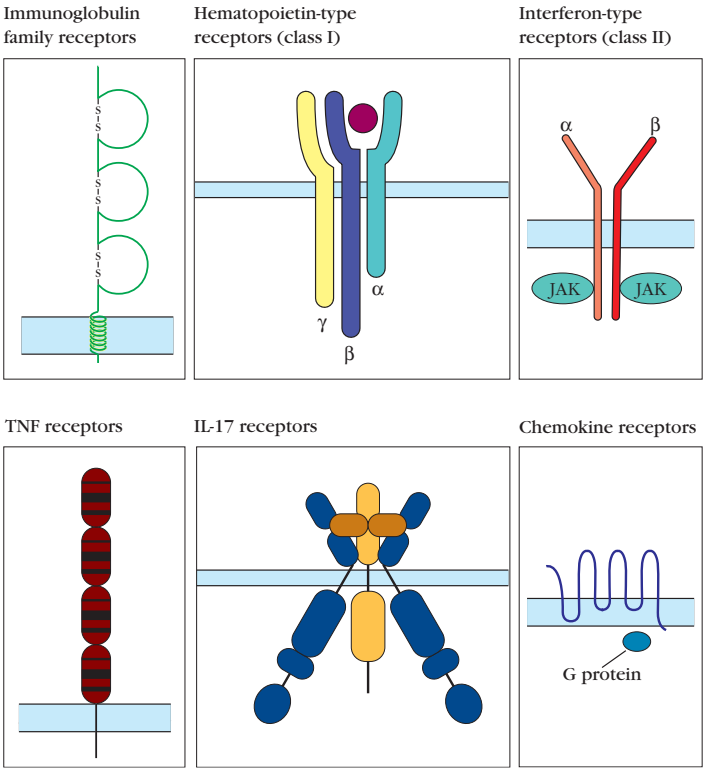
4

Receptors and Signaling: Cytokines and Chemokines



Cytokine and Chemokine Receptor Families

- General Properties of Cytokines and Chemokines
- Six Families of Cytokines and Associated Receptor Molecules
- Cytokine Antagonists
- Cytokine-Related Diseases
- Cytokine-Based Therapies

The hundreds of millions of cells that comprise the vertebrate immune system are distributed throughout the body of the host (see Chapter 2). Some cells circulate through the blood and lymph systems, whereas others are sessile (remain in place) in the primary and secondary lymphoid tissues, the skin, and the mucosa of the respiratory, alimentary, and genito-urinary tracts. The key to success for such a widely dispersed organ system is the ability of its various components to communicate quickly and efficiently with one another, so that the right cells can home to the appropriate locations and take the necessary measures to destroy invading pathogens.

Molecules that communicate among cells of the immune system are referred to as **cytokines**. In general, cytokines are soluble molecules, although some also exist in membrane-bound forms. The interaction of a cytokine with its receptor on a target cell can cause changes in the expression of adhesion molecules and chemokine receptors on the target membrane, thus allowing it to move from one location to another. Cytokines can also signal an immune cell to increase or decrease the activity of particular enzymes or to change its transcriptional program, thereby altering and enhancing its effector functions. Finally, they can instruct a cell when to survive and when to die.

In an early attempt to classify cytokines, immunologists began numbering them in the order of their discovery, and naming them **interleukins**. This name reflects the fact that interleukins communicate between (Latin, *inter*) white blood cells (*leukocytes*). Examples include interleukin 1 (IL-1), secreted by macrophages, and interleukin 2 (IL-2), secreted by activated T cells. However, many cytokines that were named prior to this attempt at rationalizing nomenclature have resisted reclassification, and so students will come across cytokines such as Tumor Necrosis Factor or Interferons, that are also "interleukins" in all but name.

Although the term *cytokine* refers to all molecules that communicate among immune cells, the name **chemokine** is used specifically to describe that subpopulation of cytokines that share the specific purpose of mobilizing immune cells from one organ, or indeed, from one part of an organ, to another. Chemokines belong to the class of molecules called **chemoattractants**, molecules that attract cells by influencing the assembly, disassembly, and contractility of cytoskeleton proteins and the expression of cell-surface adhesion molecules. Chemokines attract cells with the appropriate chemokine receptors to regions where the chemokine concentration is highest. For example, chemokines are important in attracting cells of

the innate immune system to the site of infection and inducing T cells to move toward antigen-presenting cells in the secondary lymphoid tissues. Leukocytes change their pattern of expression of chemokine receptors over the course of an immune response, first migrating to the secondary immune organs, in which they undergo differentiation to mature effector cells, and then moving out into the affected tissues to fight the infection, responding to different chemokine gradients with each movement. As we will learn in a later section, chemokines are also capable of instructing cells to alter their transcriptional programs.

The classification and nomenclature of chemokines is more logical than that of interleukins, and is based on their biochemical structures. Although chemokines technically fall under the umbrella classification of "cytokines," normal usage is evolving such that the term *chemokine* is used when referring to molecules that move immune cells from place to place, and the term *cytokine* is employed when referring to any other messenger molecule of the immune system.

Like all signaling molecules, cytokines can be further classified on the basis of the distance between the cell secreting the signaling ligand and the cell receiving that chemical signal. Cytokines that act on cells some distance away from the secreting cell, such that they must pass through the bloodstream before reaching their target, are referred to as **endocrine** (Figure 4-1). Those that act on cells near the secreting cell, such that the cytokine merely has to diffuse a few Ångstroms through tissue fluids or across an immunological synapse, are referred to as **paracrine**. Sometimes, a cell needs to receive a signal through its own membrane receptors from a cytokine that it, itself, has secreted. This type of signaling is referred to as **autocrine**. Of note, the T-cell interleukin IL-2 acts effectively in all three modes. Unlike the classical hormones, such as insulin and glucagon, that generally act at long range in an endocrine fashion, many cytokines act over a short distance in an autocrine or paracrine fashion.

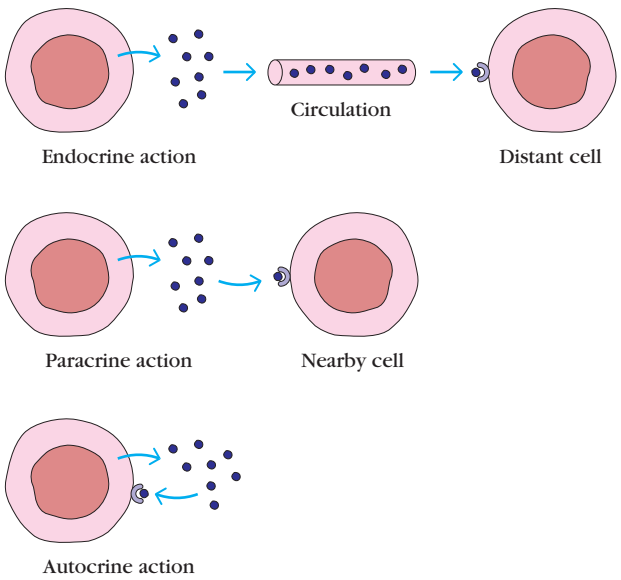


FIGURE 4-1 Most immune system cytokines exhibit autocrine and/or paracrine action; fewer exhibit endocrine action.

We begin this chapter with an introduction to the general properties of cytokines and chemokines followed by a discussion of the specific receptors and signaling pathways used by the six families of immune system cytokines and chemokines. Next, we describe the ways in which cytokine signaling can be regulated by antagonists. Finally, we turn to the role of cytokines and chemokines in disease and medicine.

General Properties of Cytokines and Chemokines

The activity of cytokines was first recognized in the mid-1960s, when supernatants derived from in vitro cultures of lymphocytes were found to contain soluble factors, usually proteins or glycoproteins, that could regulate proliferation, differentiation, and maturation of immune system cells. Production of these factors by cultured lymphocytes was induced by activation with antigens or with nonspecific **mitogens** (molecules inducing cell division, or mitosis). However, biochemical isolation and purification of cytokines was initially hampered because of their low concentrations in the culture supernatants and the absence of well-defined assay systems for individual cytokines.

The advent of hybridoma technology (see Chapter 20) allowed the production of artificially generated T-cell tumors that constitutively produced IL-2, allowing for its purification and characterization. Gene cloning techniques developed during the 1970s and 1980s then made it possible to generate pure cytokines by expressing the proteins from cloned genes derived from hybridomas or from normal leukocytes, after transfection into bacterial or yeast cells. Using these pure cytokine preparations, researchers were able to identify cell lines whose growth depended on the presence of a particular cytokine, thus providing them with biological cytokine assay systems. Since then, monoclonal antibodies specific for many cytokines have made it possible to develop rapid, quantitative, cytokine-specific immunoassays. ELISA assays measure the concentrations of cytokines in solution, Elispot assays quantitate the cytokines secreted by individual cells, and cytokine-specific antibodies can be used to identify cytokine-secreting cells using intracellular cytokine staining followed by flow cytometry or immuno-fluorescence microscopy (see Chapter 20).

Cytokines Mediate the Activation, Proliferation, and Differentiation of Target Cells

Cytokines bind to specific receptors on the membranes of target cells, triggering signal transduction pathways that ultimately alter enzyme activity and gene expression (Figure 4-2). The susceptibility of a target cell to a particular cytokine is determined by the presence of specific membrane receptors. In general, cytokines and their fully assembled receptors exhibit very high affinity for one another, with dissociation constants for cytokines and their receptors ranging from 10^{-8} to 10^{-12} M^{-1}. Because their receptor affinities are so high and because cytokines are often secreted in close proximity to their receptors, such that the cytokine concentration is not diluted by diffusion (as mentioned in Chapter 3), the secretion of very few cytokine molecules can mediate powerful biological effects.

Cytokines regulate the *intensity* and *duration* of the immune response by stimulating or inhibiting the activation, proliferation, and/or differentiation of various cells, by regulating the secretion of other cytokines or of antibodies, or in some cases by actually inducing programmed cell death in the target cell. In addition, cytokines can modulate the expression of various cell-surface receptors for chemokines, other cytokines, or even for themselves. Thus, the cytokines secreted by even a small number of antigen-activated lymphocytes can influence the activity of many different types of cells involved in the immune response.

Cytokines exhibit the attributes of pleiotropy, redundancy, synergism, antagonism, and cascade induction (Figure 4-3), which permit them to regulate cellular activity in a coordinated, interactive way. A cytokine that induces different biological effects depending on the nature of the target cells is said to have a **pleiotropic** action, whereas two or more cytokines that mediate similar functions are said to be **redundant**. Cytokine **synergy** occurs when the combined effect of two cytokines on cellular activity is greater than the additive effects of the individual cytokines. In some cases, the effects of one cytokine inhibit or **antagonize** the effects of another. **Cascade induction** occurs when the action of one cytokine on a target cell induces that cell to produce one or more additional cytokines.

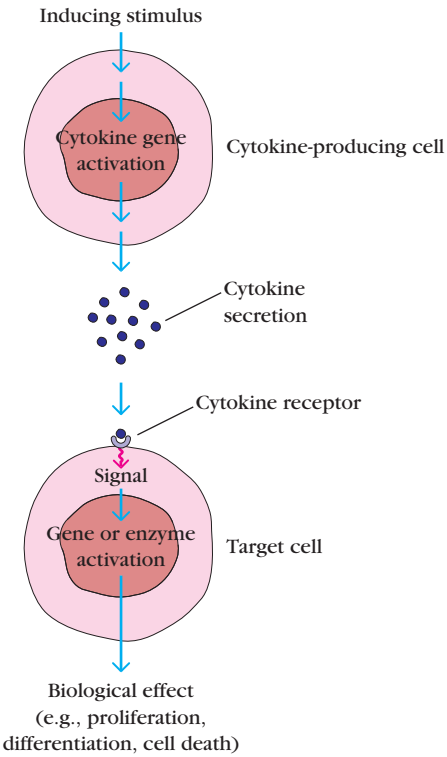


FIGURE 4-2 Overview of the induction and function of cytokines. An inducing stimulus, which may be an antigen or another cytokine, interacts with a receptor on one cell, inducing it to secrete cytokines that in turn act on receptors of a second cell, bringing about a biological consequence. In the case of IL-2, both cells may be antigen-activated T cells that secrete IL-2, which acts both on the secreting cell and on neighboring, activated T cells.

Cytokines Have Numerous Biological Functions

Although a variety of cells can secrete cytokines that instruct the immune system, the principal producers are T_H cells, dendritic cells, and macrophages. Cytokines released from these cell types are capable of activating entire networks of interacting cells (Figure 4-4). Among the numerous physiological responses that require cytokine involvement are the generation of cellular and humoral immune responses, the induction of the inflammatory response, the regulation of hematopoiesis, and wound healing.

The total number of proteins with cytokine activity grows daily as research continues to uncover new ones. Table 4-1 summarizes the activities of some commonly encountered cytokines. An expanded list of cytokines can be found in Appendix II. Note, however, that many of the listed functions have been identified from analyses of the effects of recombinant cytokines, sometimes added alone to in vitro systems at nonphysiologic concentrations. In vivo, cytokines rarely, if ever, act alone. Instead, a target cell is exposed to a milieu containing a mixture of cytokines whose combined synergistic or antagonistic effects can have a wide variety of consequences. In addition, as we have learned, cytokines often induce the synthesis of other cytokines, resulting in cascades of activity.

Cytokines Can Elicit and Support the Activation of Specific T-Cell Subpopulations

As described in Chapters 2 and 11, helper T cells can be classified into subpopulations, each of which is responsible for the support of a different set of immune functions. For example, T_H1 cells secrete cytokines that promote the differentiation and activity of macrophages and cytotoxic T cells, thus leading to a primarily cytotoxic immune response, in which cells that have been infected with viruses and

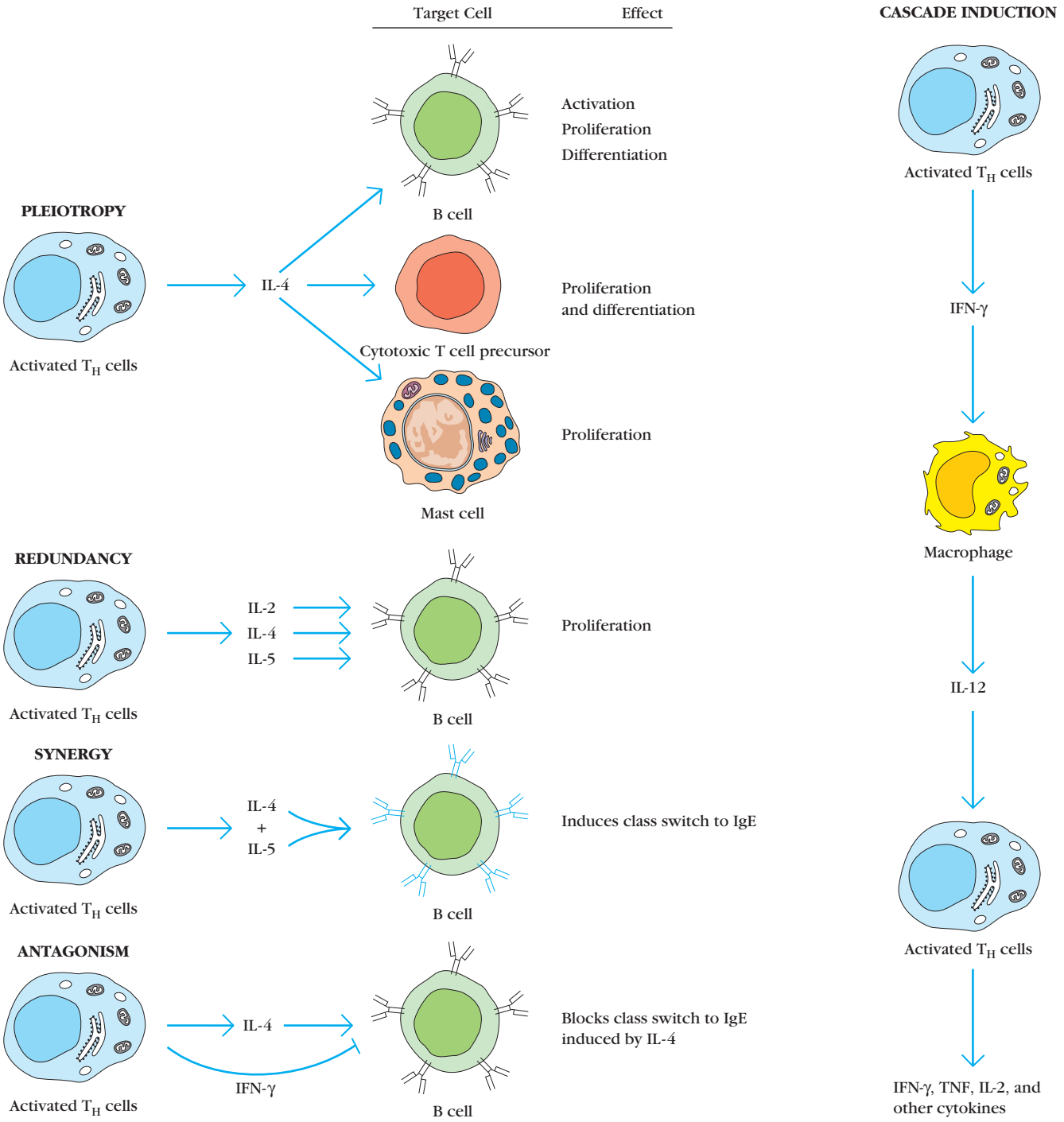


FIGURE 4-3 **Cytokine attributes of (a) pleiotropy, redundancy, synergism, antagonism, and (b) cascade induction.**

intracellular bacteria are recognized and destroyed. The cytokines IL-12 and interferon (IFN) γ induce T_H1 differentiation. In contrast, T_H2 cells activate B cells to make antibodies, which neutralize and bind extracellular pathogens, rendering them susceptible to phagocytosis and complement-mediated lysis. IL-4 and IL-5 support the generation of T_H2 cells. T_H17 cells promote the differentiation of activated macrophages and neutrophils, and support the inflammatory state; their generation is induced by IL-17 and IL-23. The differentiation and activity of each distinctive T-cell subpopulation is therefore supported by the binding of different combinations of cytokines to T-cell surface receptors, with each cytokine combination inducing its own characteristic array of intracellular signals, and sending the helper T cell down a particular differentiation pathway.

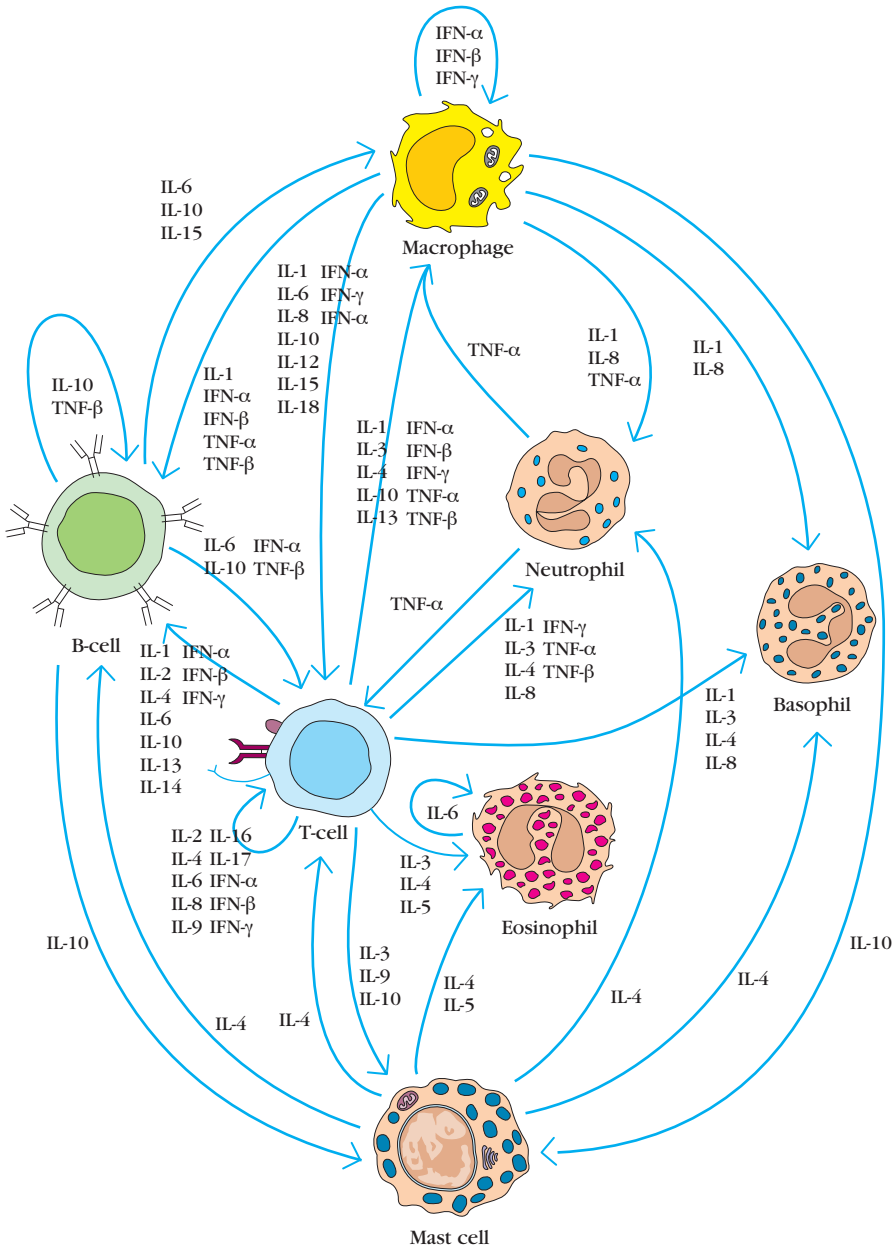


FIGURE 4-4 **The cells of the immune system are subject to control by a network of cytokine actions.**

Cell Activation May Alter the Expression of Receptors and Adhesion Molecules

The ability of cytokines to activate most, if not all, members of particular immune cell subpopulations appears to conflict with the established specificity of the immune system. What keeps cytokines from activating all T cells, for example, in a nonspecific fashion during the immune response?

