance.

- ***T cells that recognize self antigens in the absence of costimulation may activate Bim, resulting in apoptosis by the mitochondrial pathway.*** In normal immune responses, the responding lymphocytes receive signals from the TCR, costimulators, and growth factors. These signals stimulate the expression of anti-apoptotic proteins of the Bcl-2 family (Bcl-2, Bcl-X_L) and thus prevent apoptosis and promote cell survival, the necessary prelude to proliferation. When T cells avidly recognize self antigens, they may directly activate Bim, which triggers death by the mitochondrial pathway, as described earlier. At the same time, because of the relative lack of costimulation and growth factors, the anti-apoptotic members of the Bcl-2 family, Bcl-2 and Bcl-X_L, are expressed at low levels, and the actions of Bim, Bax, and Bak are thus not counteracted.

  The Bim-dependent mitochondrial pathway of apoptosis is also involved in negative selection of self-reactive T cells in the thymus (described earlier) and in the contraction phase (decline) of immune responses after the initiating antigen has been eliminated (see Chapter 9).

- ***Repeated stimulation of T cells results in the coexpression of death receptors and their ligands, and engagement of the death receptors triggers apoptotic death.*** In $CD4^+$ T cells, the most important death receptor is Fas (CD95), and its ligand is Fas ligand (FasL). Fas is a member of the TNF receptor family, and FasL is homologous to TNF. When T cells are repeatedly activated, FasL is expressed on the cell surface, and it binds to surface Fas on the same or adjacent T cells. This activates a cascade of caspases, which ultimately cause the apoptotic death of the cells. The same pathway of apoptosis may be involved in the elimination of self-reactive B lymphocytes also in the periphery (discussed later).

  Mice carrying mutations of the genes encoding Fas or Fas ligand provided the first clear evidence that failure of apoptotic cell death results in autoimmunity. These mice develop a systemic autoimmune disease with multiple autoantibodies and nephritis, resembling human systemic lupus erythematosus (see Chapter 19). The lpr (for lymphoproliferation) mouse strain produces low levels of Fas protein, and the gld (for generalized lymphoproliferative disease) strain produces FasL with a point mutation that interferes with its signaling function. The cause of autoimmunity is believed to be defective peripheral deletion and accumulation of autoreactive B and helper T cells. Children with a phenotypically similar disease have been identified and shown to carry mutations in the gene encoding Fas or in genes encoding proteins in the Fas-mediated death pathway. This disease is called **autoimmune lymphoproliferative syndrome (ALPS).**

Peripheral Tolerance in $CD8^+$ T Lymphocytes

Much of our knowledge of peripheral T cell tolerance is limited to $CD4^+$ T cells, and less is known about the mechanisms of tolerance in mature $CD8^+$ T cells. It is likely that if $CD8^+$ T cells recognize class I MHC–associated peptides without costimulation or T cell help, the $CD8^+$ cells become anergic. In this situation, the $CD8^+$ T cells would encounter signal 1 (antigen) without second signals, and the mechanism of anergy would be essentially the same as for $CD4^+$ T lymphocytes. Inhibitory receptors such as PD-1 suppress the activation of $CD8^+$ T cells and may be involved in terminating their responses, in a phenomenon called exhaustion (see Chapter 11). $CD25^+$ regulatory T cells can directly inhibit the activation of $CD8^+$ T cells or suppress $CD4^+$ helper cells that are required for full $CD8^+$ T cell responses. $CD8^+$ T cells that are exposed to high concentrations of self antigens may also undergo apoptotic cell death.

Factors That Determine the Tolerogenicity of Self Antigens

Studies with a variety of experimental models have shown that many features of protein antigens determine whether these antigens will induce T cell activation or tolerance (Table 15-1). Self antigens have several properties that make them tolerogenic. These antigens are expressed in generative lymphoid organs, where they are recognized by immature lymphocytes. In peripheral

TABLE 15-1 Factors That Determine the Immunogenicity and Tolerogenicity of Protein Antigens

	Features That Favor Stimulation of Immune Responses	Features That Favor Tolerance
Persistence	Short-lived (eliminated by immune response)	Prolonged
Portal of entry; location	Subcutaneous, intradermal; absence from generative organs	Intravenous, mucosal; presence in generative organs
Presence of adjuvants	Antigens with adjuvants: stimulate helper T cells	Antigens without adjuvants: non-immunogenic or tolerogenic
Properties of antigen-presenting cells	High levels of costimulators	Low levels of costimulators and cytokines

tissues, self antigens engage antigen receptors of specific lymphocytes for prolonged periods and without inflammation or innate immunity.

The nature of the dendritic cell that displays antigens to T lymphocytes is an important determinant of the subsequent response. Dendritic cells that are resident in lymphoid organs and nonlymphoid tissues may present self antigens to T lymphocytes and maintain tolerance. Tissue dendritic cells are normally in a resting (immature) state and express few or no costimulators. Such APCs may be constantly presenting self antigens without providing activating signals, and T cells that recognize these antigens become anergic or differentiate into regulatory T lymphocytes instead of effector and memory lymphocytes. By contrast, dendritic cells that are activated by microbes are the principal APCs for initiation of T cell responses (see Chapter 6). As we will discuss later, local infections and inflammation may activate resident dendritic cells, leading to increased expression of costimulators, breakdown of tolerance, and autoimmune reactions against tissue antigens. The characteristics of dendritic cells that make them tolerogenic are not defined but presumably include low expression of costimulators. There is great interest in manipulating the properties of dendritic cells as a way of enhancing or inhibiting immune responses for therapeutic purposes.

Our understanding of the mechanisms that link the signals that a T cell receives at the time of antigen recognition with the fate of that T cell remains incomplete. These concepts are based largely on experimental models in which antigens are administered to mice or are produced by transgenes expressed in mice. One of the continuing challenges in this field is to define the mechanisms by which various normally expressed self antigens induce tolerance, especially in humans.

B LYMPHOCYTE TOLERANCE

Tolerance in B lymphocytes is necessary for maintaining unresponsiveness to thymus-independent self antigens, such as polysaccharides and lipids. B cell tolerance also plays a role in preventing antibody responses to protein antigens. Experimental studies have revealed multiple mechanisms by which encounter with self antigens may abort B cell maturation and activation.

Central B Cell Tolerance

Immature B lymphocytes that recognize self antigens in the bone marrow with high affinity either change their specificity or are deleted. The mechanisms of central B cell tolerance have been best described in experimental models (Fig. 15-9).

- ***Receptor editing.*** If immature B cells recognize self antigens that are present at high concentration in the bone marrow and especially if the antigen is displayed in multivalent form (e.g., on cell surfaces), many antigen receptors on each B cell are cross-linked, thus delivering strong signals to the cells. As discussed in Chapter 8, one consequence of such signaling is that the B cells reactivate their *RAG1* and *RAG2* genes and initiate a new round of VJ recombination in the immunoglobulin (Ig) κ light chain gene locus. A V_κ segment upstream of the already rearranged $V_\kappa J_\kappa$ unit is joined to a downstream J_κ. As a result, the previously rearranged $V_\kappa J_\kappa$ exon in the self-reactive immature B cell is deleted, and a new Ig light chain is expressed, thus creating a B cell receptor with a new specificity. This process is called **receptor editing** (see Chapter 8) and is an important mechanism for eliminating self-reactivity from the mature B cell repertoire. If the edited light chain rearrangement is non-productive, rearrangement may proceed at the κ locus on the other chromosome, and if that is non-productive, rearrangements at the λ light chain loci may follow. A B cell expressing a λ light chain is frequently a cell that has undergone receptor editing.
- ***Deletion.*** If editing fails, the immature B cells may die by apoptosis. The mechanisms of deletion are not well defined.
- ***Anergy.*** If developing B cells recognize self antigens weakly (e.g., if the antigen is soluble and does not cross-link many antigen receptors or if the B cell receptors recognize the antigen with low affinity), the cells become functionally unresponsive (anergic) and exit the bone marrow in this unresponsive state. Anergy is due to downregulation of antigen receptor expression as well as a block in antigen receptor signaling.

