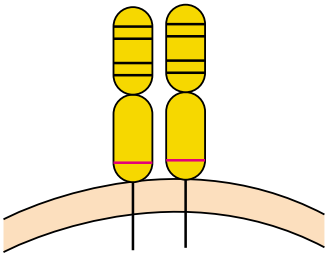
Cytokines

chapter 12



Class I Cytokine Receptors

The development of an effective immune response involves lymphoid cells, inflammatory cells, and hematopoietic cells. The complex interactions among these cells are mediated by a group of proteins collectively designated **cytokines** to denote their role in cell-to-cell communication. Cytokines are low-molecular-weight regulatory proteins or glycoproteins secreted by white blood cells and various other cells in the body in response to a number of stimuli. These proteins assist in regulating the development of immune effector cells, and some cytokines possess direct effector functions of their own.

This chapter focuses on the biological activity of cytokines, the structure of cytokines and their receptors, signal transduction by cytokine receptors, the role of cytokine abnormalities in the pathogenesis of certain diseases, and therapeutic uses of cytokines or their receptors. The important role of cytokines in the inflammatory response is described in Chapter 15.

Properties of Cytokines

Cytokines bind to specific receptors on the membrane of target cells, triggering signal-transduction pathways that ultimately alter gene expression in the target cells (Figure 12-1a). The susceptibility of the target cell to a particular cytokine is determined by the presence of specific membrane receptors. In general, the cytokines and their receptors exhibit very high affinity for each other, with dissociation constants ranging from 10^{-10} to 10^{-12} M. Because their affinities are so high, cytokines can mediate biological effects at picomolar concentrations.

A particular cytokine may bind to receptors on the membrane of the same cell that secreted it, exerting **autocrine** action; it may bind to receptors on a target cell in close proximity to the producer cell, exerting **paracrine** action; in a few cases, it may bind to target cells in distant parts of the body, exerting **endocrine** action (Figure 12-1b). Cytokines regulate the intensity and duration of the immune response by stimulating or inhibiting the activation, proliferation, and/or differentiation of various cells and by regulating the secretion of antibodies or other cytokines. As described later, binding of a given cytokine to responsive target cells generally stimulates increased expression of cytokine receptors and secretion of other cytokines, which affect other target cells in turn. Thus, the cytokines secreted by even a small number of lymphocytes activated by antigen can influence the activity of numerous cells involved in the immune response. For example, cytokines produced by activated T_H cells can influence the activity of B cells, T_C cells, natural killer cells, macrophages, granulocytes, and hematopoietic stem cells, thereby activating an entire network of interacting cells.

Cytokines exhibit the attributes of pleiotropy, redundancy, synergy, antagonism, and cascade induction, which permit them to regulate cellular activity in a coordinated, interactive way (Figure 12-2). A given cytokine that has different biological effects on different target cells has a pleiotropic action. Two or more cytokines that mediate similar functions are said to be redundant; redundancy makes it difficult to ascribe a particular activity to a single cytokine. Cytokine synergism occurs when the combined effect of two cytokines on cellular activity is greater than the additive

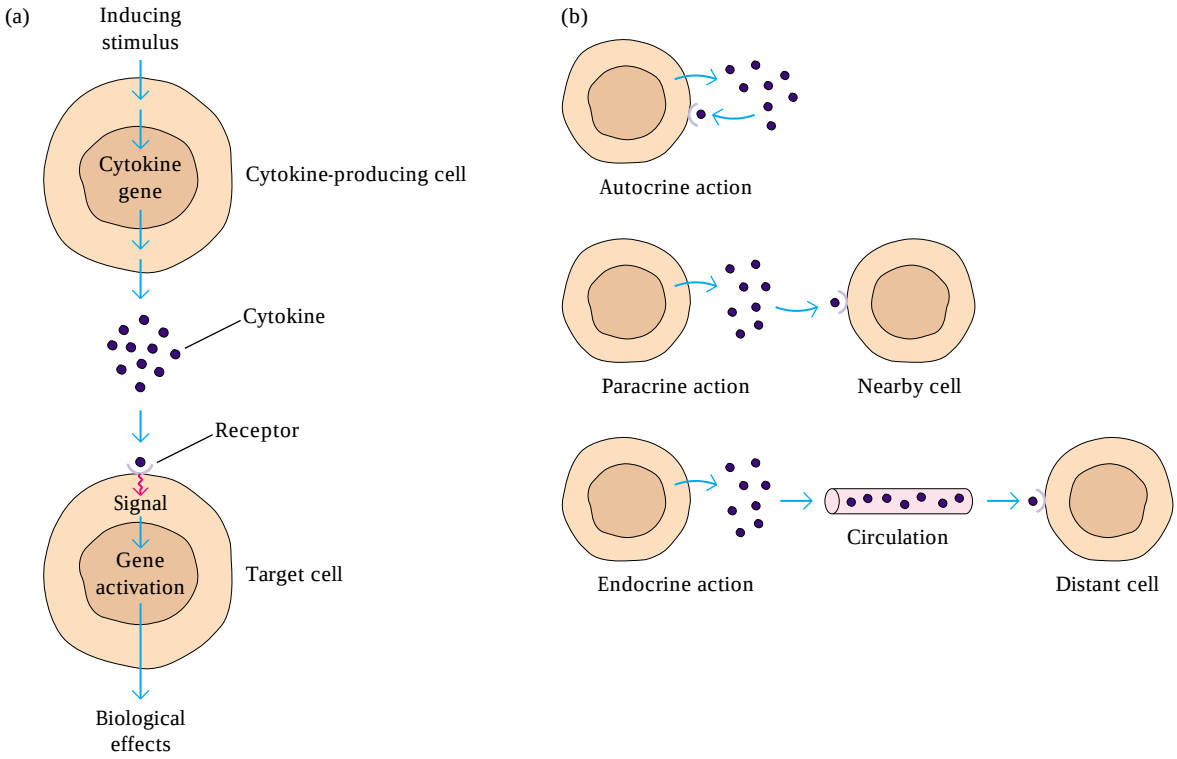


FIGURE 12-1 (a) Overview of the induction and function of cytokines. (b) Most cytokines exhibit autocrine and/or paracrine action; fewer exhibit endocrine action.

effects of the individual cytokines. In some cases, cytokines exhibit antagonism; that is, the effects of one cytokine inhibit or offset the effects of another cytokine. Cascade induction occurs when the action of one cytokine on a target cell induces that cell to produce one or more other cytokines, which in turn may induce other target cells to produce other cytokines.