In order for a cell to respond to a signaling molecule, it must express receptors for that molecule, and responsiveness to a molecular signal can thus be controlled by signal receptor expression. For example, antigen stimulation of a T cell induces alterations in the T-cell surface expression of chemokine receptors. Reception of chemokine signals through these receptors therefore instructs *only those cells that have previously been activated by antigen* to migrate to nearby lymph nodes or to the spleen. Furthermore, activation-induced changes in the adhesion molecules that are expressed on the cell membrane ensures that stimulated cells migrate to, and then remain in, the location best suited to their function. T-cell activation by antigen also up-regulates the expression of the receptors for cytokines that provide proliferative signals, such as IL-2 (as described in Chapter 3), and also for differentiative cytokines such as IL-4. In this way, following antigen encounter, only those T cells that have been activated by antigen are primed to relocate and to receive the proliferative and differentiative signals they need to function as a mature immune effector cell. This

TABLE 4-1 Functional groups of selected cytokines*

Cytokine	Secreted by†	Targets and effects
	SOME CYTOKINES OF INNATE IMMUNITY	
Interleukin 1 (IL-1)	Monocytes, macrophages, endothelial cells, epithelial cells	Vasculature (inflammation); hypothalamus (fever); liver (induction of acute phase proteins)
Tumor necrosis factor-α (TNF-α)	Macrophages, monocytes, neutrophils, activated T cells and NK cells	Vasculature (inflammation); liver (induction of acute phase proteins); loss of muscle, body fat (cachexia); induction of death in many cell types; neutrophil activation
Interleukin 12 (IL-12)	Macrophages, dendritic cells	NK cells; influences adaptive immunity (promotes T_H1 subset)
Interleukin 6 (IL-6)	Macrophages, endothelial cells, and T_H2 cells	Liver (induces acute phase proteins); influences adaptive immunity (proliferation and antibody secretion of B-cell lineage)
Interferon-α (IFN-α) (this is a family of molecules)	Macrophages dendritic cells, virus-infected cells	Induces an antiviral state in most nucleated cells; increases MHC Class I expression; activates NK cells
Interferon β (IFN-β)	Macrophages, dendritic cells, virus-infected cells	Induces an antiviral state in most nucleated cells; increases MHC Class I expression; activates NK cells
	SOME CYTOKINES OF ADAPTIVE IMMUNITY	
Interleukin 2 (IL-2)	T cells	T-cell proliferation; can promote AICD. NK cell activation and proliferation; B-cell proliferation
Interleukin 4 (IL-4)	T_H2 cells, mast cells	Promotes T_H2 differentiation; isotype switch to IgE
Interleukin 5 (IL-5)	T_H2 cells	Eosinophil activation and generation
Transforming growth factor β (TGF-β)	T cells, macrophages, other cell types	Inhibits T-cell proliferation and effector functions; inhibits B-cell proliferation; promotes isotype switch to IgA; inhibits macrophages
Interferon γ (IFN-γ)	T_H1 cells, $CD8^+$ cells, NK cells	Activates macrophages; increases expression MHC Class I and Class II molecules; increases antigen presentation

*Many cytokines play roles in more than one functional category.

†Only the major cell types providing cytokines for the indicated activity are listed; other cell types may also have the capacity to synthesize the given cytokine. Activated cells generally secrete greater amounts of cytokine than unactivated cells.

pattern of activation-induced alteration in the cell surface expression of adhesion molecules, chemokine receptors, and cytokine receptors is a common strategy employed by the immune system.

Cytokines Are Concentrated Between Secreting and Target Cells

During the process of T-cell activation by an antigen-presenting dendritic cell, or of B-cell activation by a cognate T cell, the respective pairs of cells are held in close juxtaposition for many hours (see Chapter 14). Over that time period, the cells release cytokines that bind to relevant receptors on the partner cell surface, without ever entering the general circulation. Furthermore, during this period of close cell-cell contact, the secretory apparatus of the stimulating cell is oriented so that the cytokines are released right at the region of the cell membrane that is in closest contact with the recipient cell (see Figure 3-4). The close nature of the cell-cell interaction and the directional release of cytokines by the secretory apparatus means that the effective concentration of cytokines in the region of the membrane receptors may be orders of magnitude higher than that experienced outside the contact region of the two cells. Thus, discussions of membrane receptor affinity and cytokine concentrations within tissue fluids must always take into account the biology of the responding system and the geography of the cell interactions involved. In addition, the half-life of cytokines in the bloodstream or other extracellular fluids into which they are secreted is usually very short, ensuring that cytokines usually act for only a limited time and over a short distance.

Signaling Through Multiple Receptors Can Fine Tune a Cellular Response

Cytokine and chemokine signaling in the immune response can be a strikingly complex and occasionally redundant affair. Effector molecules such as cytokines can bind to more

than one receptor, and receptors can bind to more than one signaling molecule. Nowhere is the latter concept more clearly illustrated than in the chemokine system, in which approximately 20 receptors bind to close to 50 distinct chemokines (see Appendix III). Effector molecule signaling can also cooperate with signaling through antigen-specific receptors. Signals received through more than one receptor must then be integrated at the level of the biological response, with multiple pathways acting to tune up or tune down the expression of particular transcription factors or the activity of particular enzymes. Thus, the actual biological response mounted by a cell to a particular chemical signal depends not only on the nature of the individual receptor for that signal, but also on all of the downstream adapters and enzymes present in the recipient cell.

Six Families of Cytokines and Associated Receptor Molecules

In recent years, immunologists have enjoyed an explosion of information about new cytokines and cytokine receptors as a result of advances in genomic and proteomic analysis. Advances Box 4-1 describes a recently developed proteomic approach to the search for new, secreted cytokines and illustrates the manner in which a sophisticated appreciation of the molecular and cell biology of secretory pathways aids in the identification of new cytokines. The purpose of this chapter is not to provide an exhaustive list of cytokines and their receptors (see Appendices II and III for a comprehensive and current list of cytokines and chemokines), but rather to outline

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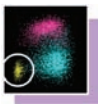

Methods Used to Map the Secretome

The related approaches of genomics and proteomics provide scientists with tools they can use to assess the complex changes that occur in gene and protein expression induced by stimuli, such as antigen or cytokine stimulation. Vast arrays of information regarding the derivation and readout of genes in different cells and organisms, and the expression of particular proteins, can be analyzed and presented in ways not available to scientists just a few years ago.

Recently, the science of proteomics has been extended to address the mapping of proteins that are secreted by various cell types. The array of proteins secreted by a cell is referred to as its *secretome*, and the secretome can be more formally defined as the "proteins released by a cell, tissue, or organisms through classical and nonclassical secretion mechanisms."

Scientists first became interested in the concept of the secretome as a way to diagnose and identify various types of cancer. They reasoned that they could use the set of proteins secreted into the serum or other tissue fluids as a biological marker for specific tumor types. If particular proteins can be shown to be secreted at high concentration only under conditions of malignancy, then rapid and inexpensive tests can be developed that have the potential to screen for tumors at an early stage, when they are still amenable to treatment. Although such tumor-specific profiles of secreted proteins are surprisingly difficult to develop, given the range of mutations associated with the generation of a cancer, the ability to diagnose a tumor at an early stage using only a serum sample provides intense motivation, and many such attempts are ongoing.

The approaches used to define a set of cancer secretomes have since been applied to studies of many other, nonmalignant cell populations for which the description of a secretome would be a useful analytical tool. These populations include stem cells, cells of the immune system, and adipose cells. Given the diversity of cytokines that can be secreted by a single cell, and the manner in which the activities of cytokines can interact at the level of the target cell, cytokine biology is a superb target for such a global approach.

A recent secretome analysis (Botto et al., 2011) addressed the question of how the human cytomegalovirus induces the formation of new blood vessels (angiogenesis). Virus-free supernatant from virus-infected endothelial cells was found to induce angiogenesis. Secretome analysis of the infected endothelial cell supernatant revealed the presence of multiple cytokines, including IL-8, GM-CSF, and IL-6. The addition of a blocking anti-IL-6 antibody at the same time as the virus-free supernatant was then shown to inhibit its angiogenic activity, thus demonstrating that it was the IL-6 activity in the supernatant that was primarily responsible for inducing the new blood vessel growth.

One difficulty that is frequently encountered in trying to analyze the secretome of a particular type of cell is the need of many cells to grow in a tissue culture fluid supplemented with serum, which is itself a complex mixture of proteins. In this case it is important to distinguish between proteins released by the cells under study and those which were originally present in the serum. Several

(continued)

ADVANCES *(continued)* BOX 4-1

techniques are available to discriminate between secreted proteins and those from the tissue culture media, including adding inhibitors of secretion to some cultures and then comparing those proteins present in the culture supernatant in the presence and absence of inhibitors. Alternatively, culturing the cells in the presence of radioisotopes that only label newly synthesized proteins, such as ^{35}S methionine, can be used to distinguish these proteins from preexisting proteins in the culture medium.

In the case, such as that described above, that a cell line is being tested to determine whether it secretes a set of cytokines for which antibody assays already exist, two different types of multiplex measurements may be used (see Figure 1). Both of these approaches utilize antibodies to the array of cytokines to be analyzed, attached to some sort of solid phase support. This support may be glass, a membrane, or a set of beads, with each antibody attached to a bead of a different color. The sample of tissue culture fluid is added to the solid phase antibody, excess fluid is washed away, and then biotinylated antibodies are added. (Biotin, a small molecule, is used because it has an extremely high affinity for a protein, streptavidin, and is therefore used to couple two molecules together in assays such as these. For more details, see Chapter 20.) After antibody binding, the excess biotinylated antibodies are removed by washing and the cytokine concentrations are assessed by the addition of fluorescent streptavidin, which will bind to the biotin. A fluorescent signal indicates the presence of the cytokine in the sample, and the level of the signal reveals its concentration Since each bead fluoresces at a different wavelength, the fluorescence associated with each cytokine can be distinguished.

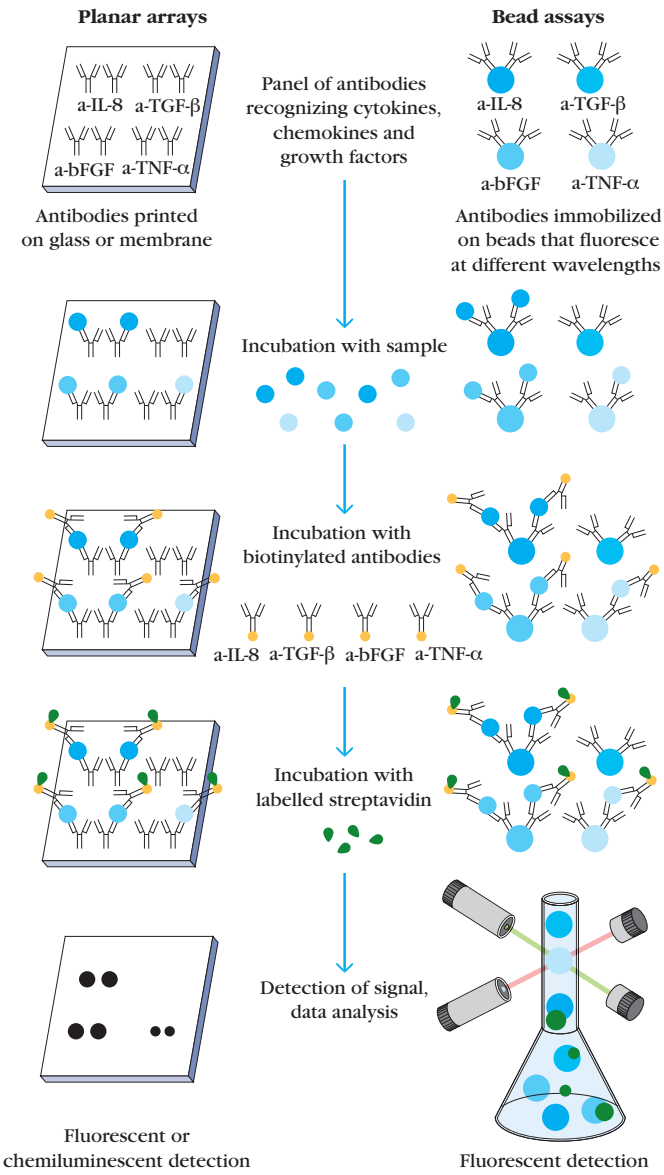

FIGURE 1
Principle of planar and bead-based multiplex detection and quantitation of cytokines, chemokines, growth factors, and other proteins. Assays use antibodies against (a-) various cytokines, and biotin (yellow)-streptavidin (green) conjugation. See text for details. *[Adapted from H. Skalnikova et al., Mapping of the secretome of primary isolates of mammalian cells, stem cells and derived cell lines, 2011,* Proteomics ***11:****691.]*

Various bioinformatics tools have been developed that have particular application to secretome analysis. These include *SignalP*, which identifies the presence of signal peptides and also shows the location of signal peptide cleavage sites in bacterial and eukaryotic proteins. In addition, *SecretomeP* can be used for the prediction of nonclassically secreted proteins. Several bioinformatics tools, including *TargetP* and *Protein Prowler*, use the protein sequence to predict its subcellular localization. Finally, *Ingenuity Pathway Analysis* allows the investigator to search for protein interaction partners and to predict the involvement of the protein of interest in functional networks.

Botto, S., D. N. Streblow, V. DeFilippis, L. White, C. N. Kreklywich, P. P. Smith, and P. Caposio. (2011). IL-6 in human cytomegalovirus secretome promotes angiogenesis and survival of endothelial cells through the stimulation of survivin. *Blood* **117**:352–361.

TABLE 4-2 Six Cytokine Families

Family name	Representative members of family	Comments
Interleukin 1 family	IL-1α, IL-1β, IL-1Ra, IL-18, IL-33	IL-1 was the first noninterferon cytokine to be identified. Members of this family include important inflammatory mediators.
Hematopoietin (Class I cytokine) family	IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-12, IL-13, IL15, IL-21, IL-23, GM–CSF, G-CSF, Growth hormone, Prolactin, Erythropoietin/hematopoietin	This large family of small cytokine molecules exhibits striking sequence and functional diversity.
Interferon (Class II cytokine) family	IFN-α, IFN-β, IFN-γ, IL-10, IL-19, IL-20, IL-22, IL-24	While the IFNs have important roles in anti-viral responses, all are important modulators of immune responses.
Tumor Necrosis Factor family	TNF-α, TNF-β, CD40L, Fas (CD95), BAFF, APRIL, LTβ	Members of this family may be either soluble or membrane bound; they are involved in immune system development, effector functions, and homeostasis.
Interleukin 17 family	IL-17 (IL17-A), IL17B, C, D, and F	This is the most recently discovered family; members function to promote neutrophil accumulation and activation, and are proinflammatory.
Chemokines (see Appendix III)	IL-8, CCL19, CCL21, RANTES, CCL2 (MCP-1), CCL3 (MIP-1α)	All serve chemoattractant function.

some general principles of cytokine and receptor architecture and function that should then enable the reader to place any cytokine into its unique biological context.

Detailed studies of cytokine structure and function have revealed common features among families of cytokines. Cytokines are relatively small proteins and generally have a molecular mass of less than 30 kDa. Many are glycosylated, and glycosylation appears to contribute to cytokine stability, although not necessarily to cytokine activity. Cytokines characterized so far belong to one of six groups: the Interleukin 1 (IL-1) family, the Hematopoietin (Class I cytokine) family, the Interferon (Class II cytokine) family, the Tumor Necrosis Factor (TNF) family, the Interleukin 17 (IL-17) family, and the Chemokine family (Table 4-2). Each of these six families of cytokines, the receptors that engage them, and the signaling pathways that transduce the message received upon cytokine binding into the appropriate biological outcome are described in the following pages.

Cytokines of the IL-1 Family Promote Proinflammatory Signals

Cytokines of the **interleukin 1 (IL-1) family** are typically secreted very early in the immune response by dendritic cells and monocytes or macrophages. IL-1 secretion is stimulated by recognition of viral, parasitic, or bacterial antigens by innate immune receptors. IL-1 family members are generally *proinflammatory*, meaning that they induce an increase in the capillary permeability at the site of cytokine secretion, along with an amplification of the level of leukocyte migration into the infected tissues. In addition, IL-1 has systemic (whole body) effects and signals the liver to produce *acute phase proteins* such as the Type I interferons (IFNs α and β), IL-6, and the chemokine CXCL8. These proteins further induce multiple protective effects, including the destruction of viral RNA and the generation of a systemic fever response (which helps to eliminate many temperature-sensitive bacterial strains). IL-1 also activates both T and B cells at the induction of the adaptive immune response.

Cytokines of the IL-1 Family

Members of the IL-1 cytokine and receptor family are shown in Figure 4-5. The canonical (most representative) members of the IL-1 family, IL-1α and IL-1β, are both synthesized as 31 kDa precursors, pro-IL-1α and pro-IL-1β. Pro IL-1α is biologically active, and often occurs in a membrane-bound form, whereas pro-IL-1β requires processing to the fully mature soluble molecule before it can function. Pro-IL-1α and β are both trimmed to their 17 kDa active forms by the proteolytic enzyme caspase-1 inside the secreting cell. Active caspase-1 is located in a complex set of proteins referred to as the *inflammasome* (see Chapter 5).

Other IL-1 family members, IL-18 and IL-33, have also been shown to be processed by caspase-1 in vitro (although there is ambiguity as to whether IL-33 requires this processing for full activity in vivo). IL-18 is related to IL-1, uses the same receptor family, and has a similar function; like IL-1, IL-18 is expressed by monocytes, macrophages, and dendritic cells and is secreted early in the immune response. In contrast, IL-33 is constitutively expressed in smooth muscle and in bronchial epithelia, and its expression can be induced by IL-1β and TNF-α in lung and skin fibroblasts. IL-33 has been shown to induce T_H2 cytokines that promote T-lymphocyte interactions with B cells, mast cells, and eosinophils. IL-33

has also been implicated in the pathology of diseases such as asthma and inflammatory airway and bowel diseases.

Two additional members of this cytokine family act as *natural inhibitors of IL-1 family function*. The soluble protein IL-1Ra (IL-1 *R*eceptor *a*ntagonist) binds to the IL-1RI receptor, but prevents its interaction with its partner receptor chain, IL-1RAcP, thus rendering it incapable of transducing a signal to the interior of the cell. IL-1Ra therefore functions as an **antagonist** ligand of IL-1. IL-18BP adopts a different strategy of inhibition, binding to IL-18 in solution and preventing IL-18 from interacting productively with its receptor. The inhibitory effect of IL-18BP is enhanced by the further binding of IL-1F7 (see Figure 4-5b).