Peripheral B Cell Tolerance

Mature B lymphocytes that recognize self antigens in peripheral tissues in the absence of specific helper T cells may be rendered functionally unresponsive or die by apoptosis

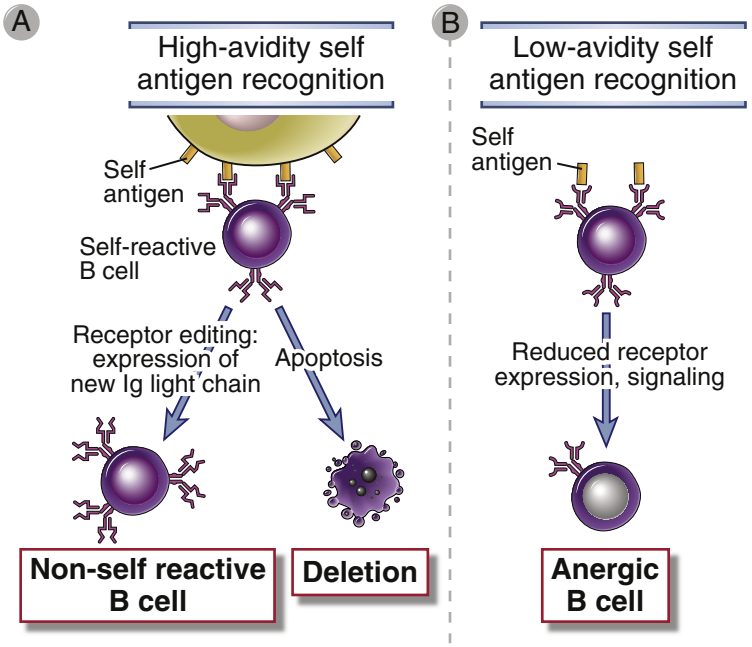

FIGURE 15-9 Central tolerance in B cells. Immature B cells that recognize self antigens in the bone marrow with high avidity (e.g., multivalent arrays of antigens on cells) die by apoptosis or change the specificity of their antigen receptors (receptor editing). Weak recognition of self antigens in the bone marrow may lead to anergy (functional inactivation) of the B cells.

(Fig. 15-10). Signals from helper T cells may be absent if these T cells are deleted or anergic or if the self antigens are non-protein antigens. Since self antigens usually do not elicit innate immune responses, B cells will also not be activated via complement receptors or pattern recognition receptors. Thus, as in T cells, antigen recognition without additional stimuli results in tolerance. Peripheral tolerance mechanisms also eliminate autoreactive B cell clones that may be generated as an unintended consequence of somatic mutation in germinal centers.

- ***Anergy and deletion.*** Some self-reactive B cells that are repeatedly stimulated by self antigens become unresponsive to further activation. These cells require high levels of the growth factor BAFF/BLys for survival (see Chapter 11) and cannot compete efficiently with less BAFF–dependent normal naive B cells for survival in lymphoid follicles. As a result, the B cells that have encountered self antigens have a shortened life span and are eliminated more rapidly than cells that have not recognized self antigens. B cells that bind with high avidity to self antigens in the periphery may also undergo apoptotic death by the mitochondrial pathway.

  The high rate of somatic mutation of Ig genes that occurs in germinal centers has the risk of generating self-reactive B cells (see Chapter 12). These B cells may be actively eliminated by the interaction of FasL on helper T cells with Fas on the activated B cells. The same interaction was described earlier as a mechanism for the death of self-reactive T cells. Failure of this pathway of peripheral B cell tolerance may contribute to the autoimmunity that is caused by mutations in the *Fas* and *FasL* genes in mice, and in patients with ALPS, discussed earlier.

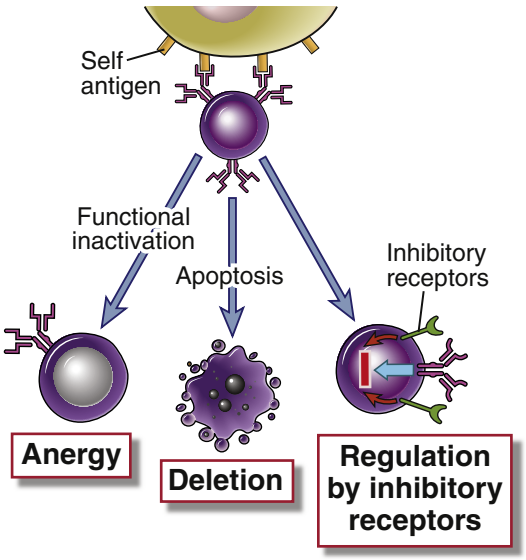

FIGURE 15-10 Peripheral tolerance in B cells. B cells that encounter self antigens in peripheral tissues become anergic or die by apoptosis. In some situations, recognition of self antigens may trigger inhibitory receptors that prevent B cell activation.

- ***Signaling by inhibitory receptors.*** B cells that recognize self antigens with low affinity may be prevented from responding by the engagement of various inhibitory receptors. The function of these inhibitory receptors is to set a threshold for B cell activation, which allows responses to foreign antigens with T cell help but does not allow responses to self antigens. This mechanism of peripheral tolerance was revealed by studies showing that mice with defects in the SHP-1 tyrosine phosphatase, the Lyn tyrosine kinase, and the CD22 inhibitory receptor develop autoimmunity. ITIM motifs in the cytoplasmic tail of CD22 are phosphorylated by Lyn, and this inhibitory receptor then recruits SHP-1, thus attenuating B cell receptor signaling. However, it is not known when inhibitory receptors such as CD22 are engaged and what ligands they recognize.

Much has been learned about the mechanisms of tolerance in T and B lymphocytes, largely from the use of animal models such as genetically modified mice. Application of this knowledge to understanding the mechanisms of tolerance to different self antigens in normal individuals and to defining why tolerance fails, giving rise to autoimmune diseases, is an area of active investigation.

TOLERANCE INDUCED BY FOREIGN PROTEIN ANTIGENS

Foreign antigens may be administered in ways that preferentially induce tolerance rather than immune responses. Understanding how to induce tolerance by antigen administration is the key to developing antigen-specific tolerance as a treatment strategy for immunologic diseases. In general, protein antigens administered cutaneously with adjuvants favor immunity, whereas high doses of antigens administered without adjuvants tend to induce tolerance. The likely reason for this is that adjuvants stimulate innate immune responses and the expression of costimulators on APCs, and in the absence of these second signals, T cells that recognize the antigen may become anergic or die or may differentiate into regulatory cells. Many other features of antigens, and how they are administered, may influence the balance between immunity and tolerance (see Table 15-1).