The term *cytokine* encompasses those cytokines secreted by lymphocytes, substances formerly known as **lymphokines,** and those secreted by monocytes and macrophages, substances formerly known as **monokines.** Although these other two terms continue to be used, they are misleading because secretion of many lymphokines and monokines is not limited to lymphocytes and monocytes as these terms imply, but extends to a broad spectrum of cells and types. For this reason, the more inclusive term *cytokine* is preferred.

Many cytokines are referred to as **interleukins,** a name indicating that they are secreted by some leukocytes and act upon other leukocytes. Interleukins 1–25 have been identified. There is reason to suppose that still other cytokines will be discovered and that the interleukin group will expand further. Some cytokines are known by common names, including the interferons and tumor necrosis factors. Recently gaining prominence is yet another another subgroup of cytokines, the **chemokines,** a group of low-molecular-weight cytokines that affect chemotaxis and other aspects of leukocyte behavior. These molecules play an important role in the inflammatory response and are described in Chapter 15.

Because cytokines share many properties with hormones and growth factors, the distinction between these three classes of mediators is often blurred. All three are secreted soluble factors that elicit their biological effects at picomolar concentrations by binding to receptors on target cells. Growth factors tend to be produced constitutively, whereas cytokines and hormones are secreted in response to discrete stimuli, and secretion is short-lived, generally ranging from a few hours to a few days. Unlike hormones, which generally act long range in an endocrine fashion, most cytokines act over a short distance in an autocrine or paracrine fashion. In addition, most hormones are produced by specialized glands and tend to have a unique action on one or a few types of target cell. In contrast, cytokines are often produced by, and bind to, a variety of cells.

The activity of cytokines was first recognized in the mid-1960s, when supernatants derived from in vitro cultures of lymphocytes were found to contain factors that could regulate proliferation, differentiation, and maturation of allogeneic

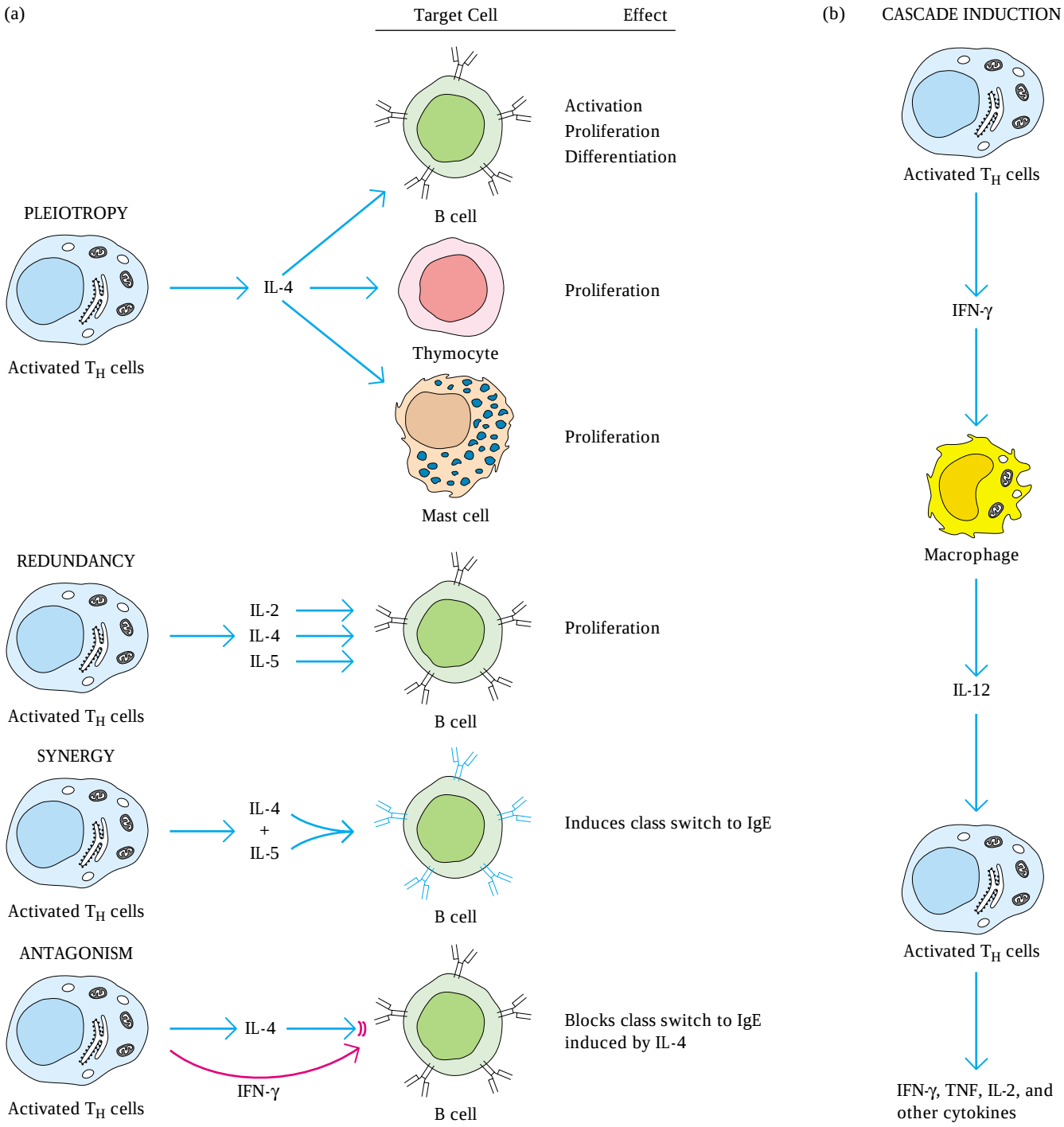


FIGURE 12-2 Cytokine attributes of (a) pleiotropy, redundancy, synergy (synergism), antagonism, and (b) cascade induction.

immune-system cells. Soon after, it was discovered that production of these factors by cultured lymphocytes was induced by activation with antigen or with nonspecific mitogens. Biochemical isolation and purification of cytokines was hampered because of their low concentration in culture supernatants and the absence of well-defined assay systems for individual cytokines. A great advance was made with the development of gene-cloning techniques during the 1970s and 1980s, which made it possible to produce pure cytokines by expressing the protein from cloned genes. The discovery of