The IL-1 Family of Cytokine Receptors

The Interleukin 1 family of receptors includes the receptors for IL-1, IL-18, and IL-33. Both forms of IL-1—IL-1α and IL-1β—bind to the same receptors and mediate the same responses. Two different receptors for IL-1 are known, and both are members of the immunoglobulin superfamily of proteins (see Chapter 3). Only the type I IL-1R (IL-1RI), which is expressed on many cell types, is able to transduce a cellular signal; the type II IL-1R (IL-1RII) is limited to B cells and is inactive. For full functioning, the Type 1 IL-1R also requires the presence of an interacting accessory protein, IL-1RAcP (IL-1 *R*eceptor *Ac*cessory *P*rotein (see Figure 4-5a).

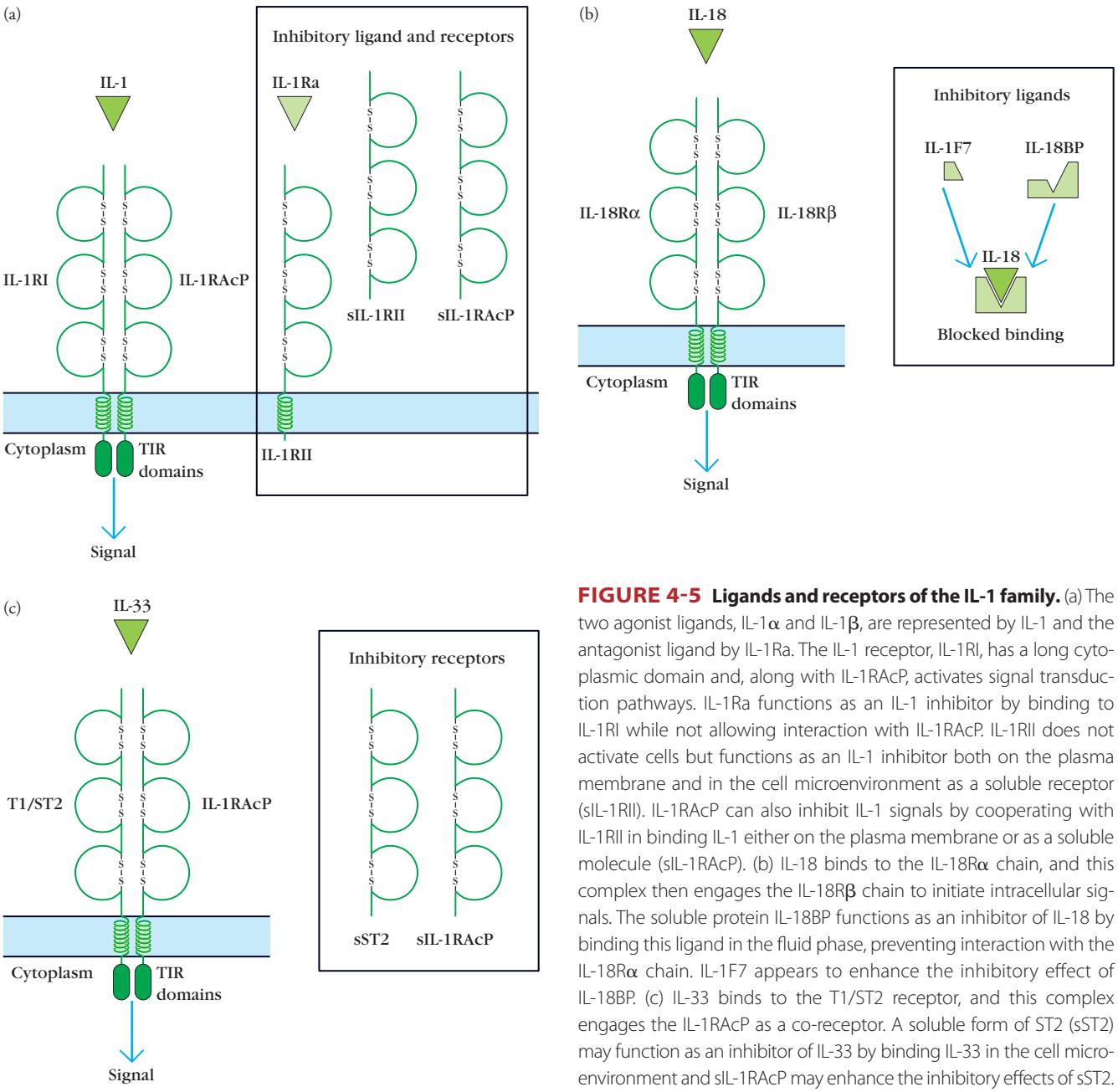

FIGURE 4-5 Ligands and receptors of the IL-1 family. (a) The two agonist ligands, IL-1α and IL-1β, are represented by IL-1 and the antagonist ligand by IL-1Ra. The IL-1 receptor, IL-1RI, has a long cytoplasmic domain and, along with IL-1RAcP, activates signal transduction pathways. IL-1Ra functions as an IL-1 inhibitor by binding to IL-1RI while not allowing interaction with IL-1RAcP. IL-1RII does not activate cells but functions as an IL-1 inhibitor both on the plasma membrane and in the cell microenvironment as a soluble receptor (sIL-1RII). IL-1RAcP can also inhibit IL-1 signals by cooperating with IL-1RII in binding IL-1 either on the plasma membrane or as a soluble molecule (sIL-1RAcP). (b) IL-18 binds to the IL-18Rα chain, and this complex then engages the IL-18Rβ chain to initiate intracellular signals. The soluble protein IL-18BP functions as an inhibitor of IL-18 by binding this ligand in the fluid phase, preventing interaction with the IL-18Rα chain. IL-1F7 appears to enhance the inhibitory effect of IL-18BP. (c) IL-33 binds to the T1/ST2 receptor, and this complex engages the IL-1RAcP as a co-receptor. A soluble form of ST2 (sST2) may function as an inhibitor of IL-33 by binding IL-33 in the cell microenvironment and sIL-1RAcP may enhance the inhibitory effects of sST2.

Note that both the IL-1RI and the IL-1RII receptor chains as well as the receptor accessory protein exist in both soluble and membrane-bound forms. However, *a full signal is transmitted only from the dimer of the membrane-bound forms of IL-1RI and IL-1RAcP*. The alternative, membrane-bound and soluble forms of IL-1 binding proteins, can "soak up" excess cytokine, but they are unable to transduce the interleukin signal. Thus, by secreting more or fewer of these inactive receptors, at different times during an immune response, the organism has the opportunity to fine-tune the cytokine signal by allowing the inactive and soluble receptors to compete with the signal-transducing receptor for available cytokine. This theme finds echoes in other immune system receptor families, and appears to be a frequently evolved strategy for controlling the strength of signals that give rise to important outcomes. In the case of IL-1, the ultimate result of successful IL-1 signaling is a global, proinflammatory state, and so the penalty paid by the host for an inappropriately strong IL-1 response would be physiologically significant and even potentially fatal.

The receptor for IL-18 is also a heterodimer, made up of IL-18Rα and IL-18Rβ. IL-33 is recognized by the IL-1RAcP in combination with a novel receptor protein, variously termed T1/ST-2 or IL-1RL1. As for IL-1, inhibitory receptors exist for IL-33 (see Figure 4-5c).

Signaling from IL-1 Receptors

Productive ligand binding to the extracellular portion of the IL-1 receptor leads to a conformational alteration in its cytoplasmic domain. This structural alteration in the receptor leads to a series of downstream signaling events (Figure 4-6). Most of the themes of these events will be familiar to the reader from Chapter 3, and we will encounter them again in the discussion of innate immune receptors in Chapter 5.

First, binding of the *adapter protein* **MyD88** to the occupied receptor allows recruitment to the receptor complex of one or more members of the ***I*L-1 *R*eceptor *A*ctivated *K*inase (IRAK)** protein family. One of these, IRAK-4, is activated by autophosphorylation and phosphorylates its fellow IRAKs, resulting in the generation of binding sites for *T*NF *R*eceptor *A*ssociated *F*actor 6 (TRAF6), which is associated with a ubiquitin-ligase complex capable of generating polyubiquitin chains. The IRAK-TRAF6 complex now dissociates from the receptor and interacts with a preformed cytosolic complex made up of the kinase *T*GFβ−*A*ssociated *K*inase 1 (TAK1) and two *TA*K1-*B*inding proteins, TAB1 and TAB2. Binding of polyubiquitin chains to the TAB proteins in the TAK1 complex activates it.

The TAK1 complex now performs two functions with which the reader should be familiar. It phosphorylates and activates the IKK complex, leading to the destruction of IκB and the resultant activation of the transcription factor NF-κB (see Figure 3-17). In addition, TRAF6 also plays a role in IKK activation by providing ubiquitination sites to which the NEMO component of IKK can bind, resulting in its further activation. TAK1 also activates downstream members of the MAP kinase cascade, which in turn activate the AP-1 transcription factor (see Figure 3-16). Binding of IL-1 family cytokines to their receptors thereby leads to a global alteration in the transcription patterns of the affected cells, which in turn results in the up-regulation of proinflammatory cytokines and adhesion molecules.

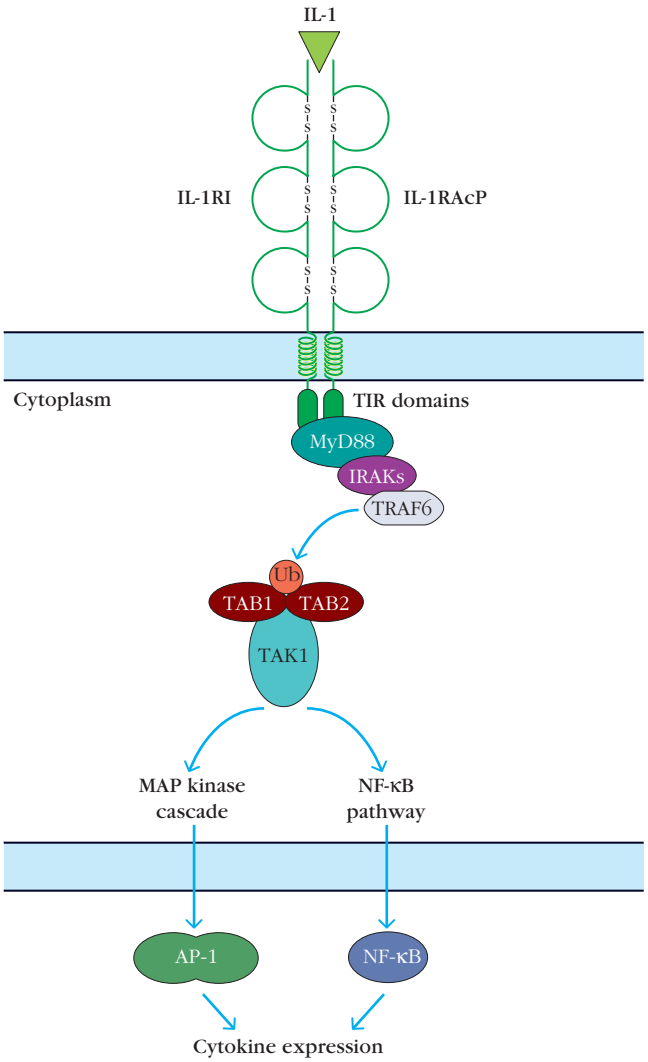

FIGURE 4-6 Signaling from members of the IL-1 receptor family. IL-1 binding to its receptor induces a conformational alteration in the receptor's Toll-IL-1R (TIR) domain that allows binding of the adapter protein MyD88 via its TIR domain. MyD88 recruits one or more IL-1 receptor activated kinases (IRAKs) to the receptor complex, which phosphorylate one another providing binding sites for TRAF6. The IRAK-TRAF6 complex dissociates from the receptor complex and interacts with the cytoplasmic protein TAK1 and its two binding proteins, TABs 1 and 2. TRAF6, together with a ubiquitin-ligase complex, catalyzes the generation of polyubiquitin chains that activate the TAK1 complex. TAK1 activates downstream events leading to the activation and nuclear localization of the transcription factor NF-κB. TAK1 also activates downstream members of the MAP kinase cascade, leading to activation of the AP-1 transcription factor.

Hematopoietin (Class I) Family Cytokines Share Three-Dimensional Structural Motifs, but Induce a Diversity of Functions in Target Cells

Members of the **hematopoietin (Class I) cytokine family** are small, soluble cytokines that communicate between and among cells of the immune system. Their name is somewhat misleading in that not all members of this family are implicated in hematopoietic (blood-cell forming) functions per se. However, some of the earliest members of this family to be characterized indeed have hematopoietic functions, and the cytokine family was then defined on the basis of structural similarities among all the participants. Because the hematopoietin family contains some of the earliest cytokines to be structurally characterized, it is sometimes also referred to as the *Class I cytokine family*.

Cytokines of the Hematopoietin (Class I) Family

As more hematopoietin family members have been defined, it has become clear that their cellular origins and target cells are as diverse as their ultimate functions, which range from signaling the onset of T- and B-cell proliferation (e.g., IL-2), to signaling the onset of B-cell differentiation to plasma cells and antibody secretion (e.g., IL-6), to signaling the differentiation of a T helper cell along one particular differentiation pathway versus another (e.g., IL-4 vs. IL-12) and, finally, to initiating the differentiation of particular leukocyte lineages (e.g., GM-CSF, G-CSF). Appendix II lists the cytokines described in this book, along with their cells of origin, their target cells, and the functions they induce.

Significant homology in the three-dimensional structure of hematopoietin family cytokines defines them as members of a single protein family, despite a relatively high degree of amino acid sequence diversity. The defining structural feature of this class of cytokines is a four-helix bundle motif, organized into four anti-parallel helices (Figure 4-7). Members of this family can then be further subclassified on the basis of helical length. Cytokines such as IL-2, IL-4, and IL-3 typically have short helices of 8 to 10 residues in length. In contrast, the so-called long-chain cytokines, which include IL-6 and IL-12, typically have helical lengths of 10 to 20 residues.

The Hematopoietin or Class I Receptor Family

Most hematopoietin cytokine receptors include two types of protein domains: an immunoglobulin-like domain, made up of β sheets, as described in Chapter 3, and domains that bear structural homology to the FNIII domain of the extracellular matrix protein fibronectin. Binding sites for most cytokines are to be found in a structure made up of two, tandem (side-by-side) FNIII domains referred to as ***C*ytokine-binding *H*omology *R*egions (CHRs)**. As we will see, the CHR motif is common to cytokine receptors from several families.

A feature common to most of the Hematopoietin and Interferon cytokine receptor families is the presence of multiple subunits. Table 4-3 lists the three subfamilies of hematopoietin

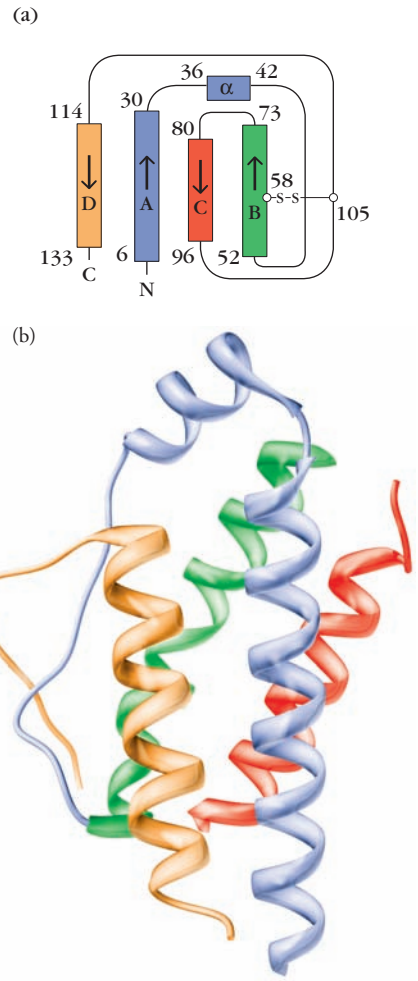

FIGURE 4-7 The four-helix bundle is the defining structural feature of the Hematopoietin family of cytokines. Structure of interleukin 2—the defining member of the Hematopoietin family—showing the four α-helices of the hematopoietin cytokines point in alternating directions. (a) Topographical representation of the primary structure of IL-2 showing α-helical regions (α and A-D) and connecting chains of the molecule. (b) Ribbon representation of the crystallographic structure of human Il-2. *[Part (b) PDB ID 1M47.]*

receptors, each subfamily being defined by a receptor subunit that is shared among all members of that family.

The γ-Chain Bearing, or IL-2 Receptor, Subfamily

Expression of a common γ chain defines the IL-2 receptor subfamily, which includes receptors for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21. The IL-2 and the IL-15 receptors are heterotrimers, consisting of a cytokine-specific α chain and two chains—β and γ—responsible for both signal transduction and cytokine recognition. The IL-2 receptor γ chain also functions as the signal-transducing subunit for the other receptors in this subfamily, which are all dimers. Congenital **X-linked severe combined immunodeficiency (XSCID)** results from a defect in the γ-chain gene, which maps to the X chromosome. The immunodeficiency observed in this disorder, which includes deficiencies in both T-cell and

TABLE 4-3 Subfamilies of hematopoietin family cytokine receptors share common subunits

Common cytokine receptor subunit	Cytokines recognized by receptors bearing that common subunit
γ	IL-2, IL-4, IL-7, IL-9, IL-15, IL-12
β	IL-3, IL-5, GM-CSF
gp130	IL-6, IL-11, LIF, OSM, CNTF, IL-27

NK-cell activity, results from the loss of all the cytokine functions mediated by the IL-2 subfamily receptors.