The oral administration of a protein antigen often leads to suppression of systemic humoral and cell-mediated immune responses to immunization with the same antigen. This phenomenon, called **oral tolerance**, was discussed in Chapter 14.

MECHANISMS OF AUTOIMMUNITY

The possibility that an individual's immune system may react against autologous antigens and cause tissue injury was appreciated by immunologists from the time that the specificity of the immune system for foreign antigens was recognized. In the early 1900s, Paul Ehrlich coined the rather melodramatic phrase *horror autotoxicus* for harmful (toxic) immune reactions against self. Autoimmunity is an important cause of disease in humans and is estimated to affect at least 2% to 5% of the U.S. population. The term *autoimmunity* is often erroneously used for any disease in which immune reactions accompany tissue injury, even though it may be difficult or impossible to establish a role for immune responses against self antigens in causing these disorders. Because inflammation is a prominent component of these disorders, they are sometimes grouped under *immune-mediated inflammatory diseases,* which does not imply that the pathologic response is directed against self antigens (see Chapter 19).

The fundamental questions about autoimmunity are how self-tolerance fails and how self-reactive lymphocytes are activated. Answers to these questions are needed to understand the etiology and pathogenesis of autoimmune diseases, which is a major challenge in immunology. Our understanding of autoimmunity has improved greatly during the past two decades, mainly because of the development of informative animal models of these diseases, the identification of genes that may predispose to autoimmunity, and improved methods for analyzing immune responses in humans. Several important general concepts have emerged from studies of autoimmunity.

The factors that contribute to the development of autoimmunity are genetic susceptibility and environmental triggers, such as infections and local tissue injury. Susceptibility genes may disrupt self-tolerance mechanisms, and infection or necrosis in tissues promotes the influx of autoreactive lymphocytes and activation of these cells, resulting in tissue injury (Fig. 15-11). Infections and tissue injury may also alter the way in which self antigens are displayed to the immune system, leading to failure of self-tolerance and activation of self-reactive lymphocytes. The roles of these factors in the development of autoimmunity are discussed later. Other factors such as changes in the host microbiome and epigenetic alterations in immune cells may play important roles in pathogenesis, but studies on these topics are in their infancy.

General Features of Autoimmune Disorders

Autoimmune diseases have several general characteristics that are relevant to defining their underlying mechanisms.

- ***Autoimmune diseases may be either systemic or organ specific, depending on the distribution of the autoantigens that are recognized.*** For instance, the formation of circulating immune complexes composed of self nucleoproteins and specific antibodies typically produces systemic diseases, such as systemic lupus erythematosus (SLE). In contrast, autoantibody or T cell responses against self antigens with restricted tissue distribution lead to organ-specific diseases, such as myasthenia gravis, type 1 diabetes, and multiple sclerosis.
- ***Various effector mechanisms are responsible for tissue injury in different autoimmune diseases.*** These mechanisms include immune complexes, circulating autoantibodies, and autoreactive T lymphocytes and are discussed in Chapter 19. The clinical and pathologic features of the disease are usually determined by the nature of the dominant autoimmune response.
- ***Autoimmune diseases tend to be chronic, progressive, and self-perpetuating.*** The reasons for these features are that the self antigens that trigger these reactions are persistent, and once an immune response starts, many amplification mechanisms are activated that perpetuate the response. In addition, a response initiated against one self antigen that injures tissues may result in the release and alterations of other tissue antigens, activation of lymphocytes specific for these other antigens, and exacerbation of the disease. This phenomenon is called epitope spreading, and it may explain why once an autoimmune disease has developed, it may become prolonged and self-perpetuating.

Immunologic Abnormalities Leading to Autoimmunity

Autoimmunity results from some combination of three main immunologic aberrations.

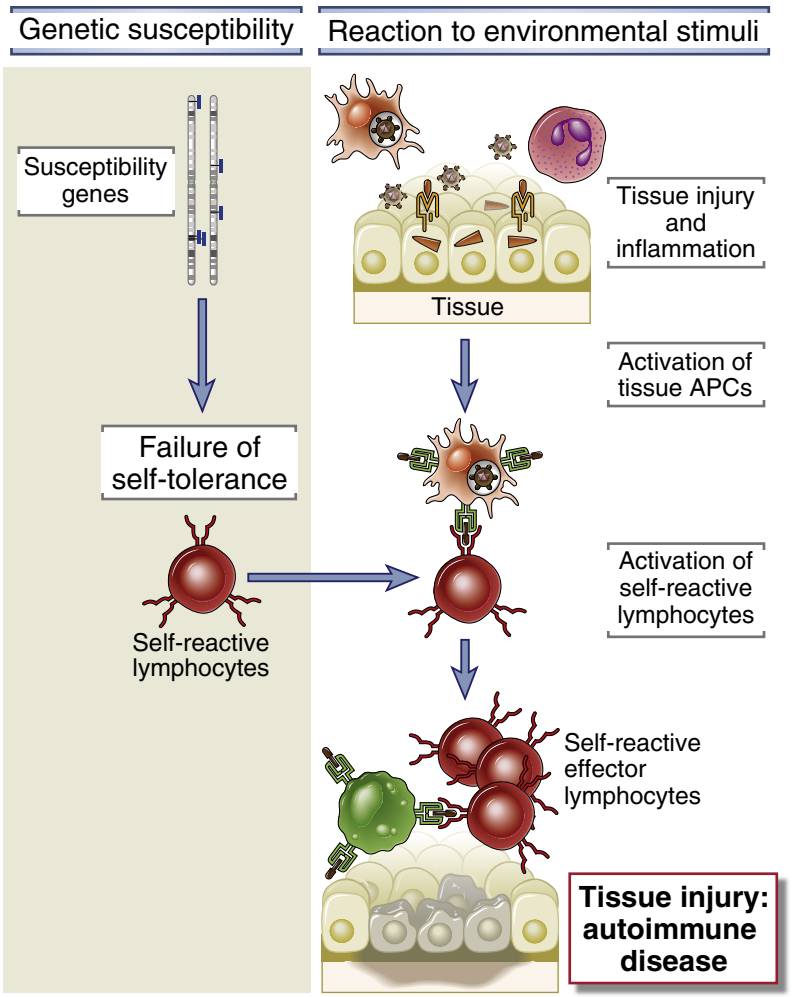

FIGURE 15-11 Postulated mechanisms of autoimmunity. In this proposed model of an organ-specific T cell–mediated autoimmune disease, various genetic loci may confer susceptibility to autoimmunity, in part by influencing the maintenance of self-tolerance. Environmental triggers, such as infections and other inflammatory stimuli, promote the influx of lymphocytes into tissues and the activation of self-reactive T cells, resulting in tissue injury.