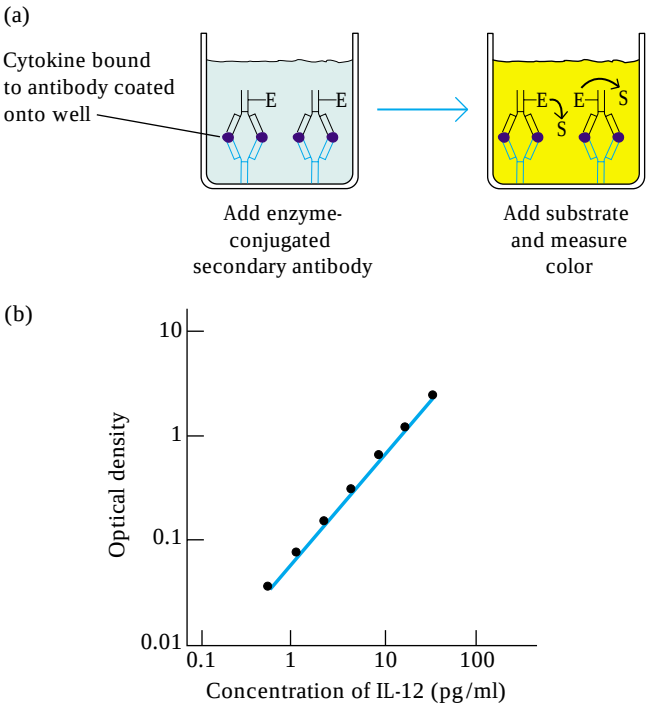


FIGURE 12-3 ELISA assay of a cytokine. (a) The sample containing the cytokine of interest is captured by specific antibody (blue) coated onto wells of a microtiter plate. A second specific antibody (blue), conjugated to an enzyme (E) such as horseradish peroxidase, forms a sandwich with the captured cytokine, immobilizing the enzyme in the microtiter well. A chromogenic substrate (S) is added, and the enzyme generates a color whose intensity is proportional to the amount of cytokine bound to the capture antibody. The optical density of this color produced by the unknown is compared with values on an appropriately determined standard curve. (b) The standard curve shown here is for human interleukin 12 (IL-12). It is clear that this assay is sufficiently sensitive to detect as little as 1 picogram of IL-12. *[Part (b) courtesy of R&D Systems.]*

cell lines whose growth depended on the presence of a particular cytokine provided researchers with the first simple assay systems. The derivation of monoclonal antibodies specific for each of the more important cytokines has made it possible to develop rapid quantitative immunoassays for each of them (Figure 12-3).

Cytokines Belong to Four Structural Families

Once the genes encoding various cytokines had been cloned, sufficient quantities of purified preparations became available for detailed studies on their structure and function. Cytokines generally have a molecular mass of less than 30 kDa. Structural studies have shown that the cytokines characterized so far belong to one of four groups: the hematopoietin family, the interferon family, the chemokine family, or the tumor necrosis factor family.

The structures of two members of the hematopoietin family, IL-2 and IL-4, are depicted in Figure 12-4. Although the amino acid sequences of these family members differ considerably, all of them have a high degree of α-helical structure and little or no β-sheet structure. The molecules

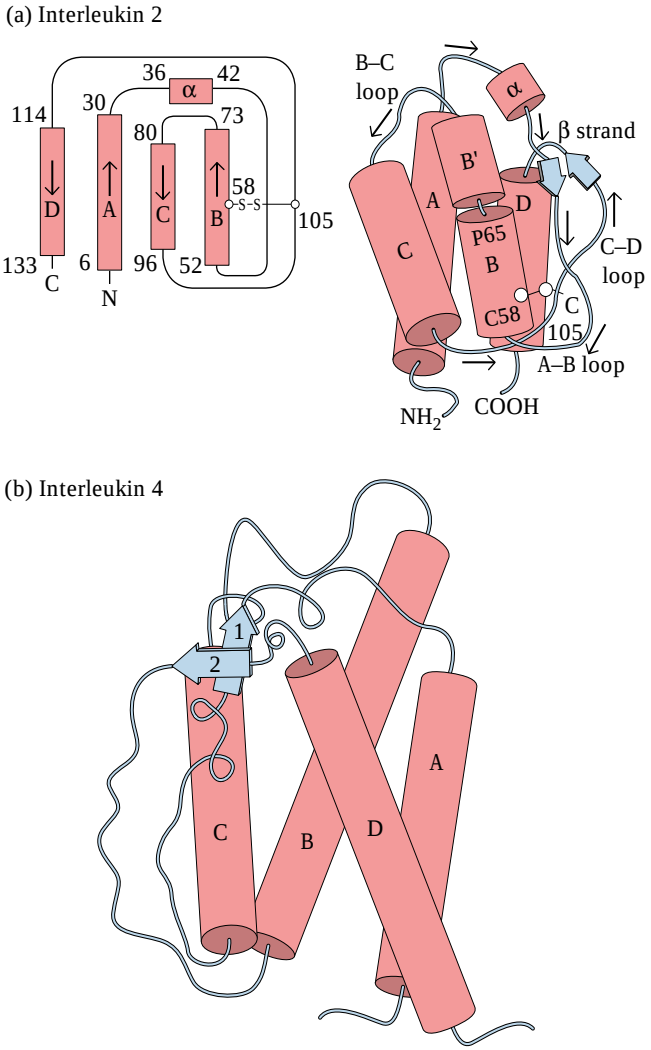


FIGURE 12-4 Several representations of structures in the hematopoietin family. (a) *Left:* Topographical representation of the primary structure of IL-2 showing α-helical regions (α and A–D) and connecting chains of the molecule. *Right:* Proposed three-dimensional model of IL-2. (b) Ribbon model of IL-4 deduced from x-ray crystallographic analysis of the molecule. In (a) and (b) the α helices are shown in red and the β sheets in blue. The structures of other cytokines belonging to the hematopoietin family are thought to be generally similar. *[Part (b) from J. L. Boulay and W. E. Paul, 1993,* Curr. Biol. ***3:****573.]*