The IL-2 receptor occurs in three forms, each exhibiting a different affinity for IL-2: the low-affinity monomeric IL-2Rα (CD25) (which can bind to IL-2, but is incapable of transducing a signal from it), the intermediate-affinity dimeric IL-2Rβγ (which is capable of signal transduction), and the high-affinity trimeric IL-2Rαβγ (which is responsible for most physiologically relevant IL-2 signaling) (Figure 4-8a). A recent x-ray crystallographic structure of the high-affinity trimeric form of the IL-2 receptor with an IL-2 molecule in its binding site reveals that IL-2 binds in a pocket formed by the β and γ chains

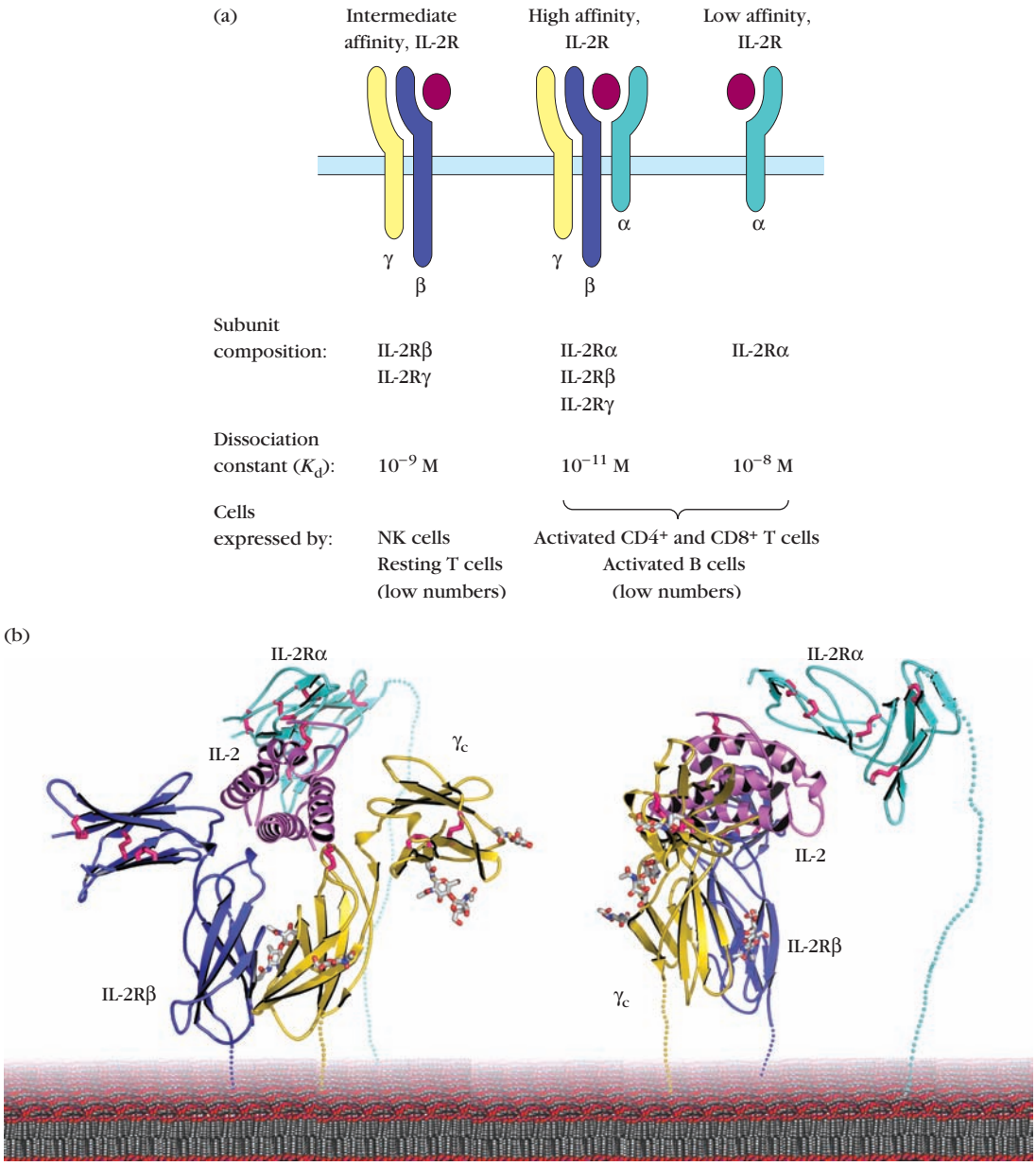

FIGURE 4-8 Comparison of the three forms of the IL-2 receptor. (a) Schematic of the three forms of the receptor and listing of dissociation constants and properties for each. Signal transduction is mediated by the β and γ chains, but all three chains are required for high-affinity binding of IL-2. (b) Three-dimensional structure of the three-chain form of the IL-2 receptor with bound IL-2 (views rotated by 90°). Note that the α chain completes the pocket to which IL-2 binds, accounting for the higher affinity of the trimeric form. *[From X. Wang, M. Rickert, and K. C. Garcia, 2005, Structure of the quaternary complex of interleukin–2 with its α, β, and γ_c receptors.* Science ***310:****1159.]*

(Figure 4-8b). Important additional contacts with the IL-2 ligand are contributed when the α chain is present, accounting for the higher affinity of binding by the trimer.

The expression of the three chains of the IL-2 receptor varies among cell types and in different activation states. The intermediate affinity (βγ) IL-2 receptors are expressed on resting T cells and on NK cells, whereas activated T and B cells express both the low-affinity (α) and the high-affinity (αβγ) receptor forms (see Figure 4-8a). Since there are approximately ten times as many low-affinity as high-affinity receptors on activated T cells (50,000 vs. 5000), one must ask what the function of the low-affinity receptor might be, and two possible ideas have been advanced. It may serve to concentrate IL-2 onto the recipient cell surface for passage to the high-affinity receptor. Conversely, it may reduce the local concentration of available IL-2, ensuring that only cells bearing the high-affinity receptor are capable of being activated. Whatever the answer to this question may be, the restriction of the high-affinity IL-2 receptor expression to activated T cells ensures that only antigen-activated $CD4^+$ and $CD8^+$ T cells will proliferate in response to physiologic levels of IL-2.

The β−Chain Bearing, or GM-CSF, Receptor Subfamily

Members of the GM-CSF receptor subfamily, which includes the receptors for IL-3, IL-5, and GM-CSF, share the β signaling subunit. Each of these cytokines binds with relatively low affinity to a unique, cytokine-specific receptor protein, the α subunit of a dimeric receptor. All three low-affinity subunits associate noncovalently with the common signal-transducing β subunit. The resulting αβ dimeric receptor has a higher affinity for the cytokine than the specific α chain alone, and is also capable of transducing a signal across the membrane upon cytokine binding (Figure 4-9a).

IL-3, IL-5, and GM-CSF exhibit redundant activities. IL-3 and GM-CSF both act on hematopoietic stem cells and progenitor cells, activate monocytes, and induce megakaryocyte differentiation, and all three of these cytokines induce eosinophil proliferation and basophil degranulation with release of histamine.

Since the receptors for IL-3, IL-5, and GM-CSF share a common signal-transducing β subunit, each of these cytokines would be expected to transduce a similar activation signal, accounting for the redundancy seen among their biological effects, and indeed, all three cytokines induce the same patterns of protein phosphorylation upon cell activation. However, when introduced simultaneously to a cell culture, IL-3 and GM-CSF appear to antagonize one another; the binding of IL-3 is inhibited by GM-CSF, and binding of GM-CSF is inhibited by IL-3. This antagonism is caused by competition for a limited number of β subunits available to associate with the cytokine-specific α subunits of the dimeric receptors (Figure 4-9b).

The gp130 Receptor Subfamily

The importance of the gp130 cytokine receptor family to the development and health of the individual is underscored by the results of deletion studies which have demonstrated that

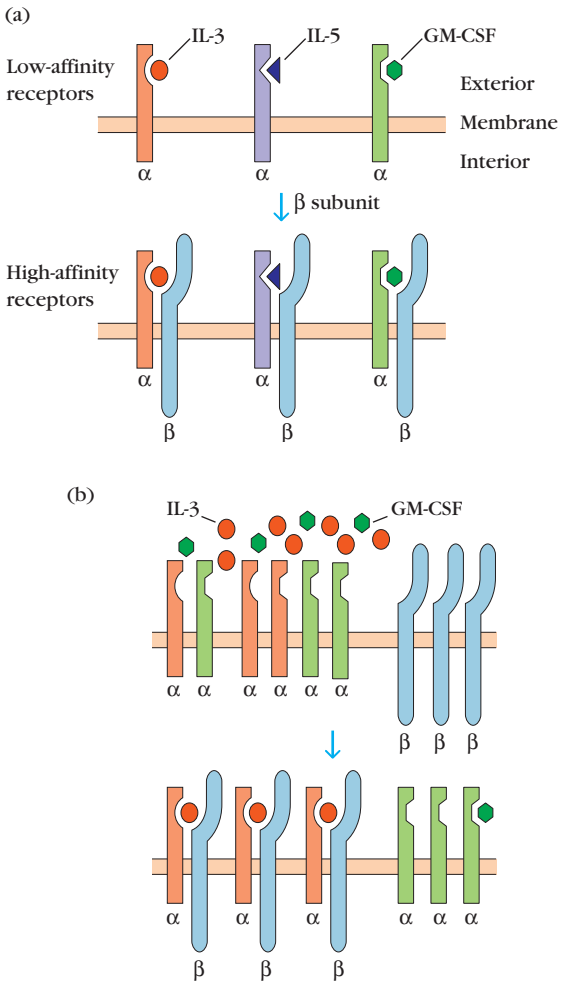

FIGURE 4-9 Interactions between cytokine-specific subunits and a common signal-transducing subunit of the β-chain family of cytokine receptors. (a) Schematic diagram of the low-affinity and high-affinity receptors for IL-3, IL-5, and GM-CSF. The cytokine-specific subunits exhibit low-affinity binding and cannot transduce an activation signal. Noncovalent association of each subunit with a common β subunit yields a high-affinity dimeric receptor that can transduce a signal across the membrane. (b) Competition of ligand-binding chains of different receptors for a common subunit can produce antagonistic effects between cytokines. Here binding of IL-3 by α subunits of the IL-3 receptor allows them to outcompete α chains of the GM-CSF receptor for β subunits. *[Part a adapted from T. Kishimoto et al., 1992, Interleukin-6 and its receptor: A paradigm for cytokines,* Science ***258:****593.]*

the targeted disruption of gp130 in mice during embryonic development is lethal. Receptors in this family include those for IL-6, important in the initiation of the immune response, and IL-12, critical for signaling differentiation of helper T cells along the T_H1 pathway. Targeted disruption of individual cytokine receptors, as well as of the cytokines themselves, has provided much functional information about signaling via these cytokine family members.

Cytokine specificity of the gp130 family of receptors is determined by the regulated expression of ligand-specific chains in dimers, or trimers, with the gp130 component. The

gp130 subunit family of cytokine receptors is further subdivided into receptors specific for *monomeric cytokines*, *such as IL-6*, and those which bind the *dimeric cytokines*, *such as IL-12*.

Because the Hematopoietin (Class I) and Interferon (Class II) cytokine receptor families utilize similar signaling pathways, we will first describe the Interferons and their receptors and then consider the signaling pathways used by the two families together.

The Interferon (Class II) Cytokine Family Was the First to Be Discovered

In the late 1950s, investigators studying two different viral systems in two laboratories half a world apart almost simultaneously discovered interferons. Yasu-Ichi Nagano and Yashuhiko Kojima, Japanese virologists, were using a rabbit skin and testes tissue culture model to develop a vaccine against smallpox. They noted that immunization with a UV-inactivated form of the cowpox virus resulted in the localized inhibition of viral growth, following a subsequent injection of the same virus. Viral growth inhibition was restricted to a small area of skin close to the site of the original immunization, and the scientists postulated that the initial injection had resulted in the production of a "viral inhibitory factor." After showing that their "inhibitory factor" was not simply antibody, they published a series of papers about it. With hindsight, scientists now believe that their protective effect was mediated by interferons. However, the technical complexity of their system, and the fact that their papers were published in French, rather than in English, delayed the dissemination of their findings to the broader scientific community.

Meanwhile, in London, Alick Isaacs and Jean Lindenman were growing live influenza virus on chick egg chorioallantoic membranes (a method that is still used today), and noticed that exposure of their membranes to a heat-inactivated form of influenza interfered with subsequent growth of a live virus preparation on that surface preparation. They proved that the growth inhibition resulted from the production of an inhibiting molecule by the chick membrane. They named it "interferon" because of its ability to "interfere" with the growth of the live virus. Their more straightforward in vitro assay system enabled them to rapidly characterize the biological effects of the molecule involved, and they wrote a series of papers describing the biological effects of interferon(s) in the late 1950s. However, since interferons are active at very low concentrations, it was not until 1978 that they were produced in quantities sufficient for biochemical and crystallographic analysis. Since that time, investigators have shown that there are two major types of interferons, Types 1 and 2, and that Type 1 interferons can be subdivided into two subgroups.

Interferons

Type I interferons are composed of *Interferons* α, a family of about 20 related proteins, and *interferon*-β, which are secreted by activated macrophages and dendritic cells, as well as by virus-infected cells. Interferons α and β are also secreted by virally infected cells after recognition of viral components by **pattern recognition receptors (PRRs)** located either at the cell surface, or inside the cell (see Chapter 5). Intracellular PRRs may interact with virally derived nucleic acids or with endocytosed viral particles. The secreted Type I interferons then interact in turn with membrane-bound interferon receptors on the surfaces of many different cell types. The results of their interaction with these receptors are discussed in detail in Chapter 5, but they include the induction of ribonucleases that destroy viral (and cellular) RNA, and the cessation of cellular protein synthesis. Thus, interferons prevent virally infected cells from replicating and from making new viral particles. However, they simultaneously inhibit normal cellular functions and destroy virally infected cells so that the infection cannot spread.

Type I interferons are dimers of 18 to 20 kDa polypeptides, predominantly helical in structure, and some members of this family are naturally glycosylated. Type I interferons are used in the treatment of a variety of human diseases, most notably hepatitis infections.

Type II interferon, otherwise known as *interferon*-γ, is produced by activated T and NK cells and is released as a dimer (Figure 4-10). Interferon-γ is a powerful modulator of

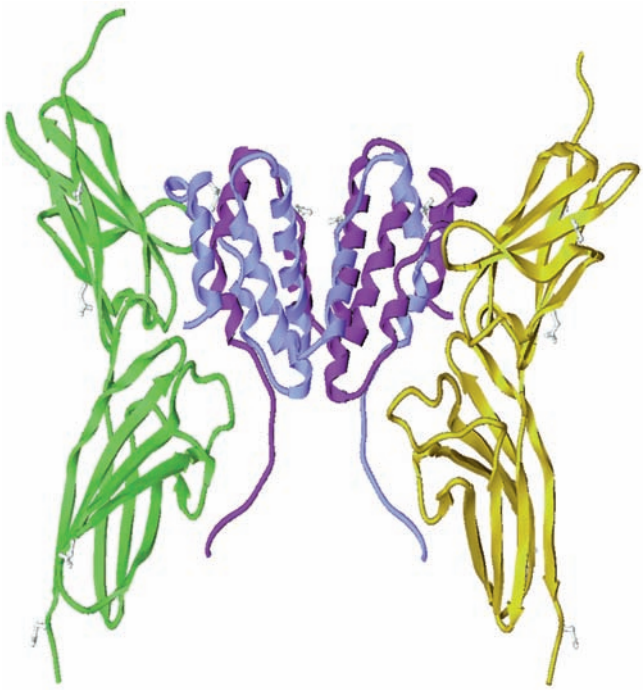

FIGURE 4-10 The complex between IFN-γ and the ligand-binding chains of its receptor. This model is based on the x-ray crystallographic analysis of a crystalline complex of interferon-γ (dark and light purple) bound to ligand-binding α chains of the receptors (green and yellow). Note that IFN-γ is shown in its native dimeric form; each member of the dimer engages the α chain of an IFN-γ receptor, thereby bringing about receptor dimerization and signal transduction. *[From M. R. Walter et al., 1995, Crystal structure of a complex between interferon- and its soluble high-affinity receptor.* Nature **376***:230, courtesy M. Walter, University of Alabama.]*

CLINICAL FOCUS

Therapy with Interferons

Interferons are an extraordinary group of proteins with important effects on the immune system. Their actions affect both the adaptive and the innate arms of the immune system and include the induction of increases in the expression of both Class I and Class II MHC molecules and the augmentation of NK-cell activity. Cloning of the genes that encode IFN-α, IFN-β, and IFN-γ has made it possible for the biotechnology industry to produce large amounts of each of these interferons at costs that make their clinical use practical (Table 1).

IFN-α (also known by its trade names Roferon and Intron A) has been used for the treatment of hepatitis C and hepatitis B. It has also been found useful in a number of different applications in cancer therapy. A type of B-cell leukemia known as hairy-cell leukemia (because the cells are covered with fine, hairlike cytoplasmic projections) responds well to IFN-α. Chronic myelogenous leukemia, a disease characterized by increased numbers of granulocytes, begins with a slowly developing chronic phase that changes to an accelerated phase and terminates in a blast phase, which is usually resistant to treatment. IFN-α is an effective treatment for this leukemia in the chronic phase (70% response rates have been reported), and some patients (as many as 20% in some studies) undergo complete remission. Kaposi's sarcoma, the cancer most often seen in AIDS patients in the United States, also responds to treatment with IFN-α, and there are reports of a trend toward longer survival and fewer opportunistic infections in patients treated with this agent. Most of the effects mentioned above have been obtained in clinical studies that used IFN-α alone, but certain applications such as hepatitis C therapy commonly use it with an antiviral drug such as ribavirin. The clearance time of IFN-α is lengthened by using it in a form complexed with polyethylene glycol (PEG) called pegylated interferon.

IFN-β has emerged as the first drug capable of producing clinical improvement in multiple sclerosis (MS). Young adults are the primary target of this autoimmune neurologic disease, in which nerves in the central nervous system (CNS) undergo demyelination. This results in progressive neurologic dysfunction, leading to significant and, in many cases, severe disability. This disease is often characterized by periods of nonprogression and remission alternating with periods of relapse. Treatment with IFN-β provides longer periods of remission and reduces the severity of relapses. Furthermore, magnetic resonance imaging (MRI) studies of CNS damage in treated and untreated patients revealed that MS-induced damage was less severe in a group of IFN-β–treated patients than in untreated ones.

IFN-γ has been used, with varying degrees of success, to treat a variety of malignancies that include non-Hodgkin's lymphoma, cutaneous T-cell lymphoma, and multiple myeloma. A more successful clinical application of IFN-γ in the clinic is in the treatment of the hereditary immunodeficiency chronic granulomatous disease (CGD; see Chapter 18). CGD features a serious impairment of the ability of phagocytic cells to kill ingested microbes, and patients with CGD suffer recurring infections by a number of bacteria (*Staphylococcus aureus*, *Klebsiella*, *Pseudomonas*, and others) and fungi such as *Aspergillus* and *Candida*. Before interferon therapy, standard treatment for the disease included attempts to avoid infection, aggressive administration of antibiotics, and surgical drainage of abscesses. A failure to generate microbicidal oxidants (H_2O_2, superoxide, and others) is the basis of CGD, and the administration of IFN-γ significantly reverses this defect. Therapy of CGD patients with IFN-γ significantly reduces the incidence of infections. Also, the infections that are contracted are less severe, and the average number of days spent by patients in the hospital is reduced.

IFN-γ has also been shown to be effective in the treatment of osteopetrosis (*not osteoporosis*), a life-threatening congenital disorder characterized by overgrowth of bone that results in blindness and deafness. Another problem presented by this disease is that the buildup of bone reduces the amount of space available for bone marrow, and the decrease in hematopoiesis results in fewer red blood cells and anemia. The decreased generation of white blood cells causes an increased susceptibility to infection.

The use of interferons in clinical practice is likely to expand as more is learned about their effects in combination with other therapeutic agents.

the adaptive immune response, biasing T cell help toward the T_H1 type and inducing the activation of macrophages, with subsequent destruction of any intracellular pathogens and the differentiation of cytotoxic T cells. All three interferons increase the expression of MHC complex proteins on the surface of cells, thus enhancing their antigen-presentation capabilities.

Interferon-γ is used medically to bias the adaptive immune system toward a cytotoxic response in diseases such as leprosy and toxoplasmosis, in which antibody responses are less effective than those that destroy infected cells. Clinical Focus Box 4-2 describes additional roles of interferons in the clinic.

Minority members of the Interferon family of cytokines include IL-10, secreted by monocytes and by T, B, and

BOX 4-2

TABLE 1 Cytokine-based therapies in clinical use

Agent	Nature of agent	Clinical application
Enbrel	Chimeric TNF-receptor/IgG constant region	Rheumatoid arthritis
Remicade or Humira	Monoclonal antibody against TNF-α receptor	Rheumatoid arthritis, Crohn's disease
Roferon	Interferon-α-2a*	Hepatitis B, Hairy-cell leukemia, Kaposi's sarcoma, Hepatitis C†
Intron A	Interferon-α–2b	Melanoma
Betaseron	Interferon-β–1b	Multiple sclerosis
Avonex	Interferon-β–1a	Multiple sclerosis
Actimmune	Interferon-γ–1b	Chronic granulomatous disease (CGD), Osteopetrosis
Neupogen	G-CSF (hematopoietic cytokine)	Stimulates production of neutrophils; reduction of infection in cancer patients treated with chemotherapy, AIDS patients
Leukine	GM-CSF (hematopoietic cytokine)	Stimulates production of myeloid cells after bone marrow transplantation
Neumega or Neulasta	Interleukin 11 (IL-11), a hematopoietic cytokine	Stimulates production of platelets
Epogen	Erythropoietin (hematopoietic cytokine)	Stimulates red-blood-cell production
Ankinra (kineret)	Recombinant IL-1Ra	Rheumatoid arthritis
Daclizumab (Zenapax)	Humanized monoclonal antibody against IL-2R	Prevents rejection after transplantation
Basiliximab (Simulect)	Human/mouse chimeric monoclonal antibody against IL-2R	Prevents transplant rejection

*Interferon-α–2a is also licensed for veterinary use to combat feline leukemia.

† Normally used in combination with an antiviral drug (ribavirin) for hepatitis C treatment.