- ***Defective tolerance or regulation. Failure of the mechanisms of self-tolerance in T or B cells, leading to an imbalance between lymphocyte activation and control, is the underlying cause of all autoimmune diseases.*** The potential for autoimmunity exists in all individuals because some of the randomly generated specificities of clones of developing lymphocytes may be for self antigens, and many self antigens are readily accessible to lymphocytes. As discussed earlier, tolerance to self antigens is normally maintained by selection processes that prevent the maturation of some self antigen–specific lymphocytes and by mechanisms that inactivate or delete self-reactive lymphocytes that do mature. Loss of self-tolerance may result if self-reactive lymphocytes are not deleted or inactivated during or after their maturation and if APCs are activated so that self antigens are presented to the immune system in an immunogenic manner. Experimental models and limited studies in humans have shown that any of the following mechanisms may contribute to the failure of self-tolerance:
  - Defects in deletion (negative selection) of T or B cells or receptor editing in B cells during the maturation of these cells in the generative lymphoid organs
  - Defective numbers and functions of regulatory T lymphocytes
  - Defective apoptosis of mature self-reactive lymphocytes
  - Inadequate function of inhibitory receptors
- ***Abnormal display of self antigens.*** Abnormalities may include increased expression and persistence of self antigens that are normally cleared, or structural changes in these antigens resulting from enzymatic modifications or from cellular stress or injury. If these changes lead to the display of antigenic epitopes that are not present normally, the immune system may not be tolerant to these epitopes, thus allowing anti-self responses to develop.
- ***Inflammation or an initial innate immune response.*** As we have discussed in previous chapters, the innate immune response is a strong stimulus for the subsequent activation of lymphocytes and the generation of adaptive immune responses. Infections or cell injury may elicit local innate immune reactions with inflammation. These may contribute to the development of autoimmune disease, perhaps by activating APCs, which overcomes regulatory mechanisms and results in excessive T cell activation.

Much recent attention has focused on the role of T cells in autoimmunity for two main reasons. First, helper T cells are the key regulators of all immune responses

to proteins, and most self antigens implicated in autoimmune diseases are proteins. Second, several autoimmune diseases are genetically linked to the MHC (the HLA complex in humans), and the function of MHC molecules is to present peptide antigens to T cells. Failure of self-tolerance in T lymphocytes may result in autoimmune diseases in which tissue damage is caused by cell-mediated immune reactions. Helper T cell abnormalities may also lead to autoantibody production because helper T cells are necessary for the production of high-affinity antibodies against protein antigens.

In the following section, we describe the general principles of the pathogenesis of autoimmune diseases, with an emphasis on susceptibility genes, infections, and other factors that contribute to the development of autoimmunity. We will describe the pathogenesis and features of some illustrative autoimmune diseases in Chapter 19.

Genetic Basis of Autoimmunity

From the earliest studies of autoimmune diseases in patients and experimental animals, it has been appreciated that these diseases have a strong genetic component. For instance, type 1 diabetes shows a concordance of 35% to 50% in monozygotic twins and 5% to 6% in dizygotic twins, and other autoimmune diseases show similar evidence of a genetic contribution. Linkage analyses in families, genome-wide association studies, and large-scale sequencing efforts are revealing new information about the genes that may play causal roles in the development of autoimmunity and chronic inflammatory disorders. From these studies, several general features of genetic susceptibility have become apparent.

Most autoimmune diseases are complex polygenic traits in which affected individuals inherit multiple genetic polymorphisms that contribute to disease susceptibility, and these genes act with environmental factors to cause the diseases. Some of these polymorphisms are associated with several autoimmune diseases, suggesting that the causative genes influence general mechanisms of immune regulation and self-tolerance. Other loci are associated with particular diseases, suggesting that they may affect organ damage or autoreactive lymphocytes of particular specificities. Each genetic polymorphism makes a small contribution to the development of particular autoimmune diseases and is also found in healthy individuals but at a lower frequency than in patients with the diseases. It is postulated that in individual patients, multiple such polymorphisms are coinherited and together account for development of the disease. Understanding the interplay of multiple genes with one another and with environmental factors is one of the continuing challenges in the field.

The best-characterized genes associated with autoimmune diseases and our current understanding of how they may contribute to loss of self-tolerance are described here.

Association of MHC Alleles with Autoimmunity

Among the genes that are associated with autoimmunity, the strongest associations are with MHC genes. In fact, in many autoimmune diseases, such as type 1 diabetes, 20 or 30 disease-associated genes have been identified; in most of these diseases, the HLA locus alone contributes half or more of the genetic susceptibility. HLA typing of large groups of patients with various autoimmune diseases has shown that some HLA alleles occur at higher frequency in these patients than in the general population. From such studies, one can calculate the odds ratio for development of a disease in individuals who inherit various HLA alleles (often referred to as the relative risk) (Table 15-2). The strongest such association is between ankylosing spondylitis, an inflammatory, presumably autoimmune, disease of vertebral joints, and the class I HLA allele B27. Individuals who are HLA-B27 positive are over 100 times more likely to develop ankylosing spondylitis than individuals who are B27-negative. Neither the mechanism of this disease nor the basis of its association with HLA-B27 is known. The association of class II HLA-DR and HLA-DQ alleles with autoimmune diseases has received great attention, mainly because class II MHC molecules are involved in the selection and activation of $CD4^+$ T cells, and $CD4^+$ T cells regulate both humoral and cell-mediated immune responses to protein antigens.

TABLE 15-2 Association of HLA Alleles with Autoimmune Disease

Disease	HLA Allele	Odds Ratio[1]
Rheumatoid arthritis (anti-CCP Ab positive)[2]	*DRB1, 1 SE* allele[3]	4
	DRB1, 2 SE alleles	12
Type 1 diabetes	*DRB1*0301-DQA1*0501-DQB1*0201* haplotype	4
	*DRB1*0401-DQA1*0301-DQB1*0302* haplotype	8
	*DRB1*0301/0401* heterozygotes	35
Multiple sclerosis	*DRB1*1501*	3
Systemic lupus erythematosus	*DRB1*0301*	2
	*DRB1*1501*	1.3
Ankylosing spondylitis	*B*27* (mainly *B*2705* and *B*2702*)	100-200
Celiac disease	*DQA1*0501-DQB1*0201* haplotype	7

[1]The odds ratio approximates values of increased risk of the disease associated with inheritance of particular HLA alleles. The data are from populations of European ancestry. Alleles of individual MHC genes (e.g., DRB1) are indicated by 4 numbers (e.g., 0301), based on serologic and molecular typing.
[2]Anti-CCP Ab, antibodies directed against cyclic citrullinated peptides. Data are from patients who test positive for these antibodies in the serum.
[3]SE refers to shared epitope, so called because it is a consensus sequence in the DRB1 protein (positions 70-74) present in multiple *DRB1* alleles.
(Courtesy of Dr. Michelle Fernando, Kings College, London.)

Several features of the association of HLA alleles with autoimmune diseases are noteworthy.

- An HLA-disease association may be identified by serologic typing of one HLA locus, but the actual association may be with other alleles that are linked to the typed allele and inherited together. For instance, individuals

with a particular HLA-DR allele (hypothetically DR1) may show a higher probability of inheriting a particular HLA-DQ allele (hypothetically DQ2) than the probability of inheriting these alleles separately and randomly (i.e., at equilibrium) in the population. Such inheritance is an example of linkage disequilibrium. A disease may be found to be DR1 associated by HLA typing, but the causal association may actually be with the coinherited DQ2. This realization has emphasized the concept of extended HLA haplotypes, which refers to sets of linked genes, both classical HLA and adjacent non-HLA genes, that tend to be inherited together as a single unit.