VISUALIZING CONCEPTS

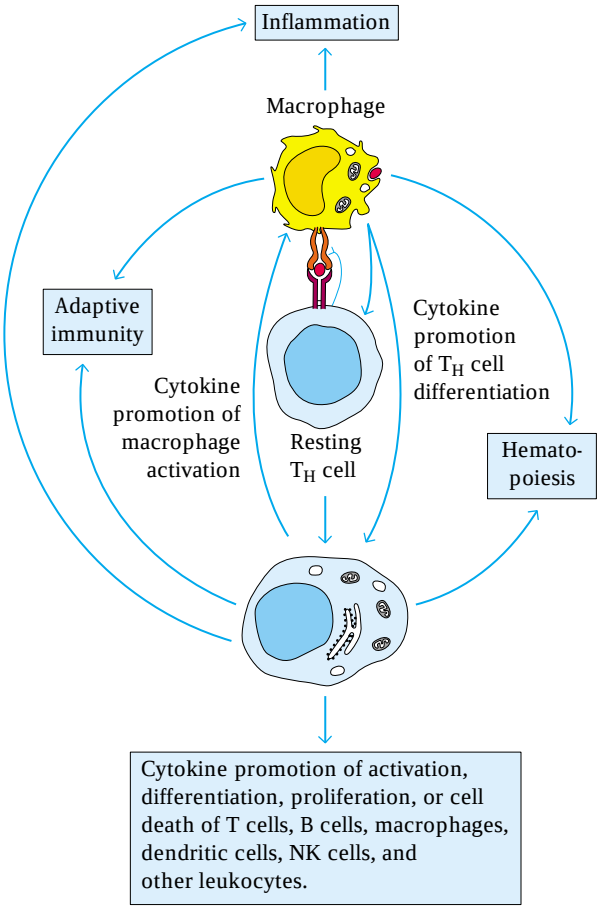


FIGURE 12-5 Interaction of antigen with macrophages and the subsequent activation of resting T_H cells leads to release of numerous cytokines (blue arrows), generating a complex network of interacting cells in the immune response.

share a similar polypeptide fold, with four α-helical regions (A–D) in which the first and second helices and the third and fourth helices run roughly parallel to one another and are connected by loops.

Cytokines Have Numerous Biological Functions

Although a variety of cells can secrete cytokines, the two principal producers are the T_H cell and the macrophage. Cytokines released from these two cell types activate an entire network of interacting cells (Figure 12-5). Among the numerous physiologic responses that require cytokine involvement are development of cellular and humoral immune responses, induction of the inflammatory response, regulation of hematopoiesis, control of cellular proliferation and differentiation, and the healing of wounds. Although the immune response to a specific antigen may include the production of cytokines, it is important to remember that cytokines act in an antigen-nonspecific manner. That is, they affect whatever cells they encounter that bear appropriate receptors and are in a physiological state that allows them to respond.

Cytokines are involved in a staggeringly broad array of biological activities including innate immunity, adaptive immunity, inflammation, and hematopoiesis. Altogether, the total number of proteins with cytokine activity easily exceeds 100 and research continues to uncover new ones. Table 12-1 summarizes the activities of some cytokines and places them into functional groups. An expanded list of cytokines can be found in the Appendix. It should be kept in mind that most of the listed functions have been identified from analysis of

TABLE 12-1 Functional groups of selected cytokines[1]

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); l iver (induction of acute phase proteins)
Tumor Necrosis Factor-α (TNF-α)	Macrophages	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	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	Induces an antiviral state in most nucleated cells; increases MHC class I expression; activates NK cells
Interferon β (IFN-β)	Fibroblasts	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
Interleukin 25 (IL-25)	Unknown	Induces secretion of T_H2 cytokine profile
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 IgE; 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

[1]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.
**Also note that activated cells generally secrete greater amounts of cytokine than unactivated cells.

the effects of recombinant cytokines, often at nonphysiologic concentrations, added individually to in vitro systems. In vivo, however, 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 very different consequences. In addition, cytokines often induce the synthesis of other cytokines, resulting in cascades of activity.

The nonspecificity of cytokines seemingly conflicts with the established specificity of the immune system. What keeps the nonspecific cytokines from activating cells in a nonspecific fashion during the immune response? One way in which specificity is maintained is by careful regulation of the expression of cytokine receptors on cells. Often cytokine receptors are expressed on a cell only after that cell has interacted with antigen. In this way cytokine activation is limited to antigen-activated lymphocytes. Another means of maintaining specificity may be a requirement for direct interaction between the cytokine-producing cell and the target cell to trigger cytokine secretion, thus ensuring that effective concentrations of the cytokine are released only in the vicinity of the intended target. In the case of the T_H cell, a major producer of cytokines, close cellular interaction occurs when the T-cell receptor recognizes an antigen-MHC complex on an appropriate antigen-presenting cell, such as a macrophage, dendritic cell, or B lymphocyte. Cytokines secreted at the junction of these interacting cells reach high enough local concentrations to affect the target APC but not more distant cells. 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 they act for only a limited period of time and thus over a short distance.

Cytokine Receptors

As noted already, to exert their biological effects, cytokines must first bind to specific receptors expressed on the membrane of responsive target cells. Because these receptors are expressed by many types of cells, the cytokines can affect a diverse array of cells. Biochemical characterization of cytokine receptors initially progressed at a very slow pace because their levels on the membrane of responsive cells is quite low. As with the cytokines themselves, cloning of the genes encoding cytokine receptors has led to rapid advances in the identification and characterization of these receptors.

Cytokine Receptors Fall Within Five Families

Receptors for the various cytokines are quite diverse structurally, but almost all belong to one of five families of receptor proteins (Figure 12-6):

- Immunoglobulin superfamily receptors
- Class I cytokine receptor family (also known as the hematopoietin receptor family)
- Class II cytokine receptor family (also known as the interferon receptor family)
- TNF receptor family
- Chemokine receptor family

Many of the cytokine-binding receptors that function in the immune and hematopoietic systems belong to the class I cytokine receptor family. The members of this receptor family have conserved amino acid sequence motifs in the extracellular domain consisting of four positionally conserved cysteine residues (CCCC) and a conserved sequence of tryptophan-serine-(any amino acid)-tryptophan-serine (WSXWS, where X is the nonconserved amino acid). The receptors for all the cytokines classified as hematopoietins belong to the class I cytokine receptor family, which also is called the hematopoietin receptor family. The class II cytokine receptors possess the conserved CCCC motifs, but lack the WSXWS motif present in class I cytokine receptors. Initially only the three interferons, α, β, and γ, were thought to be ligands for these receptors. However, recent work has shown that the IL-10 receptor is also a member of this group.