Although interferons, in common with other cytokines, are powerful modifiers of biological responses, the side effects accompanying their use are fortunately relatively mild. Typical side effects include flu-like symptoms, such as headache, fever, chills, and fatigue. These symptoms can largely be managed with acetaminophen (Tylenol) and diminish in intensity during continued treatment. Although interferon toxicity is usually not severe, treatment is sometimes associated with serious manifestations such as anemia and depressed platelet and white-blood-cell counts.

dendritic cells that regulates immune responses. IL-10 shares structural similarities with interferon-γ, and these similarities enable it to bind to the same class of receptors. In addition, a third class of interferons, the so-called interferon-λ, or *type III Interferon family*, was discovered in 2003. There are currently three members of this family: interferon-λ1 (IL-29), interferon-λ2 (IL-28A), and interferon-λ3 (IL-28B). Like Type I interferons, the Type III interferons up-regulate the expression of genes controlling viral replication and host cell proliferation.

Interferon Receptors

Members of the Interferon receptor family are heterodimers that share similarly located, conserved cysteine residues with

members of the Hematopoietin receptor family. Initially, only interferon-α, -β, and -γ were thought to be ligands for these receptors. However, recent work has shown that the receptor family consists of 12 receptor chains that, in their various assortments, bind no fewer than 27 different cytokines, including six members of the IL-10 family, 17 Type I interferons, one Type II interferon, and three members of the recently described interferon–λ family, including IL-28A, IL-28B, and IL-29.

The JAK-STAT Signaling Pathway

Early experiments in cytokine signaling demonstrated that a series of protein tyrosine phosphorylations rapidly followed the interaction of a cytokine with a receptor from the Class I or Class II cytokine receptor families. These results were initially puzzling, since the cytokine receptors lack the immunotyrosine activation motifs (ITAMs) characteristic of B- and T-cell receptors. However, studies of the molecular events triggered by binding of interferon gamma (IFN-γ) to its receptor shed light on the mode of signal transduction used by members of both the Hematopoietin and Interferon cytokine families.

In the absence of cytokine, the receptor subunits are associated only loosely with one another in the plane of the membrane, and the cytoplasmic region of each of the receptor subunits is associated noncovalently with inactive tyrosine kinases named **Janus Activated Kinases (JAKs)**. (Some members of this family of kinases retain their earlier name of **Tyk**, but share structural and functional properties with the JAK family of kinases.) The process of signal transduction from Class I and Class II cytokine receptors has been shown to proceed according to the following steps (Figure 4-11):

- Cytokine binding induces the association of the two separate cytokine receptor subunits and activation of the receptor-associated JAKs.
- The receptor-associated JAKs phosphorylate specific tyrosines in the receptor subunits.
- These phosphorylated tyrosine residues serve as docking sites for inactive transcription factors known as **Signal *T*ransducers and *A*ctivators of *T*ranscription (STATs)**.
- The inactive STATs are phosphorylated by JAK and Tyk kinases.
- Phosphorylated STAT transcription factors dimerize, binding to one another via SH2/phosphotyrosine interactions.
- Phosphorylation also results in a conformational change in the STAT dimer that reveals a nuclear localization signal.
- The STAT dimer translocates into the nucleus, where it initiates the transcription of specific genes.

Currently, we know of seven STAT proteins (STAT 1-4, 5A, 5B, and 6) and four JAK proteins (JAK 1–3 and Tyk2) in mammals. Specific STATs play essential roles in the signaling pathways of a wide variety of cytokines (Table 4-4).

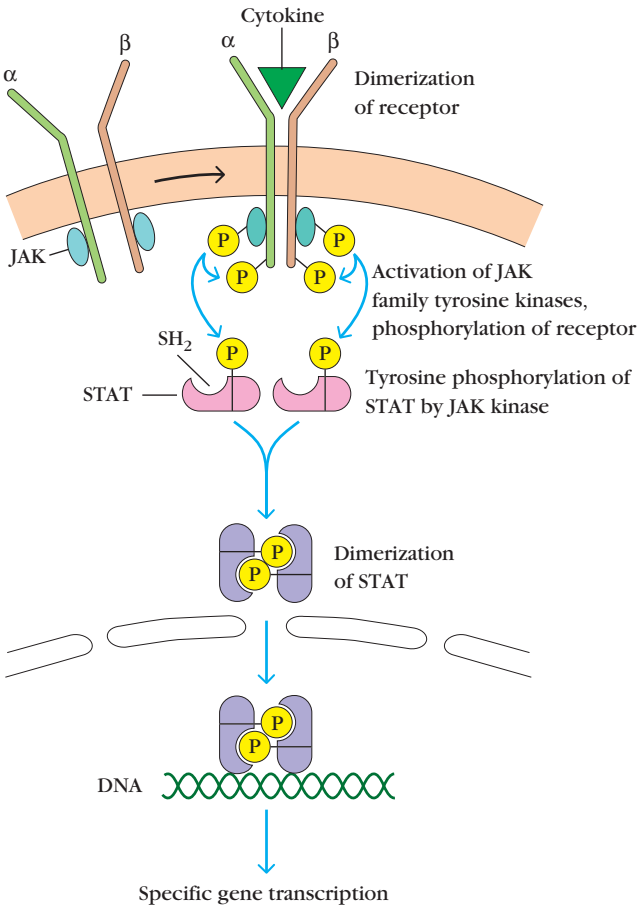

FIGURE 4-11 General model of signal transduction mediated by most Class I and Class II cytokine receptors. Binding of a cytokine induces dimerization of the receptor subunits, which leads to the activation of receptor subunit-associated JAK tyrosine kinases by reciprocal phosphorylation. Subsequently, the activated JAKs phosphorylate various tyrosine residues, resulting in the creation of docking sites for STATs on the receptor and the activation of one or more STAT transcription factors. The phosphorylated STATs dimerize and translocate to the nucleus, where they activate transcription of specific genes.

Given the generality of this pathway among Class I and Class II cytokines, how does the immune system induce a specific response to each cytokine? First, there is exquisite specificity in the binding of cytokines to their receptors. Secondly, particular cytokine receptors are bound to specific partner JAK enzymes that in turn activate unique STAT transcription factors. Third, the transcriptional activity of activated STATs is specific because a particular STAT homodimer or heterodimer will only recognize certain sequence motifs and thus can interact only with the promoters of certain genes. Finally, only those target genes whose expression is permitted by a particular cell type can be activated within that variety of cell. For example, promoter regions in some cell types may be caught up in heterochromatin and

TABLE 4-4 STAT and JAK interaction with selected cytokine receptors during signal transduction

Each cytokine receptor must signal through a pair of Janus kinases. The JAKs may operate as either homo- or heterodimers.

Cytokine receptor	Janus kinase	STAT
IFN-α/-β	JAK 1, Tyk 2*	STATs 1 and 2
IFN-γ	JAK 1, JAK 2	STAT 1
IL-2	JAK 1, JAK 3	Mainly STATs 3 and 5. Also STAT 1.
IL-4	JAK 1, JAK 3	Mainly STAT 6. Also STAT 5.
IL-6	JAK 1, JAK 2	STAT 3
IL-7	JAK 1, JAK 3	STATs 5 and 3
IL-12	JAK 2, Tyk2	STATS 2, 3, 4, and 5
IL-15	JAK 1, JAK 3	STAT 5
IL-21	JAK 1, JAK 3	Mainly STATs 1 and 3; also STAT 5

* Despite its name, Tyk2 is also a Janus kinase.

inaccessible to transcription factors. In this way, the Class I cytokine IL-4 can induce one set of genes in T cells, another in B cells, and yet a third in eosinophils.

JAK-STAT pathways are not unique to the immune system. Among the many genes known to be regulated by mammalian STAT proteins are those encoding cell survival factors such as the Bcl-2 family members, those involved in cell proliferation such as *cyclin D1* and *myc*, and those implicated in angiogenesis or metastasis such as vascular endothelial growth factor, or *VEGF*.

At the close of cytokine signaling, negative regulators of the STAT pathway, such as *p*rotein *i*nhibitor of *a*ctivated *S*TAT (PIAS), *s*uppressor *o*f *c*ytokine *s*ignaling (SOCS), and protein tyrosine phosphatases are believed to be responsible for turning off JAK-STAT signaling and returning the cell to a quiescent state.

Members of the TNF Cytokine Family Can Signal Development, Activation, or Death

The ***T*umor *N*ecrosis *F*amily (TNF) family** of cytokines regulates the development, effector function, and homeostasis of cells participating in the skeletal, neuronal, and immune systems, among others.

Cytokines of the TNF Family Can Be Soluble or Membrane Bound

TNF-related cytokines are unusual in that they are often firmly anchored into the cell membrane. Generally they are Type 2 transmembrane proteins with a short, intracytoplasmic N-terminal region, and a longer, extracellular C-terminal region. The extracellular region typically contains a canonical TNF-homology domain responsible for interaction with the cytokine receptors. Members of the TNF family can also act as soluble mediators, following cleavage of their extracellular regions, and in some cases, the same cytokine exists in both soluble and membrane-bound forms.

There are two eponymous (having the same name as) members of the TNF family: TNF-α and TNF-β, though TNF-β is more commonly known as Lymphotoxin-α, or *LT*-α. Both of these are secreted as soluble proteins. TNF-α (frequently referred to simply as TNF) is a proinflammatory cytokine, produced primarily by activated macrophages, but also by other cell types including lymphocytes, fibroblasts, and keratinocytes (skin cells), in response to infection, inflammation, and environmental stressors. TNF elicits its biological effects by binding to its receptors, TNF-R1 or TNF-R2, which are described below. Lymphotoxin-α is produced by activated lymphocytes and can deliver a variety of signals. On binding to neutrophils, endothelial cells, and osteoclasts (bone cells), Lymphotoxin-α delivers activation signals; in other cells, binding of Lymphotoxin-α can lead to increased expression of MHC glycoproteins and of adhesion molecules.

We will also encounter five physiologically significant, membrane-bound members of the TNF cytokine family throughout this book. *Lymphotoxin*-β, a membrane-bound cytokine, is important in lymphocyte differentiation. We will learn about BAFF and APRIL in the context of B-cell development and homeostasis (Chapter 10). CD40L is a cytokine expressed on the surface of T cells that is required to signal for B-cell differentiation (Chapter 12). Fas ligand (FasL), or CD95L, induces apoptosis on binding to its cognate receptor, **Fas**, or CD95.

Whether membrane-bound or in soluble form, active cytokines of the TNF family assemble into trimers. Although in most cases they are homotrimeric, heterotrimeric cytokines do form between the TNF family members Lymphotoxin-α and Lymphotoxin-β and between APRIL and BAFF. Crystallographic analysis of TNF family members has revealed that

FIGURE 4-12 The TNF-family members act as trimers in vivo. *[PDB ID 1TNF]*

they have a conserved tertiary structure and fold into a β-sheet sandwich. The conserved residues direct the folding in the internal β strands that, in turn, promote the trimer formation (Figure 4-12).

TNF Receptors

Members of the TNF receptor superfamily are defined by the presence of *C*ysteine-*R*ich *D*omains (CRDs) in the extracellular, ligand-binding domain. Each CRD typically contains six cysteine residues, which form three disulfide bonded loops, and individual members of the superfamily can contain from one to six CRDs.

Although most TNF receptors are Type 1 membrane proteins (their N-terminals are outside the cell), a few family members are cleaved from the membrane to form soluble receptor variants. Alternatively, some lack a membrane anchoring domain at all, or are linked to the membrane only by covalently bound, glycolipid anchors. These soluble forms of TNF family receptors are known as "decoy receptors," as they are capable of intercepting the signal from the ligand before it can reach a cell, effectively blocking the signal. This is a theme that we have encountered before in our consideration of the IL-1 receptor family.

Signaling Through TNF Superfamily Receptors

The work of delineating the precise pathways of signaling through TNF family receptors is ongoing, and some important questions still await resolution. One reason that these pathways have been so difficult to define is that the same receptor, TNF-R1, can transduce both activating and death-promoting signals, depending on the local cellular and molecular environment in which the signal is received, and investigators have yet to determine the trigger that shifts the signaling program from life to death. However, much is known about how each of these signaling pathways work, once that all-important decision has been made.

We will start by describing the proapoptotic (death-inducing) pathway that is initiated when the membrane-bound TNF family member FasL on one cell binds to a Fas receptor on a second cell, leading to death in the cell bearing the Fas receptor. With this as our foundation, we will then illustrate how the TNF-R1 receptor mediates both life- and death- promoting signals. Signaling through other TNF-R family members, such as CD40, BAFF, and April, will be described in later chapters in the context of the various immune responses in which they are involved.

Signaling Through the Fas Receptor

At the close of an immune response, when the pathogen is safely demolished and the immune system needs to eliminate the extra lymphocytes it has generated to deal with the invader, responding lymphocytes begin to express the TNF family receptor Fas on their cell surfaces. Fas, and its ligand FasL, are specialized members of the TNF receptor and the TNF cytokine families, respectively, and they work together to promote lymphocyte homeostasis. Mice with mutations in either the *fas* (mrl/lpr mice) or the *fasL* (gld mice) genes consequently suffer from severe lympho-proliferative disorders, indicative of their inability to eliminate lymphocytes that are no longer serving a useful purpose.

On interaction with other immune cells bearing FasL, the Fas receptor trimerizes and transduces a signal to the interior of the Fas-bearing cell that results in its elimination by **apoptosis**. Apoptosis, or **programmed cell death**, is a mechanism of cell death in which the cell dies from within and is fragmented into membrane-bound vesicles that can be rapidly phagocytosed by neighboring macrophages (Figure 4-13a). By using such well-controlled apoptotic pathways, the organism ensures that minimal inflammation is associated with the natural end of an immune response. Activation of the apoptotic pathway invokes the activation of caspases; these are proteases, bearing *C*ysteine residues at their active sites, which cleave after *ASP*artic acid residues.

Binding of Fas to FasL results in the clustering of the Fas receptors (Figure 4-13b). This, in turn, promotes interaction between their cytoplasmic regions, which include domains common to a number of proapoptotic signaling molecules called **death domains**. This type of interaction, between homologous protein domains expressing affinity for one another, is referred to as a *homotypic interaction*. As they bind to one another, the clustered Fas protein death domains incorporate death domains from the adapter

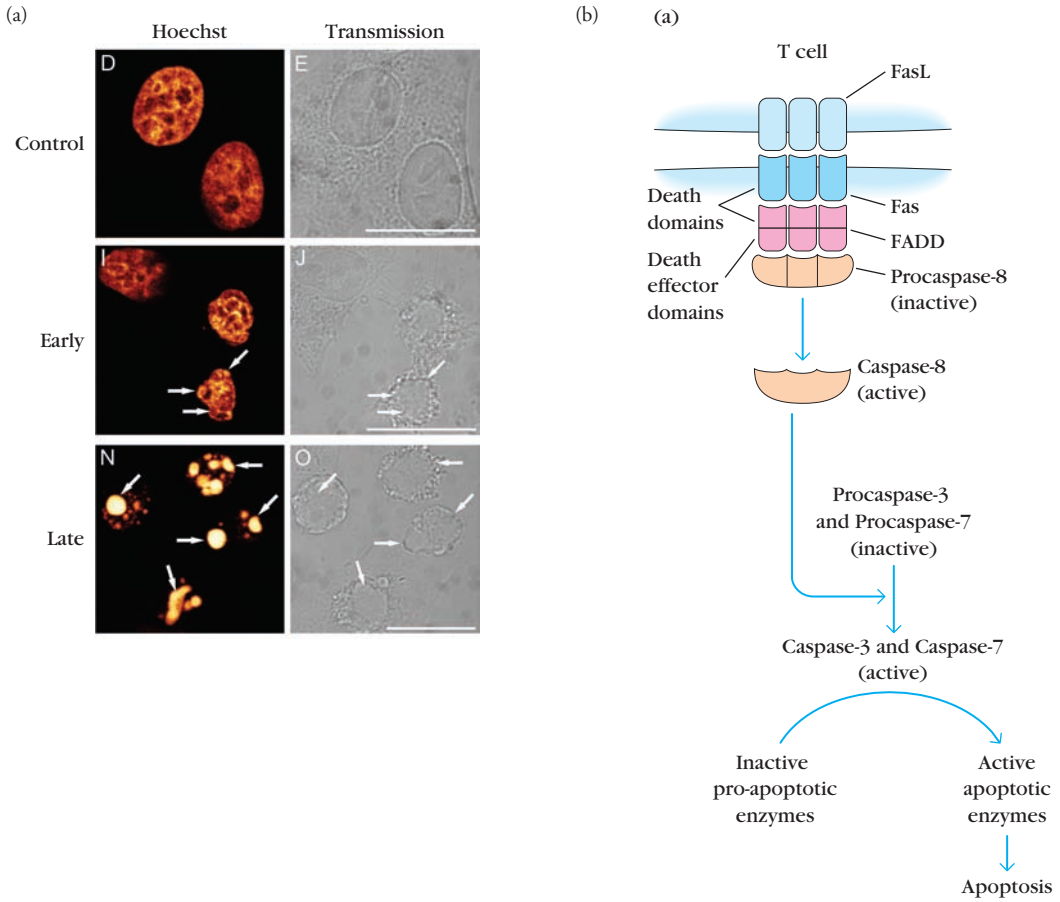

FIGURE 4-13 Apoptotic signaling through Fas receptors. (a) Human HeLa cells were activated to undergo apoptosis through the Fas receptors. Hoechst-stained cells show the gradual condensation of nuclear DNA into membrane-bounded blebs, as the cell breaks up into vesicular packages that are recognized and phagocytosed by macrophages in the absence of inflammation. The same cells are also shown under transmission microscopy. Arrows show staining of the Nuclear Mitotic Apparatus protein, an early nuclear caspase target in apoptosis. (b) Signaling from Fas leads to apoptosis. Binding of FasL to Fas induces clustering of the Fas receptors and corresponding clustering of the Fas Death Domains (DDs). The DDs of the adapter protein FADD bind to the clustered Fas DDs via a homotypic interaction. Death effector domains also located on the FADD adapter proteins incorporate the DED domains of procaspase-8 into the membrane complex. Clustering of procaspase-8 induces cleavage of the pro domains of procaspase-8, leading to the release of the active caspase-8 protease. Caspase-8 cleaves the pro domains from the executioner caspases, caspase-3 and caspase-7, which in turn cleave and activate nucleases leading to the degradation of nuclear DNA. Caspase-8 also cleaves and activates the proapoptotic Bcl-2 family member protein, BID. [Taimen, Pekka and Kallajoki, Markku. NuMA and nuclear lamins behave differently in Fasmediated apoptosis J Cell Sci 2003 116:(3):571-583; Advance Online Publication December 11, 2002, doi:10.1242/jcs.00227. Reproduced with permission of Journal of Cell S]

protein FADD (*F*as-*A*ssociated *D*eath *D*omain-containing protein). FADD contains not only death domains, but also a related type of domain called a *D*eath *E*ffector *D*omain (DED). This, in turn, binds homotypically to the DED domains of procaspase-8, resulting in the clustering of procaspase-8 molecules. Procaspase-8 molecules contain the active caspase-8 enzyme, held in an inactive state by binding to prodomains.

The multimerization of procaspase-8 molecules results in mutual cleavage of their prodomains and induces capsase-8 activation. Caspase-8 then cleaves many target proteins critical to the generation of apoptosis. The target proteins of caspase-8 include the executioner caspases, -3 and -7 (which cleave and activate nucleases leading to the degradation of nuclear DNA), and the proapoptotic Bcl-2 family member BID. The complex of Fas, FADD, and procaspase-8 is referred to as the ***Death-Inducing Signaling Complex* (DISC)**. The ultimate result of activation of this cascade is the condensation of nuclear material (see Figure 4-13a), the degradation of nuclear DNA into 240 base pair, nucleic acid fragments, and the subsequent breakdown of the cell into "easily digestible" membrane-bound fragments.

Signaling Through the TNF-R1 Receptor

The TNF-R1 receptor is present on the surface of all vertebrate cells and, like Fas, has an intracytoplasmic death domain (DD). Although this receptor is capable of binding to both TNF-α and Lymphotoxin-α, we will focus on the signaling that is elicited by TNF-α (TNF). TNF binding to the TNF-R1 receptor can lead to two very different outcomes: apoptosis (death) or survival (life). How it does so is still the focus of intensive investigation, but the story as it is unfolding is already a fascinating one.