- In many autoimmune diseases, the disease-associated nucleotide polymorphisms encode amino acids in the peptide-binding clefts of the MHC molecules. This observation is not surprising because polymorphic residues of MHC molecules are located within and adjacent to the clefts, and the structure of the clefts is the key determinant of both functions of MHC molecules, namely, antigen presentation and recognition by T cells (see Chapter 6).
- Disease-associated HLA sequences are found in healthy individuals. In fact, if all individuals bearing a particular disease-associated HLA allele are monitored prospectively, most will never develop the disease. Therefore, expression of a particular HLA gene is not by itself the cause of any autoimmune disease, but it may be one of several factors that contribute to autoimmunity.

The mechanisms underlying the association of different HLA alleles with various autoimmune diseases are still not clear. In diseases in which particular MHC alleles increase the risk of disease, the disease-associated MHC molecule may present a self peptide and activate pathogenic T cells, and this has been established in a few cases. When a particular allele is shown to be protective, it is hypothesized that this allele might induce negative selection of some potentially pathogenic T cells, or it might promote the development of regulatory T cells.

Polymorphisms in Non-HLA Genes Associated with Autoimmunity

Linkage analyses of autoimmune diseases identified a few disease-associated genes and many chromosomal regions in which the identity of the associated genes was suspected but not established. The technique of genome-wide association studies led to the putative identification of nucleotide polymorphisms (variants) of several genes that are associated with autoimmune diseases, and this has been greatly extended by more recent genome sequencing efforts (Table 15-3). Before the genes that are most clearly validated are discussed, it is important to summarize some of the general features of these genes.

- It is likely that combinations of multiple inherited genetic polymorphisms interacting with environmental factors induce the immunologic abnormalities that lead to autoimmunity. There are, however, examples of rare gene variants that make much larger individual contributions to particular diseases.
- Many of the polymorphisms associated with various autoimmune diseases are in genes that influence the development and regulation of immune responses. Although this conclusion appears predictable, it has reinforced the utility of the approaches being used to identify disease-associated genes.
- Different polymorphisms may either protect against disease development or increase the incidence of the disease. The statistical methods used for genome-wide association studies have revealed both types of associations.
- Disease-associated polymorphisms are often located in noncoding regions of the genes. This suggests that many of the polymorphisms may affect the expression of the encoded proteins.

Some of the genes associated with human autoimmune diseases, which have been defined by linkage analyses, genome-wide association studies, and whole genome sequencing, are briefly described next.

- ***PTPN22.*** A variant of the protein tyrosine phosphatase PTPN22, in which arginine at position 620 is replaced

TABLE 15-3 Selected Non-HLA Genetic Polymorphisms Associated with Autoimmune Diseases

Gene of Interest	Function	Diseases
Genes Involved in Immune Regulation		
PTPN22	Protein tyrosine phosphatase; role in T and B cell receptor signaling	RA, T1D, IBD
CD2/CD58	Costimulation of T cells	RA, MS
IL23R	Component of IL-23 receptor; role in generation and maintenance of T_H17 cells	IBD, PS, AS
IL10	Downregulates expression of costimulators, MHC molecules, IL-12 in dendritic cells; inhibits T_H1 responses	IBD, SLE, T1D
CTLA4	Inhibitory receptor of T cells, effector molecule of regulatory T cells	T1D, RA
IL2/IL21	Growth and differentiation factors for T cells; IL-2 is involved in maintenance of functional Tregs	IBD, CeD, RA, T1D, MS
IL12B	p40 subunit of IL-12 (T_H1-inducing cytokine) and IL-23 (T_H17-inducing cytokine)	IBD, PS
BLK	B lymphocyte tyrosine kinase, involved in B cell activation	SLE, RA
IL2RA	IL-2 receptor α chain (CD25); role in T cell activation and maintenance of regulatory T cells	MS, T1D
Genes Involved in Responses to Microbes		
NOD2	Cytoplasmic sensor of bacteria	IBD
ATG16	Autophagy (destruction of microbes, maintenance of epithelial cell integrity)	IBD
IRF5, IFIH1	Type I interferon responses to viruses	SLE

AS, ankylosing spondylitis; *CeD,* celiac disease; *IBD,* inflammatory bowel disease; *MS,* multiple sclerosis; *PS,* psoriasis; *RA,* rheumatoid arthritis; *SLE,* systemic lupus erythematosus; *T1D,* type 1 diabetes.

Data from Zenewicz L, Abraham C, Flavell RA, Cho J: Unraveling the genetics of autoimmunity, *Cell* 140:791-797, 2010, with permission of the publisher.

TABLE 15-4 Examples of Single-Gene Mutations That Cause Autoimmune Diseases

Gene	Phenotype of Mutant or Knockout Mouse	Mechanism of Failure of Tolerance	Human Disease?
AIRE	Destruction of endocrine organs by antibodies, lymphocytes	Failure of central tolerance	Autoimmune polyendocrine syndrome (APS)
C4	SLE	Defective clearance of immune complexes; failure of B cell tolerance	SLE
CTLA4	Lymphoproliferation; T cell infiltrates in multiple organs, especially heart; lethal by 3-4 weeks	Failure of anergy in $CD4^+$ T cells; defective function of regulatory T cells	CTLA-4 polymorphisms associated with several autoimmune diseases
FAS/FASL	Anti-DNA and other autoantibodies; immune complex nephritis; arthritis; lymphoproliferation	Defective deletion of anergic self-reactive B cells; reduced deletion of mature $CD4^+$ T cells	Autoimmune lymphoproliferative syndrome (ALPS)
FOXP3	Multiorgan lymphocytic infiltrates, wasting	Deficiency of functional regulatory T cells	IPEX
IL2, IL2Rα/β	Inflammatory bowel disease; anti-erythrocyte and anti-DNA autoantibodies	Defective development, survival, or function of regulatory T cells	None known
SHP1	Multiple autoantibodies	Failure of negative regulation of B cells	None known

AIRE, autoimmune regulator gene; *IL-2,* interleukin-2; *IPEX,* immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome; *SHP-1,* SH2-containing phosphatase 1; SLE, systemic lupus erythematosus.

with a tryptophan, is associated with rheumatoid arthritis, type 1 diabetes, autoimmune thyroiditis, and other autoimmune diseases. The disease-associated variant causes complex signaling alterations in multiple immune cell populations. Precisely how these changes lead to autoimmunity is not known.