Another feature common to most of the hematopoietin (class I cytokine) and the class II cytokine receptor families is

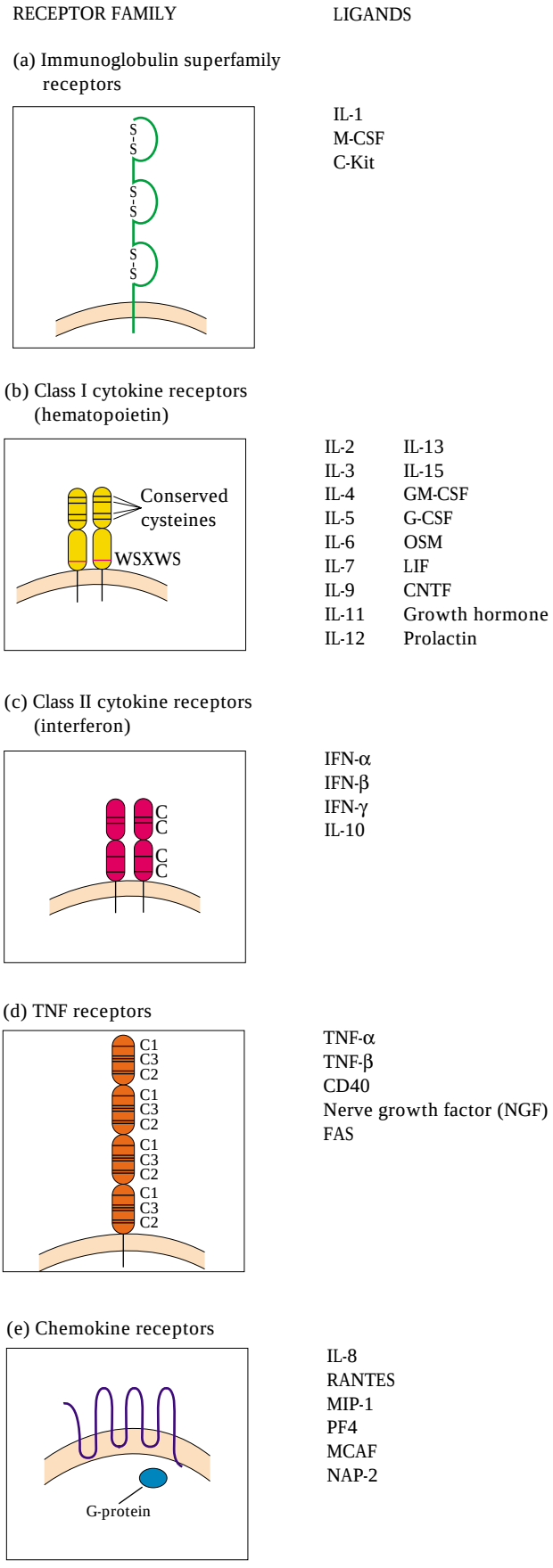


FIGURE 12-6 Schematic diagrams showing the structural features that define the five types of receptor proteins to which most cytokines bind. The receptors for most of the interleukins belong to the class I cytokine receptor family. C refers to conserved cysteine.

multiple subunits, often including one subunit that binds specific cytokine molecules and another that mediates signal transduction. Note, however, that these two functions are not always confined to one subunit or the other. Engagement of all of the class I and class II cytokine receptors studied to date has been shown to induce tyrosine phosphorylation of the receptor through the activity of protein tyrosine kinases closely associated with the cytosolic domain of the receptors.

Subfamilies of Class I Cytokine Receptors Have Signaling Subunits in Common

Several subfamilies of class I cytokine receptors have been identified, with all the receptors in a subfamily having an identical signal-transducing subunit. Figure 12-7 schematically illustrates the members of three receptor subfamilies, named after GM-CSF, IL-2, and IL-6.

The sharing of signal-transducing subunits among receptors explains the redundancy and antagonism exhibited by some cytokines. Consider the GM-CSF receptor subfamily, which includes the receptors for IL-3, IL-5, and GM-CSF (see Figure 12-7a). Each of these cytokines binds to a unique low-affinity, cytokine-specific receptor consisting of an α subunit only. All three low-affinity subunits can associate noncovalently with a common signal-transducing β subunit. The resulting dimeric receptor not only exhibits increased affinity for the cytokine but also can transduce a signal across the membrane after binding the cytokine (Figure 12-8a). Interestingly, IL-3, IL-5, and GM-CSF exhibit considerable redundancy. IL-3 and GM-CSF both act upon hematopoietic stem cells and progenitor cells, activate monocytes, and induce megakaryocyte differentiation. 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 among their biological effects (Figure 12-8b). In fact, all three cytokines induce the same patterns of protein phosphorylation. Furthermore, IL-3 and GM-CSF exhibit antagonism; IL-3 binding has been shown to be inhibited by GM-CSF, and conversely, binding