The mechanism by which TNF binding leads to apoptosis is slightly different from that which follows Fas-FasL binding. Like FasL, binding of TNF to the TNF-R1 receptor induces trimerization of the receptor as well as an alteration in its conformation, and these together result in the binding of a DD-containing adapter molecule, in this case TRADD, to the internal face of the receptor molecule (Figure 4-14). The TRADD adapter molecule provides additional binding sites for the components RIP1 (a serine/threonine kinase, rather evocatively named *R*est *I*n *P*eace 1), which binds via its own DDs and TRAF2, the *T*NF *R*eceptor *A*ssociated *F*actor 2. This is known as *complex I*. Intracellular localization experiments have shown that this complex can dissociate from the TNF-R at the membrane, and migrate to the cytoplasm where it binds to the now familiar DD-containing protein FADD. FADD recruits procaspase-8, as described above, resulting in the generation of an apoptotic signal. The proapoptotic cytoplasmic complex generated upon TNF-R1 receptor binding is shown in Figure 4-14a as *complex II*.

Counterintuitively, binding of this same molecule, TNF, to the same receptor, TNF-R1, can result in the delivery of survival as well as of proapoptotic signals. How can the same cytokine, acting through the same receptor, bring about two apparently opposing actions?

In the TNF-mediated survival pathway (Figure 4-14b), the generation of the original membrane complex appears to initiate in the same general manner as for the proapoptotic pathway. However, in the case of the prosurvival pathway, the TRADD-containing complex does not dissociate from the membrane, but rather remains at the cell surface and recruits a number of other components, including the ubiquitin ligases cIAP1 and cIAP2.

Once cIAP1 and cIAP2 join the TNF-R1 complex at the cell membrane, they recruit the LUBAC proteins, which attach linear ubiquitin chains to RIP1. Polyubiquitinated RIP1 then binds to the NEMO component of IKK as well as to TAK1, which is already complexed with its associated TAB proteins as described above. RIP1 and TAK1 activate the IKK complex, leading to IκB phosphorylation and destruction, and subsequent activation of NF-κB. Once NF-κB is fully activated, it turns on the expression of the cFLIP protein that then inhibits the activity of caspase-8. This effectively shuts down the antagonistic, proapoptotic pathway (Figure 4-14a). As previously described, the TAK1 complex also acts to activate the MAP kinase pathway, which further enhances survival signaling.

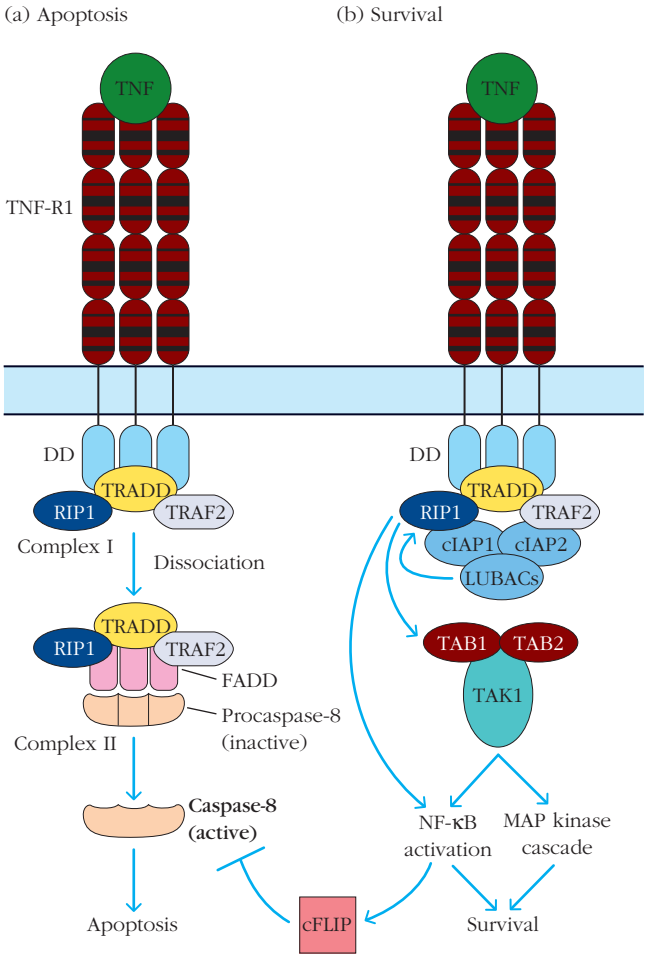

FIGURE 4-14 Signaling through TNF-R family receptors. Signaling through TNF-R family receptors can lead to pro- or anti-apoptotic outcomes depending on the nature of the signal, the receptor, and the cellular context. (a) Apoptosis. Binding of TNF to TNF-R1 induces trimerization of the receptor and conformational alteration in its cytoplasmic domain, resulting in the recruitment of the DD-containing adapter molecule TRADD to the cytoplasmic face of the receptor. TRADD binds to the serine-threonine kinase RIP1 and the TNF receptor associated factor TRAF2. This complex of TRADD, RIP1, and TRAF2, known as complex I, dissociates from the receptor and migrates to the cytoplasm where it binds to the adapter protein FADD. FADD recruits procaspase-8, leading to apoptosis as described in Figure 4-13. The proapoptotic complex generated upon TNF-R1 receptor binding is shown as Complex II. (b). Survival. As for the apoptotic pathway, TNF ligation results in receptor trimerization, TRADD binding, and RIP1 recruitment. In this case, however, TRADD also recruits the ubiquitin ligases cIAP1 and cIAP2, which in turn bind to the proteins of the *l*inear *ubi*quitin *a*ssembly *c*omplex (LUBAC) proteins. Polyubiquitination of RIP1 allows it to bind to the NEMO component of the IKK complex as well as to TAK1. TAK1 and RIP1 together activate the IKK complex, leading to IκB phosphorylation and destruction, and release of NF-κB to enter the nucleus. Among other prosurvival effects, NF-κB activates the transcription of the cFLIP protein, which inhibits caspase-8 action, thus tipping the scales in favor of survival. The TAK1 complex also activates MAP kinase signaling, which enhances cell survival.

The survival versus death decisions that are made at the level of the TNF-R1 receptor depend upon the outcome of the race between the generation of active caspase-8 on the one hand and the generation of the caspase-8 inhibitor cFLIP on the other. Although we now understand the molecular mechanisms that bring about the consequences of these decisions, we still have much to learn regarding how the cell integrates the signals received through TNF-R1 with other signals delivered to the cell in order to determine which of the two competing pathways will prevail. Since the generation of the membrane-bound complex that is capable of activating NF-κB is entirely dependent on interactions between various ubiquitinated proteins, it now appears that the life-death decision for a cell may be executed by a small protein previously thought to have only destructive intent.

The IL-17 Family Is a Recently Discovered, Proinflammatory Cytokine Cluster

The most recently described family of cytokines, the **IL-17 family**, includes interleukins 17A, 17B, 17C, 17D, and 17F. Signaling through most members of this family culminates in the generation of inflammation. IL-17 receptors are found on neutrophils, keratinocytes, and other nonlymphoid cells. Members of the IL-17 family therefore appear to occupy a location at the interface of innate and adaptive immunity. IL-17 cytokines do not share sequence similarity with other cytokines, but intriguingly the amino acid sequence of IL-17A is 58% identical to an open reading frame (ORF13) found in a T-cell–tropic herpesvirus. The significance of this sequence relationship is so far unknown; did the virus hijack the cytokine sequence for its own needs, or did the pilfering occur in the opposite direction?

IL-17 Cytokines

IL-17A, the first member of this family to be identified, is released by activated T cells and stimulates the production of factors that signal a proinflammatory state, including IL-6, CXCL8, and granulocyte colony-stimulating factor (G-CSF). As characterization of IL-17A and the T cells that secreted it progressed, it became clear that the T cells secreting this cytokine represent a new lineage, the T_H17 cell subset, which is currently the focus of intense investigation (see Chapter 11). Genomic sequencing has since led to the identification of a number of homologs of IL-17A (see Table 4-5). Most of the interleukins in the IL-17 family share the property of operating at the interface of innate and adaptive immunity, serving to coordinate the release of proinflammatory and neutrophil-mobilizing cytokines. However, IL-17E provides an exception to this general rule, instead promoting

TABLE 4-5 Expression and known functions of members of the extended IL-17 receptor family

Family member	Other common names	Receptors	Expression by which cells	Main functions
IL-17A	IL-17 and CTL-8	IL-17RA and IL-17RC	T_H17 cells, $CD8^+$ T cells, γδ T cells, NK cells, and NKT cells	Autoimmune pathology, neutrophil recruitment, and immunity to extracellular pathogens
IL-17B	NA	IL-17RB	Cells of the GI tract, pancreas, and neurons	Proinflammatory activities?
IL-17C	NA	IL-17RE	Cells of the prostate and fetal kidney	Proinflammatory activities?
IL-17D	NA	Unknown	Cells of the muscles, brain, heart, lung, pancreas, and adipose tissue	Proinflammatory activities?
IL-17E	IL-25	IL-17RA and IL-17RB	Intraepithelial lymphocytes, lung epithelial cells, alveolar macrophages, eosinophils, basophils, NKT cells, T_H2 cells, mast cells, and cells of the gastrointestinal tract and uterus	Induces T_H2 responses and suppresses T_H17 responses
IL-17F	NA	IL-17RA and IL-17RC	T_H17 cells, $CD8^+$ T cells, γδ T cells, NK cells, and NKT cells	Neutrophil recruitment and immunity to extracellular pathogens
IL-17A/IL-17F heterodimer	NA	IL-17RA and IL-17RC	T_H17 cells, $CD8^+$ T cells, γδ T cells, NK cells, and NKT cells	Neutrophil recruitment and immunity to extracellular pathogens
vIL-17	ORF13	IL-17RA (and IL-17RC?)	*Herpesvirus saimiri*	Unknown

Adapted from Gaffen, S. L. 2009. Structure and signalling in the IL-17 receptor family. *Nature Reviews Immunology* **9**:556–567.

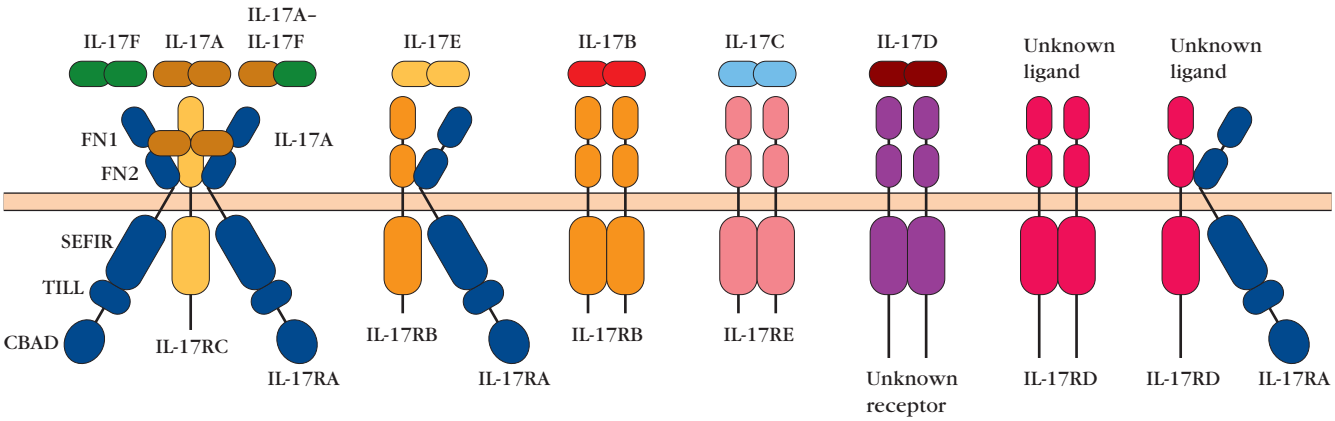

FIGURE 4-15 The IL-17 family of cytokines and their associated receptors. The cytokines that form the IL-17 family share a highly conserved structure, with four conserved cysteines. Only one of the proteins has so far been subject to x-ray analysis, which demonstrates that the structure is that of a "cysteine knot," a tightly folded protein that exists naturally as a dimer. The five proteins that make up the IL-17 receptor family are IL-17RA, IL-17RB, IL-17RC, IL-17RD, and IL-17RE. These are arranged into homo- and hetero- dimers and trimers to create the complete receptor molecules shown. Each receptor protein includes one or more fibronectin (FN) domains, as well as a cytoplasmic *SEF*/IL-17*R* (SEFIR) domain that is important in mediating downstream signaling events. The IL-17RA protein also includes a *TIR-like loop* domain (TILL), similar to that found in Toll-like receptors and IL-1 receptors, as well as a C/EBPβ activation domain, capable of interacting with the downstream transcription factor C/EBP. *[Adapted from S. Gaffen, 2009, Structure and signalling in the IL-17 receptor family,* Nature Reviews Immunology, **9**:*556.]*

the differentiation of the anti-inflammatory T_H2 subclass, while suppressing further T_H17 cell responses, in what amounts to a negative feedback loop.

In general, members of the IL-17 family exist as homodimers, but heterodimers of IL-17A and IL-17F have been described. Monomeric units of IL-17 family members range in molecular weight from 17.3 to 22.8 kDa, and crystallographic analysis has revealed that they share a structure that is primarily β sheet in nature, stabilized by intrachain disulfide bonds.

The IL-17 Family Receptors

The IL-17 receptor family is composed of five protein chains—IL-17RA, IL-17RB, IL-17RC, IL-17RD, and IL-17RE—which are variously arranged into homo- and hetero- dimeric and trimeric units to form the complete receptor molecules (Figure 4-15). Members of the IL-17 receptor family share fibronectin domains with the Hematopoietin and Interferon family cytokine receptors and are single transmembrane proteins. They all contain cytoplasmic *SEF*/*IL-17R* (Similar *E*xpression to *F*ibroblast growth factor *interleukin* 17 *R*eceptor, or SEFIR) domains, responsible for mediating the protein-protein interactions of the IL-17R signal transduction pathway. The IL-17RA chain also contains a *TIR-like loop* (TILL) domain, analogous to structures found in the Toll and IL-1 receptor molecules, as well as a *C/EBPβ activation domain* (CBAD), capable of activating the C/EBPβ transcription factor.

Signaling Through IL-17 Receptors

Analogous to signaling through IL-1 receptors, signaling through most IL-17 receptors results in an inflammatory response, and so it should not come as a surprise to learn that signaling through the IL-17 receptor results in activation of NF-κB, a hallmark transcription factor of inflammation. Details of the signaling pathways that emanate from the IL-17R are still being worked out, but Figure 4-16 illustrates the major features of our current knowledge.

1. *NF-κB activation via IL-17RA and IL-17RC.* Binding of IL-17A to the receptor molecules IL-17RA and IL-17RC results in the recruitment of the adapter protein ACT1 to the SEFIR domain. ACT1 binds other proteins, including TRAF3 and TRAF6, which then engage with the TAK1 complex. TAK1 activation results in the phosphorylation and inactivation of the inhibitor of NF-κB (IκB), allowing NF-κB activation and nuclear migration.
2. *Activation of MAP kinase pathway and cytokine mRNA stabilization.* Adapter proteins bound to the receptor also recruit components of the MAP kinase pathway, resulting in the activation of MAP kinases, including the extracellular signal-regulated kinase Erk1. Though unusual, it appears that the most important role of Erk1 in IL-17 signaling is not in the generation of phosphorylated transcription factors (as is the case for its involvement in TCR- and BCR-mediated cell signaling), but rather in *controlling the stability of cytokine mRNA transcripts.* Many of the target genes of IL-17 signaling are cytokines and chemokines whose transcription is up-regulated on receipt of an IL-17 signal. The levels of cytokine mRNA are controlled in part by binding of the cytoplasmic protein tristetraprolin to *AU-rich elements* (AREs) in the 3′-untranslated regions of mRNA transcripts. Tristetraprolin then delivers the cytokine mRNAs to the exosome complexes of the cells, where they are degraded. However, phosphorylation of tristetraprolin by MAP kinases inhibits

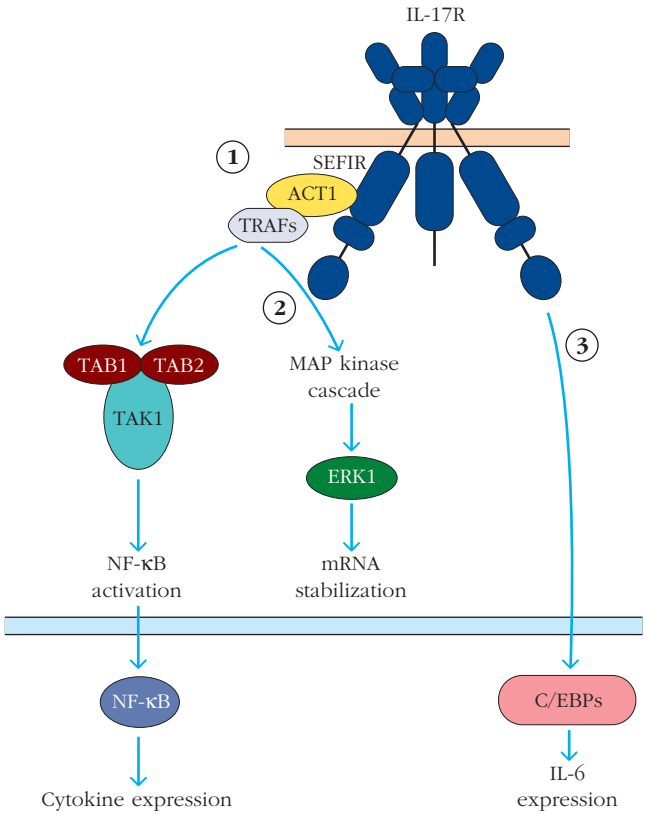

FIGURE 4-16 Signaling from the IL-17 receptor. Binding of IL-17 to its receptor initiates three signaling pathways. (1) Binding of IL-17 to its receptor results in the recruitment of the adapter protein ACT1 to the cytoplasmic region of the receptor. ACT1 then serves as a docking point for TRAF proteins 3 and 6, which in turn recruit members of the TAK1 complex, consisting of the TAK1 kinase and TAK1 binding proteins. TAK1 activation results in the phosphorylation and activation of the IKK complex and resultant NF-κB activation, as described previously. (2) Adapter proteins bound to the SEFIR (Similar *E*xpression to *F*ibroblast growth factor *interleukin* 17 *R*eceptor) domain also recruit components of the MAP kinase pathway. The MAP kinase Erk1 phosphorylates the cytoplasmic protein tristetraprolin, and inhibits its ability to bind to AU-rich elements on mRNA encoding cytokines. Since tristetraprolin binding results in mRNA degradation, activation of this arm of the pathway results in enhancing the stability of cytokine mRNA. (3) IL-17 binding to its receptor also results in the activation of transcription factors of the C/EBP family, which promote the expression of the inflammatory cytokine IL-6.

its ability to recruit the degradative machinery and hence results in increased stability of the cytokine and chemokine mRNAs.

3. *Activation of the transcription factors C/EBP*β *and C/EBP*δ In addition to up-regulation of NF-κB and members of the MAP kinase pathway, signaling through IL-17RA and IL-17RC proteins has also been shown to activate the transcription factors C/EBPβ and C/EBPδ, which promote expression of IL-6, one of the quintessential inflammatory cytokines.

Chemokines Direct the Migration of Leukocytes Through the Body

Chemokines are a structurally related family of small cytokines that bind to cell-surface receptors and induce the movement of leukocytes up a concentration gradient and toward the chemokine source. This soluble factor-directed cell movement is known as **chemotaxis**, and molecules that can elicit such movement are referred to as chemoattractants (Box 4-3). Some chemokines display innate affinity for the carbohydrates named glycosaminoglycans, located on the surfaces of endothelial cells, a property that enables them to bind to the inner surfaces of blood vessels and set up a cell-bound chemoattractant gradient along blood vessel walls, directing leukocyte movement.

Chemokine Structure

Chemokines are relatively low in molecular weight (7.5–12.5kDa) and structurally homologous. The tertiary structure of chemokines is constrained by a set of highly conserved disulfide bonds; the positions of the cysteine residues determine the classification of the chemokines into six different structural categories (Figure 4-17). Within any one category, chemokines may share 30% to 99% sequence identity.