- ***NOD2.*** Polymorphisms in this gene are associated with Crohn's disease, one type of inflammatory bowel disease. NOD2 is a cytoplasmic sensor of bacterial peptidoglycans (see Chapter 4) and is expressed in multiple cell types, including intestinal epithelial cells. It is thought that the disease-associated polymorphism reduces the function of NOD2, which cannot provide effective defense against certain intestinal microbes. As a result, these microbes are able to traverse the epithelium and initiate a chronic inflammatory reaction in the intestinal wall, which is a hallmark of inflammatory bowel disease (see Chapter 14).
- ***Insulin.*** Polymorphisms in the insulin gene that encode variable numbers of repeat sequences are associated with type 1 diabetes. These polymorphisms may affect the thymic expression of insulin. It is postulated that if the protein is expressed at low levels in the thymus because of a genetic polymorphism, developing T cells specific for insulin may not be negatively selected. These cells survive in the mature immune repertoire and are capable of attacking insulin-producing islet β cells and causing diabetes.
- ***CD25.*** Polymorphisms affecting the expression or function of CD25, the α chain of the IL-2 receptor, are associated with multiple sclerosis, type 1 diabetes, and other autoimmune diseases. These changes in CD25 likely affect the generation or function of regulatory T cells, although there is no definitive evidence for a causal link between the CD25 abnormality, regulatory T cell defects, and the autoimmune disease.
- ***IL-23 receptor (IL-23R).*** Some polymorphisms in the receptor for IL-23 are associated with increased susceptibility to inflammatory bowel disease and the skin disease psoriasis, while other polymorphisms protect against development of these diseases. IL-23 is one of the cytokines involved in the development of T_H17 cells, which stimulate inflammatory reactions (see Chapter 10).
- ***ATG16L1.*** A loss of function polymorphism in this gene that replaces a threonine in position 300 with an alanine is associated with inflammatory bowel disease. ATG16L1 is one of a family of proteins involved in autophagy, a cellular response to infection, nutrient deprivation and other forms of stress. In this process, the stressed cell eats its own organelles to provide substrates for energy generation and metabolism or an infected cell captures intracellular microbes and targets them to lysosomes. Autophagy may play a role in the maintenance of intact intestinal epithelial cells or the destruction of microbes that have entered the cytoplasm. It is also a mechanism for delivering cytosolic contents to the class II MHC pathway in antigen-presenting cells. A susceptibility allele of *ATG16L1* encodes a protein that is more rapidly destroyed in conditions of stress, and this results in defective autophagic clearance of intracellular microbes. How this polymorphism contributes to inflammatory bowel disease is not known.

Although many genetic associations with autoimmune diseases have been reported, a continuing challenge is to correlate the genetic polymorphisms with the pathogenesis of the diseases. It is also possible that epigenetic changes may regulate gene expression and thus contribute to disease onset. This possibility remains to be established.

Inherited Single-Gene (Mendelian) Abnormalities That Cause Autoimmunity

Studies with mouse models and patients have identified several genes that strongly influence the maintenance of tolerance to self antigens (Table 15-4). Unlike the complex polymorphisms described previously, these

single-gene defects are examples of Mendelian disorders in which the mutation is rare but has a high penetrance, so that most individuals carrying the mutation are affected. We mentioned many of these genes earlier in the chapter, when we discussed the mechanisms of self-tolerance. Although these genes are associated with rare autoimmune diseases, their identification has provided valuable information about the importance of various molecular pathways in the maintenance of self-tolerance. The known genes contribute to the established mechanisms of central tolerance *(AIRE)*, generation of regulatory T cells *(FOXP3, IL2, IL2R)*, anergy and the function of regulatory T cells *(CTLA4)*, and peripheral deletion of T and B lymphocytes *(FAS, FASL)*. Here we describe two other genes that are associated with autoimmune diseases in humans.

- ***Genes encoding complement proteins.*** Genetic deficiencies of several complement proteins, including C1q, C2, and C4 (see Chapter 13), are associated with lupus-like autoimmune diseases. The postulated mechanism of this association is that complement activation promotes the clearance of circulating immune complexes and apoptotic cell bodies, and in the absence of complement proteins, these complexes accumulate in the blood and are deposited in tissues and the antigens of dead cells persist.
- ***FcγRIIB.*** A polymorphism altering an isolecine to a threonine in the transmembrane domain of this inhibitory Fc receptor (see Chapter 12) impairs inhibitory signaling and is associated with SLE in humans. Genetic deletion of this receptor in mice also results in a lupus-like autoimmune disease. The likely mechanism of the disease is a failure to control antibody-mediated feedback inhibition of B cells.

Role of Infections in Autoimmunity

Viral and bacterial infections may contribute to the development and exacerbation of autoimmunity. In patients and in some animal models, the onset of autoimmune diseases is often associated with or preceded by infections. In most of these cases, the infectious microorganism is not present in lesions and is not even detectable in the individual when autoimmunity develops. Therefore, the lesions of autoimmunity are not due to the infectious agent itself but result from host immune responses that may be triggered or dysregulated by the microbe.

Infections may promote the development of autoimmunity by two principal mechanisms (Fig. 15-12).

- Infections of particular tissues may induce local innate immune responses that recruit leukocytes into the tissues and result in the activation of tissue APCs. These APCs begin to express costimulators and secrete T cell–activating cytokines, resulting in the breakdown of T cell tolerance. Thus, the infection results in the activation of T cells that are not specific for the infectious pathogen; this type of response is called **bystander activation.** The importance of aberrant expression of costimulators is suggested by experimental evidence that immunization of mice with self antigens together with strong adjuvants (which mimic microbes) results in the breakdown of self-tolerance and the development of autoimmune disease. In other experimental models, viral antigens expressed in tissues such as islet β cells induce T cell tolerance, but systemic infection of the mice with the virus results in the failure of tolerance and autoimmune destruction of the insulin-producing cells.

  Microbes may also engage Toll-like receptors (TLRs) on dendritic cells, leading to the production of lymphocyte-activating cytokines, and on autoreactive B cells, leading to autoantibody production. A role of TLR signaling in autoimmunity has been demonstrated in mouse models of SLE.
- Infectious microbes may contain antigens that cross-react with self antigens, so immune responses to the microbes may result in reactions against self antigens. This phenomenon is called **molecular mimicry** because the antigens of the microbe cross-react with, or mimic, self antigens. One example of an immunologic cross-reaction between microbial and self antigens is rheumatic fever, which develops after streptococcal infections and is caused by anti-streptococcal antibodies that cross-react with myocardial proteins. These antibodies are deposited in the heart and cause myocarditis. Molecular sequencing has revealed numerous short stretches of homologies between myocardial proteins and streptococcal proteins. However, the significance of limited homologies between microbial and self antigens in common autoimmune diseases remains to be established.

Some infections may protect against the development of autoimmunity. Epidemiologic studies suggest that reducing infections increases the incidence of type 1 diabetes and multiple sclerosis, and experimental studies show that diabetes in NOD mice is greatly retarded if the mice are infected. It seems paradoxical that infections can be triggers of autoimmunity and also inhibit autoimmune diseases. How they may reduce the incidence of autoimmune diseases is unknown.

The intestinal and cutaneous microbiome may influence the development of autoimmune diseases. As we discussed in Chapter 14, there is great interest in the idea that humans are colonized by commensal microbes that have significant effects on the maturation and activation of the immune system. It is not surprising that alterations in the microbiome also affect the incidence and severity of autoimmune diseases in experimental models. How this idea can be exploited to treat autoimmunity is a topic of great interest.

Other Factors in Autoimmunity

The development of autoimmunity is related to several factors in addition to susceptibility genes and infections.