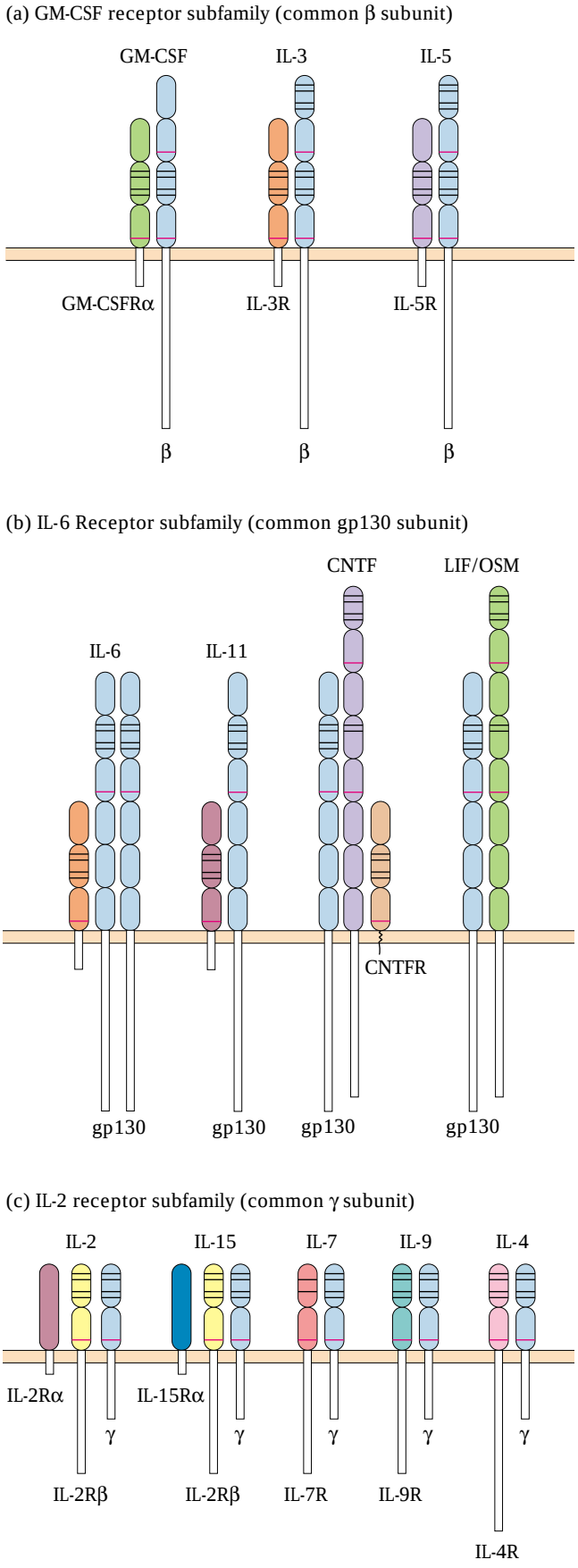


FIGURE 12-7 Schematic diagrams of the three subfamilies of class I cytokine receptors. All members of a subfamily have a common signal-transducing subunit (blue), but a unique cytokine-specific subunit. In addition to the conserved cysteines (double black lines) and WSXWS motifs (red lines) that characterize class I cytokine receptors, immunoglobulin-like domains are present in some of these receptors. CNTF = ciliary neurotrophic factor; LIF/OSM = leukemia-inhibitory factor/oncostatin. *[Adapted from K. Sugamura et al., 1996,* Annu. Rev. Immunol. ***14****:179.]*

of GM-CSF has been shown to be inhibited by IL-3. Since the signal-transducing β subunit is shared between the receptors for these two cytokines, their antagonism is due to competition for a limited number of β subunits by the cytokine-specific α subunits of the receptors (Figure 12-8c).

A similar situation is found among the IL-6 receptor subfamily, which includes the receptors for IL-6, IL-11, leukemia-inhibitory factor (LIF), oncostatin M (OSM), and ciliary neurotrophic factor (CNTF) (see Figure 12-7b). In this case, a common signal-transducing subunit called gp130 associates with one or two different cytokine-specific subunits. LIF and OSM, which must share certain structural features, both bind to the same α subunit. As expected, the cytokines that bind to receptors in this subfamily display overlapping biological activities: IL-6, OSM, and LIF induce synthesis of acute-phase proteins by liver hepatocytes and differentiation of myeloid leukemia cells into macrophages; IL-6, LIF, and CNTF affect neuronal development, and IL-6, IL-11, and OSM stimulate megakaryocyte maturation and platelet production. The presence of gp130 in all receptors of the IL-6 subfamily explains their common signaling pathways as well as the binding competition for limited gp130 molecules that is observed among these cytokines.

A third signal-transducing subunit defines the IL-2 receptor subfamily, which includes receptors for IL-2, IL-4, IL-7, IL-9, and IL-15 (see Figure 12-7c). 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 signal transduction. The IL-2 receptor γ chain functions as the signal-transducing subunit in the other receptors in this subfamily, which are all dimers. Recently, it has been shown that congenital **X-linked severe combined immunodeficiency (XSCID)** results from a defect in the γ-chain gene, which maps to the X chromosome. The immunodeficiencies observed in this disorder are due to the loss of all the cytokine functions mediated by the IL-2 subfamily receptors.

The IL-2R Is One of the Most Thoroughly Studied Cytokine Receptors

Because of the central role of IL-2 and its receptor in the clonal proliferation of T cells, the IL-2 receptor has received intensive study. As noted in the previous section, the complete trimeric receptor comprises three distinct subunits—the α, β, and γ chains. The β and γ chains belong to the class I cytokine receptor family, containing the characteristic CCCC and WSXWS motifs, whereas the α chain has a quite different structure and is not a member of this receptor family (see Figure 12-7c).

The IL-2 receptor occurs in three forms that exhibit different affinities for IL-2: the low-affinity monomeric IL-2Rα, the intermediate-affinity dimeric IL-2Rβγ, and the high-affinity trimeric IL-2Rαβγ (Figure 12-9). Because the α chain is expressed only by activated T cells, it is often referred to as the TAC (T-cell activation) antigen. A monoclonal anti-

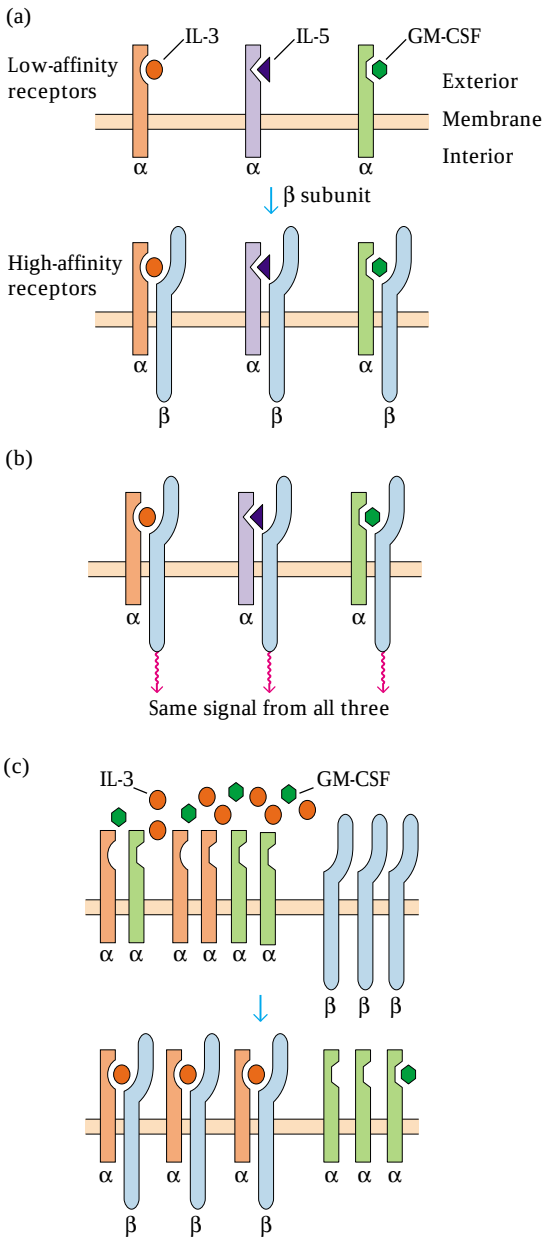
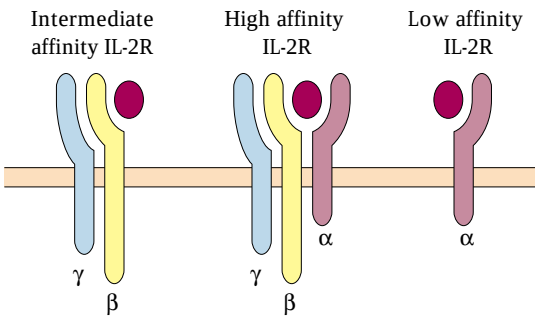