The grouping of chemokines into the subclasses shown in Figure 4-17 has functional, as well as structural, significance. For example, the seven human CXC chemokines within the ELR subclass share the same receptor (CXCR2), attract neutrophils, are angiogenic, and have greater than 40% sequence identity. (A substance is *angiogenic* if it promotes the formation of new blood vessels; it is *angiostatic* if it prevents the formation of new blood vessels.) The non-ELR, CXCL chemokines CXCL9, CXCL10, and CXCL11, are also more than 40% identical to one another; however, this group is angiostatic, not angiogenic, and utilizes the CXCR4 receptor. Members of the two, structurally distinct CC groups are chemoattractants that attract monocytes and macrophages (although not neutrophils) to the site of infection. See Appendix III for a more comprehensive tabulation of chemokines and their immunologic roles.

Chemokine Receptors

In the 1950s, investigations of the mechanisms by which glucagon and adrenaline signaling led to an increase in the rate of glycogen metabolism revealed the existence of a class of receptors that threads through the membrane seven times and transduces the ligand signal via interactions with a polymeric GTP/GDP-binding "G protein." This class of ***G-Protein–Coupled Receptors* (GPCRs)** is used in the recognition of many types of signals, including those mediated by chemokines. Certain essential features of this pathway are conserved in all GPCR-type responses. (These larger, polymeric, seven membrane pass receptor-associated G proteins are different from the small, monomeric G proteins such as ras, which participate farther downstream in intracellular signaling pathways. Although both types of G proteins are

Class	Structural signature	Names	Number (n) in class
CX3C	.....CXXXC.....C.....C.....	CX3CL1	1
Non-ELR CXC	.....CX__C.....C.....C.....	CXCL#	9
ELR CXC	...ELR....CX__C.....C.....C.....	CXCL#	7
4C CC	.....C___C.....C.....C.....	CCL#	19
6C CC	.....C___C.....C.....C.....C....C...	CCL#	5
C	.....C.....C	XCL#	2

FIGURE 4-17 Disulfide bridges in chemokine structures. A schematic of the locations of cysteine residues in chemokines that shows how the locations of cysteines determine chemokine class. Chemokines are proteins of small molecular weight which share two, four, or six conserved cysteine residues at particular points in their sequence that form intrachain disulfide bonds. The number of cysteines as well as the positions of the disulfide bonds determine the subclass of these cytokines as shown. The overscores indicate the cysteines between which disulfide bonds are made. The naming of chemokines in part reflects the cysteine-determined class (see Appendix III). *[Adapted from W. E. Paul, 2008,* Fundamental Immunology, *6th ed. Lippincott Williams & Wilkins, Philadelphia, Figure 26.1]*

activated by GTP binding, their structures and functions are quite different).

The GPCRs are classified according to the type of chemokine they bind. For example, the CC receptors (CCRs) recognize CC chemokines, the CXCRs recognize CXCL chemokines, and so on. Chemokine receptors bind to their respective ligands quite tightly (Kd $\cong 10^{-9}$ M). Interestingly, the intrinsic specificity of the receptors is balanced by the capacity of many receptors to bind more than one chemokine from a particular family and of several chemokines to bind to more than one receptor. For example, the receptor CXCR2 recognizes seven different chemokines, and CCL5 can bind to both CCR3 and CCR5.

Signaling Through Chemokine Receptors

The cytoplasmic faces of seven membrane pass GPCRs associate with intracellular, trimeric **GTP-binding proteins** consisting of Gα, Gβ, and Gγ subunits (Figure 4-18a). When the receptor site is unoccupied, the Gα subunit of the trimeric G protein binds to GDP. Chemokine ligation to the receptor results in a conformational change that is transmitted to the G protein and in turn induces an exchange of GDP for GTP at the Gα binding site that is analogous to what occurs upon GTP binding to the small GTP-binding proteins such as Ras. This results in the dissociation of the G protein into a Gα-GTP monomer and a Gβγ dimer. In the case of the chemokine receptor associated G proteins, chemokine signaling is mediated by both the dissociated Gβγ dimer (which has no nucleotide binding site) and the Gα-GTP subunit.

Just as for the small G-protein–coupled pathways, the duration of signaling through the chemokine receptor is limited by the intrinsic GTPase activity of the Gα subunit, which in turn can be increased by ***G*TPase *A*ctivating *P*roteins (GAPs)**, also known as *R*egulators of *G*-protein *S*ignaling (RGSs). Because the pathway is active only when the protein binds to GTP, GAPs *down-regulate* the activity mediated by the receptor (see Figure 3-15). Once the GTP in the Gα binding site is hydrolyzed to GDP, Gα re-associates with the Gβγ dimers, effectively terminating signaling. There are multiple subtypes of Gα and Gβ subunits that vary in representation between different cell types. Signaling through different subunits can give rise to different consequences, depending on the downstream pathways that are elicited. Just as for the small G protein, there are several different large polymeric G proteins, which vary in their cellular distribution and receptor partners.

Once released from its Gα partner, the Gβγ subunit of the trimeric G protein activates a variety of downstream effector molecules, including those of the Ras/MAP kinase pathway (path 1 in Figure 4-18). Full activation of MAP kinase is further facilitated by tyrosine phosphorylation mediated by Gα-GTP-activated tyrosine kinases (not shown). Activation of the Ras pathway culminates in the initiation of transcription as well as in up-regulation of integrin adhesion molecules on the cell membrane. Gβγ signaling also cooperates with signaling mediated by Gα-GTP to activate one isoform of phospholipase C, PLCβ, resulting in an increase in the activity of the transcription factor NF-κB (path 2).

The GαGTP complex also activates a signaling pathway that is initiated by the small G protein, Rho (path 3). This pathway leads to actin polymerization and the promotion of cell migration, so it is this third pathway that is responsible for the most commonly described aspect of chemokine signaling: cell movement (see also Box 4-3). Rho signaling is also instrumental in bringing about changes in the transcriptional program of the cell. Finally, a JAK associated with

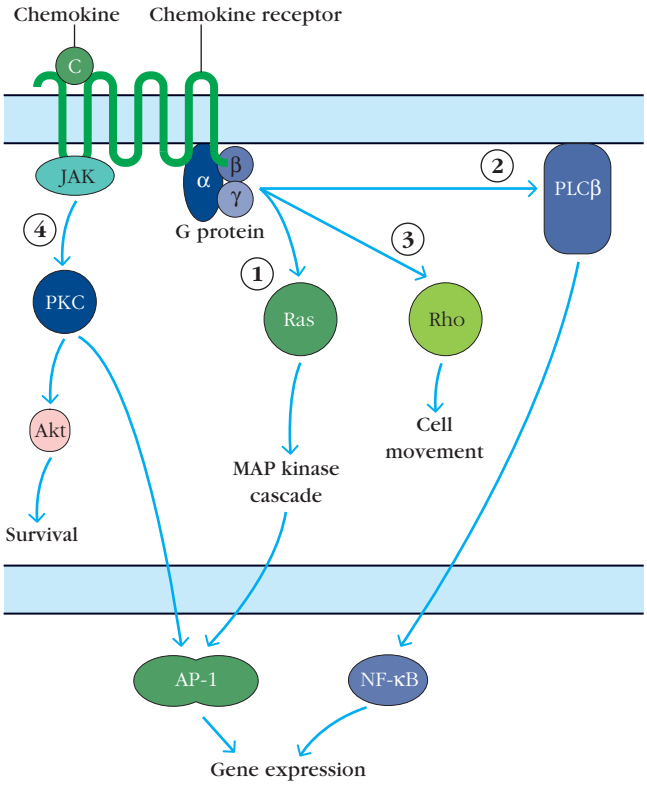

FIGURE 4-18 G-protein–coupled receptors interact with G proteins that transduce chemokine signals into the interior of the cell. Different chemokines can induce different signaling pathways. This figure therefore represents a composite of some of the most common pathways elicited by chemokine binding, which lead collectively to alterations in the transcriptional program, the enabling of cell movement, and changes in the adhesive properties of the signaled cell. (1) The Gβγ subunit binds to the adapter molecule Grb2, activating it and initiating the Ras signaling pathway that leads eventually to activation of MAP kinase and an alteration in the cell's transcriptional program, as shown in Figure 3-16. Ras pathway activation also leads eventually to activation of integrin adhesion molecules on the cell surface. Gα GTP simultaneously binds and activates a protein tyrosine kinase that phosphorylates and further activates MAP kinase (not shown). (2) Both GαGTP and Gβγ cooperate to activate PLCβ, which activates the NF-κB pathway. (3) GαGTP activates the small cytoplasmic G protein, Rho, initiating actin polymerization and cell movement. Other pathways emanate from Rho that lead to the activation of the transcription factor Serum Response Factor (SRF). (4) A JAK is stimulated by chemokine binding to the receptor, and it turns on the activity of PKC, leading eventually to the activation of the enzyme Akt. Akt affects cell survival by phosphorylating the proapoptotic genes Bax and Bad (not shown) and marking them for destruction and enhancing cell survival. It also phosphorylates and further activates the transcription factor NF-κB. JAK-mediated PKC activation can also lead to phosphorylation of the transcription factor Jun, and its dimerization with Fos to create the complete transcription factor AP-1.

the GPCR initiates signaling through PKC-mediated pathways that culminate in the activation of Akt and increased cell survival (path 4) as well as in further transcriptional alterations.

The quality of the response elicited by particular chemokines in different types of cells is dependent on the nature of the chemokine ligand, as well as on the signaling microenvironment, which in turn is determined by the range of G protein subtypes and regulatory molecules present in that cell. But from the complexity of signaling options available to the receptor, it is not difficult to see how binding of a chemokine molecule to its receptor can simultaneously bring about alterations in the location, the adhesion molecule binding capacity, and the transcriptional program of the chemokine-activated cell.

Cytokine Antagonists

A number of proteins that inhibit the biological activity of cytokines have been reported. These proteins act in one of two ways: either they bind directly to a cytokine receptor but fail to activate the cell, thus blocking the active cytokine from binding, or they bind directly to the cytokine itself, inhibiting its ability to bind to the cognate receptor. In this section, we describe some naturally occurring cytokine antagonists that modulate and refine the power of particular cytokine responses, as well as the ways in which various pathogens have hijacked cytokine responses to their own ends.

The IL-1 Receptor Antagonist Blocks the IL-1 Cytokine Receptor

The best-characterized cytokine inhibitor is the IL-1 receptor antagonist (IL-1Ra), which binds to the IL-1 receptor but does not elicit activation of the signaling pathway (see above). As previously described, ligation of IL-1Ra to the IL-1 receptor blocks the binding of both IL-1α and IL-1β, thus accounting for its inhibitory properties. IL-1Ra is synthesized by the same cells that secrete IL-1α and IL-1β, and its synthesis in the liver is up-regulated under inflammatory conditions, along with that of IL-1. Several animal and human models exist in which the levels of IL-1Ra are naturally reduced, and humans carrying an allele that decreases the expression of IL-1Ra suffer from arthritis and a variety of other autoimmune diseases. This observation suggests that the normal function of IL-1Ra is to provide for the host a means by which to modulate the numbers of receptors that are capable of mounting a physiological response to IL-1. Given the fiercely proinflammatory effects of IL-1, it makes sense that responses to this powerful cytokine should be carefully controlled. Indeed, we note quite often in biological systems that a process—if it has the potential to lead to deleterious consequences to the organism—is subject to several different means of regulation.

Recombinant IL-Ra has been used clinically, under the name of *anakrina*, for the treatment of rheumatoid arthritis. Investigations into how cells control the balance of IL-1 and IL-1Ra secretion are still ongoing, but preliminary studies suggest that the activation of different isoforms of PI3 kinase may play an important role in determining the relative amounts of IL-1 and IL-1Ra that are secreted by a stimulated monocyte.

Cytokine Antagonists Can Be Derived from Cleavage of the Cytokine Receptor

Some naturally occurring soluble antagonists arise from enzymatic cleavage of the extracellular domains of cytokine receptors. These soluble receptor components can compete with the membrane-bound receptor for cytokine binding and thus down-modulate the potential cytokine response. The best characterized of the soluble cytokine receptors consists of a segment containing the amino-terminal 192 amino acids of the IL-2Rα (CD25) subunit, which is released by proteolytic cleavage, forming a 45-kDa soluble IL-2 receptor (sIL-2R or sCD25). The shed receptor retains its ability to bind IL-2 and can therefore prevent the cytokine's productive interaction with the membrane-bound IL-2 receptor.

The origin of sIL-2R is still a matter for debate. Recently, regulatory T cells, which express high levels of CD25 on their membrane surfaces, have been shown to release sIL-2R upon activation. Since these T cells serve the function of down-regulating ongoing immune responses, it has been suggested that the soluble IL-2 receptors may serve the physiological function of soaking up excess IL-2 and thus reducing the amount of the cytokine that is available to irrelevant or even to competing effector T cells. The soluble IL-2 receptor is also found in the serum and bodily fluids of patients suffering from a number of hematologic malignancies (blood cell cancers), and high levels of sIL-2R in the blood correlate with a poor disease prognosis. However, the issue of whether the sIL-2R is released from the tumor cells per se, or whether it is released from regulatory T cells that may be acting to dampen the host anti-tumor response, has yet to be resolved.

Some Viruses Have Developed Strategies to Exploit Cytokine Activity

The cytokine antagonists described above derive from an organism's own immune system. However, as is so often the case in immunology, some pathogens have evolved ways in which to circumvent cytokine responses, by mimicking molecules and pathways used by the host. The evolution of anti-cytokine strategies by microbial pathogens provides biological evidence of the importance of cytokines in organizing and promoting effective antimicrobial immune responses. The following are among the various anti-cytokine strategies used by viruses:

- The generation of viral products that interfere with cytokine secretion
- The generation of cytokine homologs that compete with natural cytokines or inhibit anti-viral responses
- The production of soluble cytokine-binding proteins
- The expression of homologs of cytokine receptors
- The generation of viral products that interfere with intracellular signaling
- The induction of cytokine inhibitors in the host cell

Epstein-Barr virus (EBV), for example, produces an IL-10–like molecule (viral IL-10 or vIL-10) that binds to the IL-10 receptor. Just like host-derived IL-10, this viral homologue suppresses T_H1-type cell-mediated responses that would otherwise be effective in fighting a viral infection. Other cytokine mimics produced by viruses allow them to manipulate the immune response in alternative ways that aid the survival of the pathogen. For example, EBV produces an inducer of IL-1Ra, the host antagonist of IL-1. Poxviruses have also been shown to encode a soluble TNF-binding protein and a soluble IL-1-binding protein that block the ability of the bound cytokines to elicit a response. Since both TNF-α and IL-1 are critical to the early phases of an inflammatory, antiviral response, these soluble cytokine-binding proteins may allow the viruses an increased time window in which to replicate.

Yet other viruses produce molecules that inhibit the production of cytokines. One such example is the *c*ytokine *r*esponse *m*odifier (Crm) protein of the cowpox virus, which inhibits the production of caspase-1 and hence prevents the processing of IL-1 precursor proteins. Finally, some viruses produce soluble chemokines and chemokine-binding proteins that interfere with normal immune cell trafficking, and allow the producing viruses and virally infected cells to evade an immune response. Table 4-6 lists a number of viral products that inhibit cytokines, chemokines, and their activities.

Cytokine-Related Diseases

Defects in the complex regulatory networks governing the expression of cytokines and cytokine receptors have been implicated in a number of diseases. Genetic defects in cytokines, their receptors, or the molecules involved in cytokine-directed signal transduction lead to immunodeficiencies such as those described in Chapter 18. Other defects in the cytokine network can cause an inability to defend against specific families of pathogens. For example, people with a defective receptor for IFN-γ are susceptible to mycobacterial

TABLE 4-6 Viruses use many different strategies to evade cytokine-mediated immune mechanisms

Virus	Virally encoded proteins
Epstein-Barr Virus (EBV), Cytomegalovirus	IL-10 homolog
Vaccinia virus, Variola virus	Soluble IL-1 receptors
Myxoma virus	Soluble IFN-γ receptor
Variola virus	Soluble TNF receptors
Adenovirus	RID complex proteins induce internalization of Fas receptor
Measles virus	Viral hemagglutinin binds to complement receptor, CD46, signaling disruption of IL-12 production and therefore inhibition of T_H1 pathway differentiation
Herpes simplex virus	Reverses translation block induced by Type 1 interferons
Adenovirus	Blocks interferon-induced JAK/STAT signaling

infections that rarely occur in the general population. In addition to the diseases rooted in genetic defects in cytokine activity, a number of other pathologic states result from overexpression or underexpression of cytokines or cytokine receptors. Several examples of these diseases are given below, followed by an account of therapies aimed at preventing the potential harm caused by cytokine activity.

Septic Shock Is Relatively Common and Potentially Lethal

Despite the widespread use of antibiotics, bacterial infections remain a major cause of septic shock, which may develop a few hours after infection by certain bacteria, including *Staphyloccocus aureus*, *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, and *Neisseria meningitidis*.

Bacterial septic shock is one of the conditions that falls under the general heading of **sepsis**. Sepsis, in turn, may be caused not only by bacterial infection but also by trauma, injury, ischemia (decrease in blood supply to an organ or a tissue), and certain cancers. Sepsis is the most common cause of death in U.S. hospital intensive-care units and the 13th leading cause of death in the United States. A common feature of sepsis, whatever the underlying cause, is an overwhelming production of proinflammatory and fever-inducing cytokines such as TNF-α and IL-1β. The cytokine imbalance induces abnormal body temperature, alterations in the respiratory rate, and high white blood cell counts, followed by capillary leakage, tissue injury, widespread blood clotting, and lethal organ failure.

Bacterial septic shock often develops because bacterial cell wall **endotoxins** bind to innate immune system pathogen receptors, such as Toll-like receptors (see Chapter 5), on dendritic cells and macrophages, causing them to produce IL-1 and TNF-α at levels that lead to pathological capillary permeability and loss of blood pressure. A condition resembling bacterial septic shock can be produced in the absence of any bacterial infection simply by injecting mice with recombinant TNF-α. Several studies offer hope that neutralizing TNF-α or IL-1 activity with monoclonal antibodies or antagonists will prevent fatal shock from developing. In one such investigation, monoclonal antibodies to TNF-α protected animals from endotoxin-induced shock. In another, injection of a recombinant IL-1 receptor antagonist (IL-1Ra), which prevents binding of IL-1 to the IL-1 receptor as described above, resulted in a threefold reduction in mortality.

However, neutralization of TNF-α does not reverse the progression of septic shock in all cases, and antibodies against TNF-α give little benefit to patients with advanced disease. Recent studies in which the cytokine profiles of patients with septic shock were followed over time shed some light on this apparent paradox.

The increases in TNF-α and IL-1β occur rapidly in early sepsis, so neutralizing these cytokines is most beneficial early in the process. Indeed, in animal experiments, early intervention can prevent sepsis altogether. However, approximately 24 hours following the onset of sepsis, the levels of TNF-α and IL-1β fall dramatically, and other factors become more important. Cytokines critical in the later stages of sepsis include IL-6, MIF, and CCL-8. Sepsis remains an area of intense investigation, and clarification of the process involved in bacterial septic shock and other forms of sepsis can be expected to lead to advances in therapies for this major killer in the near future.

Bacterial Toxic Shock Is Caused by Superantigen Induction of T-Cell Cytokine Secretion

A variety of microorganisms produce toxins that act as **superantigens**. As described in Chapters 9 and 11, superantigens

CLINICAL FOCUS BOX 4-4

Cytokines and Obesity

Even as those in the Third World suffer from malnutrition, among the leading current causes of disease and death in developed countries are obesity and its corollary, Type 2 diabetes.

Type 1, or juvenile onset, diabetes has long been known to have an autoimmune etiology (cause). Cells of the adaptive immune system kill the β cells of the Islets of Langerhans in the pancreas, leading to the complete absence of insulin in the diabetic patient, who must rely on exogenously delivered hormone to survive.

In Type 2 diabetes, the body fails to respond to insulin in an appropriate manner, and the cells fail to take in glucose from the blood and into the tissues. Just as in Type 1 diabetes, the presence of high levels of glucose in the blood and tissue fluids facilitates nonenzymatic glucose conversions to reactive carbohydrate derivatives such as glyoxals. These derivatives cross-link proteins and carbohydrates in the membranes and walls of blood vessels and neurons and within the extracellular matrix, leading to the familiar array of diabetic symptoms: poor peripheral circulation, plaque buildup in the arteries, and heart disease. However, we are now beginning to understand that Type 2, just like Type 1, diabetes is a disease closely related to the workings of the immune system.