- ***Anatomic alterations in tissues, caused by inflammation (possibly secondary to infections), ischemic injury, or trauma, may lead to the exposure of self antigens that are normally concealed from the immune system.*** Such sequestered antigens may not have induced self-tolerance. Therefore, if previously hidden self an-

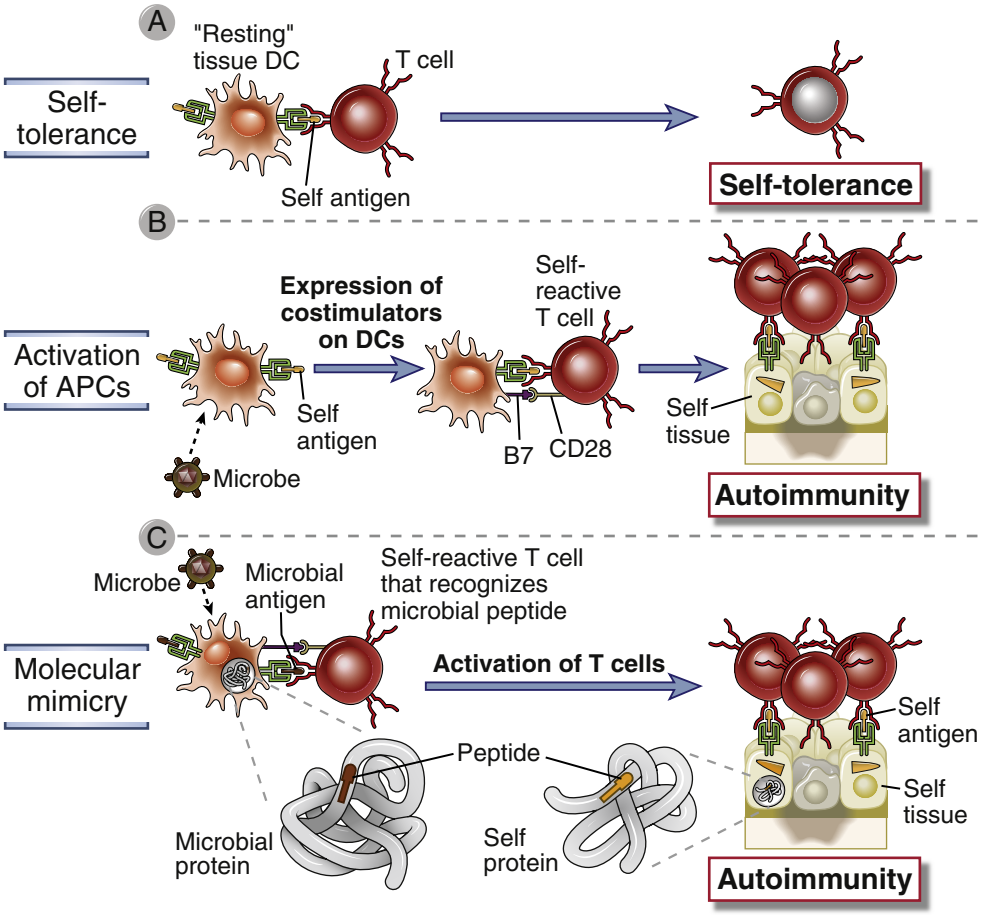

FIGURE 15-12 Role of infections in the development of autoimmunity. A, Normally, encounter of a mature self-reactive T cell with a self antigen presented by a costimulator-deficient resting tissue antigen-presenting cell (APC) results in peripheral tolerance by anergy. (Other possible mechanisms of self-tolerance are not shown.) **B,** Microbes may activate the APCs to express costimulators, and when these APCs present self antigens, the self-reactive T cells are activated rather than rendered tolerant. **C,** Some microbial antigens may cross-react with self antigens (molecular mimicry). Therefore, immune responses initiated by the microbes may activate T cells specific for self antigens.

tigens are released, they can interact with immunocompetent lymphocytes and induce specific immune responses. Examples of anatomically sequestered antigens include intraocular proteins and sperm. Posttraumatic uveitis and orchitis are thought to be due to autoimmune responses against self antigens that are released from their normal locations by trauma.

- ***Hormonal influences play a role in some autoimmune diseases.*** Many autoimmune diseases have a higher incidence in women than in men. For instance, SLE affects women about 10 times more frequently than men. The SLE-like disease of (NZB × NZW)F_1 mice develops only in females and is retarded by androgen treatment. Whether this female predominance results from the influence of sex hormones or other gender-related factors is not known.

Autoimmune diseases are among the most challenging scientific and clinical problems in immunology. The current knowledge of pathogenic mechanisms remains incomplete, so theories and hypotheses continue to outnumber facts. The application of new technical advances and the rapidly improving understanding of self-tolerance will, it is hoped, lead to clearer and more definitive answers to the enigmas of autoimmunity.

SUMMARY

- Immunologic tolerance is unresponsiveness to an antigen induced by the exposure of specific lymphocytes to that antigen. Tolerance to self antigens is a fundamental property of the normal immune system, and the failure of self-tolerance leads to autoimmune diseases. Antigens may be administered in ways that induce tolerance rather than immunity, and this may be exploited for the prevention and treatment of transplant rejection and autoimmune and allergic diseases.
- Central tolerance is induced in the generative lymphoid organs (thymus and bone marrow) when immature lymphocytes encounter self antigens

present in these organs. Peripheral tolerance occurs when mature lymphocytes recognize self antigens in peripheral tissues under particular conditions.

- In T lymphocytes, central tolerance occurs when immature thymocytes with high-affinity receptors for self antigens recognize these antigens in the thymus. Some immature T cells that encounter self antigens in the thymus die (negative selection), and others develop into FoxP3$^+$ regulatory T lymphocytes that function to control responses to self antigens in peripheral tissues.
- Several mechanisms account for peripheral tolerance in mature T cells. In CD4$^+$ T cells, anergy is induced by antigen recognition without adequate costimulation or by engagement of inhibitory receptors such as CTLA-4 and PD-1. Regulatory T cells inhibit immune responses by multiple mechanisms. T cells that encounter self antigens without other stimuli or that are repeatedly stimulated may die by apoptosis.
- In B lymphocytes, central tolerance is induced when immature B cells recognize multivalent self antigens in the bone marrow. The result is the acquisition of a new specificity, called receptor editing, or apoptotic death of the immature B cells. Mature B cells that recognize self antigens in the periphery in the absence of T cell help may be rendered anergic and ultimately die by apoptosis or become functionally unresponsive because of the activation of inhibitory receptors.
- Autoimmunity results from a failure of self-tolerance. Autoimmune reactions may be triggered by environmental stimuli, such as infections, in genetically susceptible individuals.
- Most autoimmune diseases are polygenic, and numerous susceptibility genes contribute to disease development. The greatest contribution is from MHC genes; other genes are believed to influence the selection or regulation of self-reactive lymphocytes.
- Infections may predispose to autoimmunity by several mechanisms, including enhanced expression of costimulators in tissues and cross-reactions between microbial antigens and self antigens. Some infections may protect individuals from autoimmunity, by unknown mechanisms.

SELECTED READINGS

Immunologic Tolerance, General Mechanisms

Baxter AG, Hodgkin PD: Activation rules: the two-signal theories of immune activation, *Nature Reviews Immunology* 2:439–446, 2002.

Goodnow CC, Sprent J, Fazekas de St Groth B, Vinuesa CG: Cellular and genetic mechanisms of self tolerance and autoimmunity, *Nature* 435:590–597, 2005.

Mueller DL: Mechanisms maintaining peripheral tolerance, *Nature Immunology* 11:21–27, 2010.

Parish IA, Heath WR: Too dangerous to ignore: self-tolerance and the control of ignorant autoreactive T cells, *Immunology Cell Biology* 86:146–152, 2008.