FIGURE 12-8 Interactions between cytokine-specific subunits and a common signal-transducing subunit of cytokine receptors. (a) Schematic diagram of the low-affinity and high-affinity receptors for IL-3, IL-5, and GM-CSF. The cytokine-specific subunits exhibit low-affinity binding and cannot transduce 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) Association of cytokine-specific subunits with a common signaling unit, the β subunit, allows the generation of cytokine-specific signals despite the generation of the same signal by the different cytokine receptors shown. (c) 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 out-compete α chains of the GM-CSF receptor for β subunits. *[Part (a) adapted from T. Kishimoto et al., 1992,* Science ***258:****593.]*



Subunit composition:	IL-2Rβ IL-2Rγ	IL-2Rα IL-2Rβ IL-2Rγ	IL-2Rα
Affinity constant (K_a):	$10^7 M$	$10^{11} M$	$10^8 M$
Dissociation constant (K_d):	$10^{-9} M$	$10^{-11} M$	$10^{-8} M$
Cells expressed by:	NK cells Resting T cells (low numbers)	Activated $CD4^+$ and $CD8^+$ T cells Activated B cells (low numbers)	

FIGURE 12-9 Comparison of the three forms of the IL-2 receptor. Signal transduction is mediated by the β and γ chains, but all three chains are required for high-affinity binding of IL-2.

body, designated anti-TAC, which binds to the 55-kDa α chain, is often used to identify IL-2Rα on cells. Signal transduction by the IL-2 receptor requires both the β and γ chains, but only the trimeric receptor containing the α chain as well binds IL-2 with high affinity. Although the γ chain appears to be constitutively expressed on most lymphoid cells, expression of the α and β chains is more restricted and is markedly enhanced after antigen has activated resting lymphocytes. This phenomenon ensures that only antigen-activated $CD4^+$ and $CD8^+$ T cells will express the high-affinity IL-2 receptor and proliferate in response to physiologic levels of IL-2. Activated T cells express approximately 5×10^3 high-affinity receptors and ten times as many low-affinity receptors. NK cells express the β and γ subunits constitutively, accounting for their ability to bind IL-2 with an intermediate affinity and to be activated by IL-2.

Engaged Cytokine Receptors Activate Signaling Pathways

While some important cytokine receptors lie outside the class I and class II families, the majority are included within these two families. As mentioned previously, class I and class II cytokine receptors lack signaling motifs (e.g., intrinsic tyrosine kinase domains). Yet, early observations demonstrated that one of the first events after the interaction of a cytokine with one of these receptors is a series of protein tyrosine phosphorylations. While these results were initially puzzling, they were explained when a unifying model emerged from studies of the molecular events triggered by binding of interferon gamma (IFN-γ) to its receptor, a member of the class II family.

IFN-γ was originally discovered because of its ability to induce cells to block or inhibit the replication of a wide variety of viruses. Antiviral activity is a property it shares with IFN-α and IFN-β. However, unlike these other interferons, IFN-γ plays a central role in many immunoregulatory processes, including the regulation of mononuclear phagocytes, B-cell switching to certain IgG classes, and the support or inhibition of the development of T_H-cell subsets. The discovery of the major signaling pathway invoked by interaction of IFN-γ with its receptor led to the realization that signal transduction through most, if not all, class I and class II cytokine receptors involves the following steps, which are the basis of a unifying signaling model (Figure 12-10).

- *The cytokine receptor is composed of separate subunits,* an α chain required for cytokine binding and for signal transduction and a β chain necessary for signaling but with only a minor role in binding.
- *Different inactive protein tyrosine kinases are associated with different subunits of the receptor.* The α chain of the receptor is associated with a novel family of protein tyrosine kinases, the Janus kinase (JAK)* family. The association of the JAK and the receptor subunit occurs spontaneously and does not require the binding of cytokine. However, in the absence of cytokine, JAKs lack protein tyrosine kinase activity.
- *Cytokine binding induces the association of the two separate cytokine receptor subunits and activation of the receptor-associated JAKs.* The ability of IFN-γ, which binds to a class II cytokine receptor, to bring about the association of the ligand-binding chains of its receptor has been directly demonstrated by x-ray crystallographic studies, as shown in Figure 12-11.
- *Activated JAKs create docking sites for the STAT transcription factors by phosphorylation of specific tyrosine residues on cytokine receptor subunits.* Once receptor-associated JAKs are activated, they phosphorylate specific tyrosines in the receptor subunits of the

*The Roman god Janus had two faces. Kinases of the Janus family have two sites, a binding site at which they link with the cytokine receptor subunit and a catalytic site that, when activated, has protein tyrosine kinase activity. Some biochemists, wearied by the multitude of different protein kinases that have been discovered, claim JAK means Just *A*nother *K*inase.