In 1993, a seminal publication by Hotamisligil and colleagues made the link between inflammation and metabolic conditions such as Type 2 diabetes and obesity. These authors demonstrated that adipocytes (fat cells) constitutively express the proinflammatory cytokine TNF-α, and that TNF-α expression in adipocytes of obese animals is markedly increased. Later research demonstrated that this finding could be extended to humans. The adipose tissue of human subjects was found to constitutively express TNF-α, and blood levels of TNF-α fall after weight loss.

But is this increase in TNF-α expression affecting insulin sensitivity? On binding of insulin to its receptor, tyrosine kinase activity in the cytoplasmic region of the receptor is activated. This *R*eceptor *T*yrosine *K*inase (RTK) then phosphorylates both itself (autophosphorylation) and some nearby proteins. The signaling cascade is initiated from the insulin receptor by the binding of adapter proteins such as Grb2 and IRS-1 via their SH2 domains to the phosphorylated tyrosine sites on the receptor molecule. We now know that TNF-α signaling in adipocytes inhibits the autophosphorylation of tyrosine residues of the insulin receptor and instead induces phosphorylation of serine residues of both the insulin receptor and the IRS-1 adapter. The serine phosphorylation inhibits any subsequent phosphorylation at tyrosine residues, and thus the passage of signals from insulin to the interior of the fat cell is prevented. Recently, the interleukin IL-6 has also been shown to inhibit insulin signal transduction in hepatocytes (liver cells) through a similar mechanism. The decrease in effectiveness of insulin signaling then becomes a self-reinforcing problem, as insulin signaling itself is anti-inflammatory, and so any decrease in the insulin signal can give rise to inflammatory side effects.

However, TNF-α and IL-6 are not the only cytokines implicated in the etiology of Type 2 diabetes. With the discovery and characterization of the proinflammatory cytokine family represented by IL-17, interest has arisen in the relationship between members of this family, obesity, and the control of fat cell metabolism. Since IL-6 is implicated in the differentiation of T lymphocytes to secrete IL-17, obesity and its associated inflammation tend to predispose an individual to secrete IL-17. However, again we find ourselves in a positive feedback loop, as IL-17 acts on monocytes to induce the further secretion of IL-6, thus ensuring the maintenance of an inflammatory state.

It is therefore clear that cytokine signaling plays a profound role in a disease that is emblematic of our time, and which is predicted to afflict close to 40% of the U.S. population by the middle of the next decade.

bind to MHC Class II molecules at a location in the MHC molecule that is outside the groove normally occupied by antigenic peptides (see Figure 11-6). They then bind to a part of the Vβ chain of the T-cell receptor that is outside the normal antigen-binding site, and this binding is sufficient to trigger T-cell activation. This means that a given superantigen can simultaneously activate all T cells bearing a particular Vβ domain. Because of their unique binding ability, superantigens can activate large numbers of T cells irrespective of the antigenic specificity of their canonical antigen-binding site.

Although less than 0.01% of T cells respond to a given conventional antigen (see Chapter 11), 5% or more of the T-cell population can respond to a given superantigen. Bacterial superantigens have been implicated as the causative agent of several diseases, such as bacterial toxic shock and food poisoning. Included among these bacterial superantigens are several enterotoxins, exfoliating toxins, and toxic shock syndrome toxin (TSST1) from *Staphylococcus aureus*; pyrogenic exotoxins from *Streptococcus pyrogenes*; and *Mycoplasma arthritidis* supernatant (MAS). The large number of T cells activated by these superantigens

results in excessive production of cytokines. The TSST1, for example, has been shown to induce extremely high levels of TNF-α and IL-1β. As in bacterial septic shock, these elevated concentrations of cytokines can induce systemic reactions that include fever, widespread blood clotting, and shock.

In addition to those diseases described above, in which cytokines or their receptors are directly implicated, recent information has indicated the importance of cytokine involvement in the most important public health crisis currently afflicting the developed world: the increasing incidence of Type 2 diabetes. The roles of TNF-α and IL-6 in the induction and maintenance of this disease are described in Box 4-4.

Cytokine Activity Is Implicated in Lymphoid and Myeloid Cancers

Abnormalities in the production of cytokines or their receptors have been associated with some types of cancer. For example, abnormally high levels of IL-6 are secreted by cardiac myxoma (a benign heart tumor), myeloma and plasmacytoma cells, as well as cervical and bladder cancer cells. In myeloma and plasmacytoma cells, IL-6 appears to operate in an autocrine manner to stimulate cell proliferation. When monoclonal antibodies to IL-6 are added to in vitro cultures of myeloma cells, their growth is inhibited. In contrast, transgenic mice that express high levels of IL-6 have been found to exhibit a massive, fatal, plasma-cell proliferation, called plasmacytosis. In addition, as described above, high serum concentrations of the sIL-2R are found in patients suffering from various blood cell cancers, which may impede a vigorous anti-tumor response.

Cytokine Storms May Have Caused Many Deaths in the 1918 Spanish Influenza

Occasionally, a particularly virulent infection may induce the secretion of extremely high levels of cytokines, that then feed back on the immune cells to elicit yet more cytokines. Normally, these positive feedback loops represent effective modes of immune amplification; they are themselves usually kept in check by self-regulating immune mechanisms, such as the activation of regulatory T cells (see Chapter 11). However, some viruses cause a localized, exaggerated response, resulting in the secretion of extraordinarily high levels of cytokines. If this occurs in the lungs, for example, the localized swelling, inflammation, and increase in capillary permeability can lead to the accumulation of fluids and leukocytes that block the airways, thereby causing exacerbation of symptoms, or even death, before the cytokine levels can be controlled. It is unclear why some viruses induce these **cytokine storms** and others do not.

Historical documents detailing the symptoms of the 1918 Spanish influenza suggest that the massive fatalities associated with that pandemic most likely resulted from cytokine storms, and there is some evidence that the severe acute respiratory syndrome (SARS) epidemic of 1993 may have caused a similar, unregulated, immune cell cytokine secretion. Transplant surgeons also observe this phenomenon on occasions when leukocytes associated with a graft—often a bone marrow transplant—mount an immune response against the host. Effective treatments for patients undergoing cytokine storms are still being developed. Currently, patients are offered steroidal and nonsteroidal anti-inflammatory medications, but other drugs that are more specifically directed at the reduction of cytokine secretion and/or activity are being tested.

Cytokine-Based Therapies

The availability of purified cloned cytokines, monoclonal antibodies directed against cytokines, and soluble cytokine receptors offers the prospect of specific clinical therapies to modulate the immune response. Cytokines such as interferons (see Clinical Focus Box 4-2), colony-stimulating factors such as G-CSF, and IL-2 have all been used clinically. In addition, several reagents that specifically block the proinflammatory effects of TNF-α have proven to be therapeutically useful in certain diseases. Specifically, soluble TNF-α receptor (Enbrel) and monoclonal antibodies against TNF-α (Remicade and Humira) have been used to treat rheumatoid arthritis and ankylosing spondylitis in more than a million patients. These anti–TNF-α drugs reduce proinflammatory cytokine cascades; help to alleviate pain, stiffness, and joint swelling; and promote healing and tissue repair. In addition, as described above, the recombinant form of IL-1Ra—anakinra (Kineret)—has been shown to be relatively effective in the treatment of rheumatoid arthritis. Monoclonal antibodies directed against the α chain of the IL-2R—basiliximab (Simulect) and daclizumab (Zenapax)—are also in clinical use for the prevention of transplantation rejection reactions.

As powerful as these reagents may be, interfering with the normal course of the immune response is not without its own intrinsic hazards. Reduced cytokine activity brings with it an increased risk of infection and malignancy, and the frequency of lymphoma is higher in patients who are long-term users of the first generation of TNF-α blocking drugs.

In addition, the technical problems encountered in adapting cytokines for safe, routine medical use are far from trivial. As described above, during an immune response, interacting cells may produce extremely high local concentrations of cytokines in the vicinity of target cells, but achieving such high concentrations over a clinically significant time period, when cytokines must be

administered systemically, is difficult. Furthermore, many cytokines have a very short half-life—recombinant human IL-2 has a half-life of only 7 to 10 minutes when administered intravenously—so frequent administration may be required. Finally, cytokines are extremely potent biological response modifiers, and they can cause unpredictable and undesirable side effects. The side effects from administration of recombinant IL-2, for example, range from mild (e.g., fever, chills, diarrhea, and weight gain) to serious (e.g., anemia, thrombocytopenia, shock, respiratory distress, and coma).

The use of cytokines and anti-cytokine therapies in clinical medicine holds great promise, and efforts to develop safe and effective cytokine-related strategies continue, particularly in those areas of medicine that have so far been resistant to more conventional approaches, such as inflammation, cancer, organ transplantation, and chronic allergic disease.

SUMMARY

- Cytokines are proteins that mediate the effector functions of the immune system.
- Most cytokines are soluble proteins, but some—for example, members of the TNF family, may be expressed in a membrane-bound form.
- Some cytokines are secreted following stimulation of the innate immune system (e.g., IL-1, TNF-α, CXCL8), whereas others are secreted by the T and B lymphocytes of the adaptive immune system (IL-2, IL-4, IL-17).
- Cytokines bind to receptors on the plasma membrane and elicit their effects through the activation of an intracellular signaling cascade.
- Cytokines can effect alterations in the differentiative, proliferative, and survival capacities of their target cells.
- Cytokines exhibit the properties of redundancy, pleiotropy, synergy, antagonism, and cascade induction.
- The levels of expression of cytokine receptors on the cell surface may change according to the activation status of a cell.
- There are six families of cytokines with associated receptors, distinguished on the basis of the structures of the cytokines and the receptor molecules, and on the nature of their signaling pathways.
- IL-1 family members interact with dimeric receptors to induce responses that are primarily proinflammatory. The physiological responses to some IL-1 family members are modulated by the presence of soluble forms of the receptors and soluble cytokine-binding proteins.
- The Hematopoietin (Class I cytokine) family is the largest family of cytokines, and members mediate diverse effects, including proliferation, differentiation, and antibody secretion. The Hematopoietin family members share a common, four-helix bundle structure.
- Receptors for cytokines from the Hematopoietin family are classified into three subgroups—the γ, β, or gp130 receptors—each of which shares a common signaling chain.
- The Interferon (Class II cytokine) family includes the Type I interferons (interferon α and interferon β), which were the first cytokines to be discovered and mediate early antiviral responses.
- Type II interferons (interferon γ) activate macrophages, interact with cells of the adaptive immune system and support the generation of T_H1 cells.
- The TNF family of cytokines act as trimers and may occur in either soluble or membrane-bound forms.
- FasL, a TNF family member, interacts with its receptor, Fas, to stimulate apoptosis in the recipient cell. This interaction is important at the close of the immune response.
- TNF interacts with the TNF-R1 receptor on the surface of the cell to induce either apoptosis or survival, depending on the physiological environment.
- The IL-17 family of cytokines has been defined quite recently, and its members are primarily proinflammatory in action.
- Chemokines act on GPCR-coupled receptors to promote chemoattraction, the movement of immune system cells into, within, and out of lymphoid organs.
- Naturally occurring and pathogen-derived inhibitors of cytokine function may modulate their activity in vivo.
- Levels of inflammatory cytokines such as IL-1, IL-6, and TNF may be increased in certain disease states such as rheumatoid arthritis, and such diseases are susceptible to treatment with drugs that inhibit cytokine activities.

REFERENCES

Abram, C. L., and C. A. Lowell. 2009. The ins and outs of leukocyte integrin signaling. *Annual Review of Immunology* **27**:339–362.

Ahmed, M., and S. L. Gaffen. 2010. IL-17 in obesity and adipogenesis. *Cytokine & Growth Factor Reviews* **21**:449–453.

Alcami, A. 2003. Structural basis of the herpesvirus M3-chemokine interaction. *Trends in Microbiology* **11**:191–192.

Arend, W.P., G. Palmer, and C. Gabay. 2008. IL-1, IL-18, and IL-33 families of cytokines. *Immunological Reviews* **223**:20–38.

Botto, S., et al. 2011. IL-6 in human cytomegalovirus secretome promotes angiogenesis and survival of endothelial cells through the stimulation of survivin. *Blood* **117**:352–361.

Boulanger, M. J., and K. C. Garcia. 2004. Shared cytokine signaling receptors: Structural insights from the gp130 system. *Advances in Protein Chemistry* **68**:107–146.

Crabtree, G. R., S. Gillis, K. A. Smith, and A. Munck. 1980. Mechanisms of glucocorticoid-induced immunosuppression: Inhibitory effects on expression of Fc receptors and production of T-cell growth factor. *Journal of Steroid Biochemistry* **12**:445–449.

Crispin, J. C., and G. C. Tsokos. 2009. Transcriptional regulation of IL-2 in health and autoimmunity. *Autoimmunity Reviews* **8**:190–195.

Dandona, P., A. Aljada, and A. Bandyopadhyay. 2004. Inflammation: The link between insulin resistance, obesity and diabetes. *Trends in Immunology* **25**:4–7.

Dandona, P., A. Aljada, A. Chaudhuri, P. Mohanty, and G. Rajesh. 2004. A novel view of metabolic syndrome. *Metabolic Syndrome and Related Disorders* **2**:2–8.

Eisenbarth, S. C., and R. A. Flavell. 2009. Innate instruction of adaptive immunity revisited: The inflammasome. *EMBO Molecular Medicine* **1**:92–98.

Fickenscher, H., et al. 2002. The interleukin-10 family of cytokines. *Trends in Immunology* **23:**89–96.

Gaffen, S. L. (2009). Structure and signalling in the IL-17 receptor family. *Nature Reviews. Immunology* **9**:556–567.

Gee, K., C. Guzzo, N. F. Che Mat, W. Ma, and A. Kumar. 2009. The IL-12 family of cytokines in infection, inflammation and autoimmune disorders. *Inflammation & Allergy Drug Targets* **8**:40–52.

Gillis, S., R. Mertelsmann, and M. A. Moore. 1981. T-cell growth factor (interleukin 2) control of T-lymphocyte proliferation: Possible involvement in leukemogenesis. *Transplantation Proceedings* **13**:1884–1890.

Guilherme, A., J. V. Virbasius, V. Puri, and M. P. Czech. 2008. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. *Nature Reviews. Molecular Cell Biology* **9:**367–377.

Horng, T., and G. S. Hotamisligil. 2011. Linking the inflammasome to obesity-related disease. *Nature Medicine* **17**:164–165.

Hotamisligil, G. S. 2003. Inflammatory pathways and insulin action. *International Journal of Obesity and Related Metabolic Disorders* **27:**Suppl 3, S53–55.

Hotamisligil, G. S., N. S. Shargill, and B. M. Spiegelman. 1993. Adipose expression of tumor necrosis factor-alpha: Direct role in obesity-linked insulin resistance. *Science* **259**:87–91.

Jones, L. L., and D. A. Vignali. 2011. Molecular interactions within the IL-6/IL-12 cytokine/receptor superfamily. *Immunologic Research* **51**:5–14.

Li, W. X. 2008. Canonical and non-canonical JAK-STAT signaling. *Trends in Cell Biology* **18**:545–551.

Micheau, M., and J. Tschopp. 2003. Induction of TNF Receptor 1-mediated apoptosis via two sequential signaling complexes. *Cell* **114**:181–190.

Raab, M., et al. 2010. T cell receptor "inside-out" pathway via signaling module SKAP1-RapL regulates T cell motility and interactions in lymph nodes. *Immunity* **32**:541–556.

Raman, D., T. Sobolik-Delmaire, and A. Richmond. 2011. Chemokines in health and disease. *Experimental Cell Research* **317**:5755–89.

Rochman, Y., R. Spolski, and W. J. Leonard. 2009. New insights into the regulation of T cells by gamma(c) family cytokines. *Nature Reviews. Immunology* **9**:480–490.

Rot, A., and U. H. von Andrian. 2004. Chemokines in innate and adaptive host defense: Basic chemokinese grammar for immune cells. *Annual Review of Immunollogy* **22**:891–928.

Sadler, A. J., and B. R. Williams. 2008. Interferon-inducible antiviral effectors. *Nature Reviews. Immunology* **8**:559–568.

Skalnikova, H., J. Motlik, S. J. Gadher, and H. Kovarova. Mapping of the secretome of primary isolates of mammalian cells, stem cells and derived cell lines. *Proteomics* **11**:691–708.

Skiniotis, G., P. J. Lupardus, M. Martick, T. Walz, T., and K. C. Garcia. 2008. Structural organization of a full-length gp130/LIF-R cytokine receptor transmembrane complex. *Molecular Cell* **31**:737–748.

Smith, K. A., P. E. Baker, S. Gillis, and F. W. Ruscetti. (1980). Functional and molecular characteristics of T-cell growth factor. *Molecular Immunology* **17**:579–589.

Stull, D., and S. Gillis. 1981. Constitutive production of interleukin 2 activity by a T cell hybridoma. *Journal of Immunology* **126**:1680–1683.

Walczak, H. 2011. TNF and ubiquitin at the crossroads of gene activation, cell death, inflammation and cancer. *Immunological Reviews* **244**:9–28.

Wellen, K. E., and G. S. Hotamisligil. 2003. Obesity-induced inflammatory changes in adipose tissue. *The Journal of Clinical Investigation* **112**:1785–1788.

Useful Web Sites

www.invitrogen.com

www.miltenyibiotec.com/cytokines

www.prospecbio.com/Cytokines

www.peprotech.com

www.rndsystems.com Many companies that sell recombinant cytokines or cytokine-related products provide useful information on their websites, or in print copy. The preceding are a few that are particularly helpful.

www.jakpathways.com/understandingjakpathways A useful animation of JAK-STAT signaling.

www.youtube.com/watch?v=ZUUfdP87Ssg&feature=related A rather wonderful movie of chemotaxis.

www.youtube.com/watch?v=EpC6G_DGqkI&feature=related A striking movie of a neutrophil chasing a bacterium.

www.youtube.com/watch?v=KiLJI3NwmpU An increasing number of medical animations are available on You Tube. This one shows a macrophage recognizing a pathogen and releasing cytokines in response.

www.netpath.org A curated set of pathways, with information on interacting proteins. Many interleukin pathways are included.

STUDY QUESTIONS

1. Distinguish between a hormone, a cytokine, a chemokine, and a growth factor. What functional attributes do they share, and what properties can be used to discriminate among them?

2. Measurement of the blood concentration of a particular cytokine reveals that it is rarely present above 10^{-10} M, even under the conditions of an ongoing immune response. However, when you measure the affinity of the cognate receptor, you discover that its dissociation constant is close to 10^{-8} M, implying that the receptor occupancy must rarely exceed 1%. How do you account for this discrepancy?

3. Describe how dimerization and phosphorylation of intracellular signaling molecules contribute to activation of cells by Type 1 cytokines.

4. Define the terms *pleiotropy*, *synergy*, *redundancy*, *antagonism*, and *cascade induction* as they apply to cytokine action.

5. How might receipt of a cytokine signal result in the alteration of the location of a lymphocyte?

6. Cytokines signaling through the Class I cytokine receptors can compete with one another, even though the recognition units of the receptors are different. Explain.

7. Describe one mechanism by which Type I interferons "interfere" with the production of new viral particles.

8. Signaling by tumor necrosis factor can paradoxically lead to cell activation or cell death. Explain how, by drawing diagrams of the relevant signaling pathways.

9. Describe two examples of ways in which vertebrates tune down the intensity of their own cytokine signaling.

10a. The cytokine IL-2 is capable of activating all T cells to proliferation and differentiation. How does the immune system ensure that only T cells that have been stimulated by antigen are susceptible to IL-2 signaling?

10b. The following diagram represents the results of a flow cytometry experiment in which mouse spleen cells were stained with antibodies directed against different components of the IL-2R. The more antibody that binds to the cells, the further they move along the relevant axis. The number of cells stained with fluorescein-conjugated anti-βγ IL-2R antibodies are shown along the x-axis of the flow cytometry plot, and cells that stain with phycoerythrin-labeled antibodies to the α subunit of the IL-2 receptor move along the Y-axis. We have drawn for your reference a circle that represents cells that stain with neither antibody.

On this plot, draw, as circles, and label where you would expect to find the populations representing unstimulated T cells and T cells after antigen activation, after treatment with the two fluorescent labels described above.