Probst HC, Muth S, Schild H: Regulation of tolerogenic function of steady-state DCs, *European Journal of Immunology*, 44:927-933, 2014.

Redmond WL, Sherman LA: Peripheral tolerance of CD8 T lymphocytes, *Immunity* 22:275–284, 2005.

Schwartz RH: Historical overview of immunological tolerance, *Cold Spring Harbor Perspectives in Biology* 4:a006908, 2012.

Shlomchik MJ: Sites and stages of autoreactive B cell activation and regulation, *Immunity* 28:18–28, 2008.

Steinman RM, Hawiger D, Nussenzweig MC: Tolerogenic dendritic cells, *Annual Review of Immunology* 21:685–711, 2003.

Von Boehmer H, Melchers F: Checkpoints in lymphocyte development and autoimmune disease, *Nature Immunology* 11:14–20, 2010.

Central Tolerance

Hogquist KA, Baldwin TA, Jameson SC: Central tolerance: learning self-control in the thymus, *Nature Reviews Immunology* 5:772–782, 2005.

Kyewski B, Klein L: A central role for central tolerance, *Annual Review of Immunology* 24:571–606, 2006.

Laan M, Peterson P: The many faces of Aire in central tolerance, *Frontiers in Immunology* 4:1–6, 2013.

Mathis D, Benoist C: *Aire. Annual Review of Immunology* 27: 287–312, 2009.

Nemazee D: Receptor editing in lymphocyte development and central tolerance, *Nature Reviews Immunology* 6:728–740, 2006.

Anergy; Inhibitory Receptors

Mueller DL: E3 ubiquitin ligases as T cell anergy factors, *Nature Immunology* 5:883–890, 2004.

Okazaki T, Chikuma S, Iwai Y, Fagarasan S, Honjo T: A rheostat for immune responses: the unique properties of PD-1 and their advantages for clinical applications, *Nature Immunology* 14:1212–1218, 2013.

Walker LS, Sansom DM: The emerging role of CTLA-4 as a cell-extrinsic regulator of T cell responses, *Nature Reviews Immunology* 11:852–863, 2011.

Wells AD: New insights into the molecular basis of T cell anergy: anergy factors, avoidance sensors, and epigenetic imprinting, *Journal of Immunology* 182:7331–7341, 2009.

Apoptosis

Bidere N, Su HC, Lenardo MJ: Genetic disorders of programmed cell death in the immune system, *Annual Review of Immunology* 24:321–352, 2006.

Griffith TS, Ferguson TA: Cell death in the maintenance and abrogation of tolerance: the five Ws of dying cells, *Immunity* 35:456–466, 2011.

Strasser A, Jost PJ, Nagata S: The many roles of FAS receptor signaling in the immune system, *Immunity* 30:321–326, 2009.

Strasser A, Puthalakath H, O'Reilly LA, Bouillet P: What do we know about the mechanisms of elimination of autoreactive T and B cells and what challenges remain, *Immunology and Cell Biology* 86:57–66, 2008.

Regulatory T Cells

Bilate AM, Lafaille JJ: Induced CD4+Foxp3+ regulatory T cells in immune tolerance, *Annual Review of Immunology* 30: 733–758, 2012.

Burzyn D, Benoist C, Mathis D: Regulatory T cells in nonlymphoid tissues, *Nature Immunology* 14:1007–1013, 2013.
Campbell DJ, Koch MA: Phenotypic and functional specialization of FoxP3+ regulatory T cells, *Nature Reviews Immunology* 11:119–130, 2011.
Curotto MA, Lafaille JL: Natural and adaptive Foxp3+ regulatory T cells: more of the same or a division of labor? *Immunity* 30:626–635, 2009.
Hsieh C-S, Lee M-H, Lio C-WJ: Selection of regulatory T cells in the thymus, *Nature Reviews Immunology* 12:157–167, 2012.
Josefowicz SZ, Lu L-F, Rudensky Y: Regulatory T cells: mechanisms of differentiation and function, *Annual Review of Immunology* 30:531–564, 2012.
Li MO, Flavell RA: TGF-β: a master of all T cell trades, *Cell* 134:392–404, 2008.
Liston A, Gray DHD: Homeostatic control of regulatory T cell diversity, *Nature Reviews Immunology* 14:154–165, 2014.
Liston A, Piccirillo CA: Developmental plasticity of murine and human Foxp3+ regulatory T cells, *Advances in Immunology* 119:85–106, 2013.
Ohkura N, Kitagawa Y, Sakaguchi S: Development and maintenance of regulatory T cells, *Immunity* 38:414–423, 2013.
Riley JL, June CH, Blazar BR: Human T regulatory cell therapy: take a billion or so and call me in the morning, *Immunity* 30:656–665, 2009.
Sakaguchi S, Miyara M, Costantino CM, Hafler DA: FOXP3+ regulatory T cells in the human immune system, *Nature Reviews Immunology* 10:490–500, 2010.
Sakaguchi S, Yamaguchi T, Nomura T, Ono M: Regulatory T cells and immune tolerance, *Cell* 133:775–787, 2008.
Tang Q, Bluestone JA: The Foxp3+ regulatory T cell: a jack of all trades, master of regulation, *Nature Immunology* 9:239–244, 2008.
Wing K, Sakaguchi S: Regulatory T cells exert checks and balances on self tolerance and autoimmunity, *Nature Immunology* 11:7–13, 2010.
Ziegler SF: FoxP3: of mice and men, *Annual Review of Immunology* 6:209–226, 2006.

Mechanisms of Autoimmunity: Genetics

Cheng MH, Anderson MS: Monogenic autoimmunity, *Annual Review of Immunology* 30:393–427, 2012.
Deitiker P, Atassi MZ: Non-MHC genes linked to autoimmune disease, *Crticial Reviews of Immunology* 32:193–285, 2012.
Fernando MM, Stevens CR, Walsh EC, De Jager PL, Goyette P, Plenge RM, Vyse TJ, Rioux JD: Defining the role of the MHC in autoimmunity: a review and pooled analysis, *PLoS Genetics* 4, 2008. e1000024.
Gregersen PK, Olsson LM: Recent advances in the genetics of autoimmune disease, *Annual Review of Immunology* 27: 363–391, 2009.
Pascual V, Chaussubel D, Banchereau J: A genomic approach to human autoimmune diseases, *Annual Review of Immunology* 28:535–571, 2010.
Voight BF, Cotsapas C: Human genetics offers an emerging picture of common pathways and mechanisms in autoimmunity, *Current Opinion in Immunology* 24:552–557, 2012.
Zenewicz L, Abraham C, Flavell RA, Cho J: Unraveling the genetics of autoimmunity, *Cell* 140:791–797, 2010.

Mechanisms of Autoimmunity: Environmental Factors

Belkaid Y, Hand TW: Role of the microbiota in immunity and inflammation, *Cell* 157:121–141, 2014.
Chervonsky A: Influence of microbial environment on autoimmunity, *Nature Immunology* 11:28–35, 2010.
Fourneau JM, Bach JM, van Endert PM, Bach JF: The elusive case for a role of mimicry in autoimmune diseases, *Molecular Immunology* 40:1095–1102, 2004.
Mathis D, Benoist C: Microbiota and autoimmune disease: the hosted self, *Cell Host Microbes* 10:297–301, 2011.