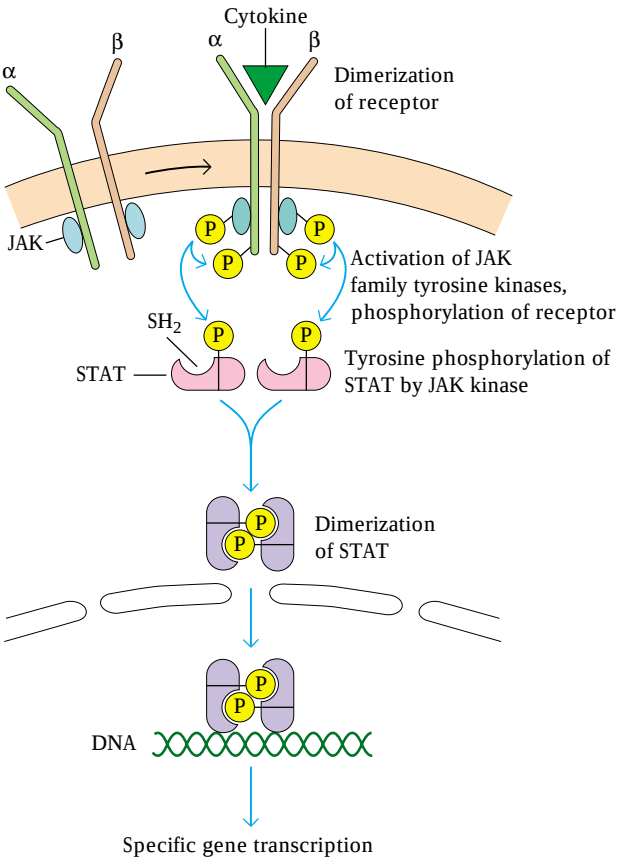


FIGURE 12-10 General model of signal transduction mediated by most class I and class II cytokine receptors. Binding of a cytokine induces dimerization of the receptor subunits, which leads to the activation of receptor-subunit-associated JAK tyrosine kinases by reciprocal phosphorylation. Subsequently, the activated JAKs phosphorylate various tyrosine residues, resulting in the creation of docking sites for STATs on the receptor and the activation of the one or more STAT transcription factors. The phosphorylated STATs dimerize and translocate to the nucleus, where they activate transcription of specific genes.

complex. Members of a family of transcription factors known as **STATs (signal transducers and activators of transcription)** bind to these phosphorylated tyrosine residues. Specific STATs (see Table 12-2) play essential roles in the signaling pathways of a wide variety of cytokines. The binding of STATs to receptor subunits is mediated by the joining of the SH2 domain on the STAT with the docking site created by the JAK-mediated phosphorylation of a particular tyrosine on receptor subunits.

- *After undergoing JAK-mediated phosphorylation, STAT transcription factors translocate from receptor docking sites at the membrane to the nucleus, where they initiate the transcription of specific genes.* While docked to receptor subunits, STATs undergo JAK-catalyzed phosphorylation of a key tyrosine. This is followed by the dissociation of the STATs from the receptor subunits and their dimerization. The STAT dimers then translocate into the nucleus and induce the expression of genes containing appropriate regulatory sequences in their promoter regions.

In addition to IFN-γ, a number of other class I and class II ligands have been shown to cause dimerization of their receptors. An important element of cytokine specificity derives from the exquisite specificity of the match between cytokines and their receptors. Another aspect of cytokine specificity is that each particular cytokine (or group of redundant cytokines) induces transcription of a specific subset of genes in a given cell type; the resulting gene products then mediate the various effects typical of that cytokine. The specificity of cytokine effects is then traceable to three factors. First, particular cytokine receptors start particular JAK-STAT pathways. Second, the transcriptional activity of activated STATs

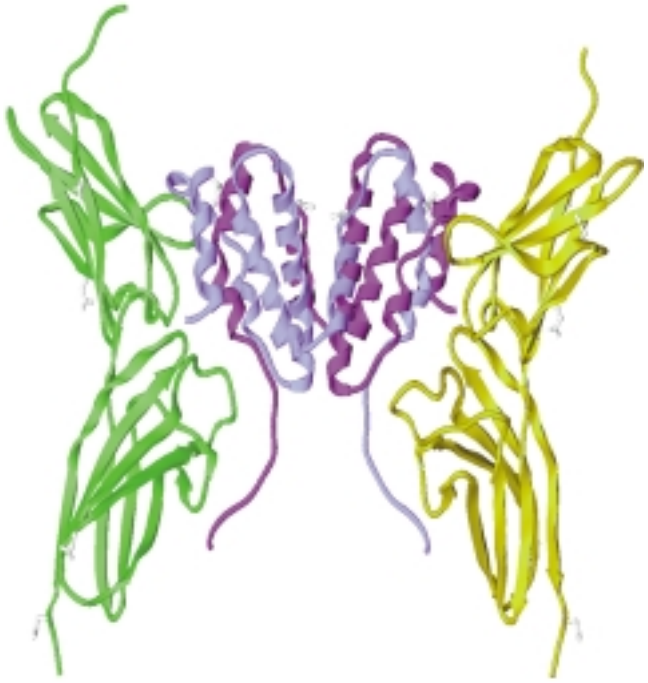


FIGURE 12-11 The complex between IFN-γ and the ligand-binding chains of its receptor. This model is based on the x-ray crystallographic analysis of a crystalline complex of interferon-γ (violet and blue) bound to ligand-binding α chains of the receptor (green and yellow). Note that IFN-γ is shown in 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,* Nature ***376:****230, courtesy M. Walter, University of Alabama.]*

TABLE 12-2 STAT and JAK interaction with selected cytokine receptors during signal transduction

Cytokine receptor	JAK	STAT
IFN-γ	JAK1 and JAK2	Stat1
IFN-α/β	JAK1 and Tyk-2	Stat2
IL-2	JAK1 and JAK3	Stat5
IL-3	JAK2	Stat5
IL-4	JAK1 and JAK3	Stat6
IL-6	JAK1 (and sometimes others)	Stat3
IL-10	JAK1 and Tyk-2*	Stat3
IL-12	JAK2 and Tyk-2*	Stat4

*Despite its name, Tyk-2 is also a Janus kinase.

SOURCE: Adapted from E. A. Bach, M. Aguet, and R. D. Schreiber, 1997, *Annu. Rev. Immun.* **15**:563.

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. Third, only those target genes whose expression is permitted by a particular cell type can be activated within that variety of cell. That is, in any given cell type only a subset of the potential target genes of a particular STAT may be permitted expression. For example, IL-4 induces one set of genes in T cells, another in B cells, and yet a third in eosinophils.

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, or they bind directly to a cytokine, inhibiting its activity. The best-characterized inhibitor is the IL-1 receptor antagonist (IL-1Ra), which binds to the IL-1 receptor but has no activity. Binding of IL-1Ra to the IL-1 receptor blocks binding of both IL-1α and IL-1β, thus accounting for its antagonistic properties. Production of IL-1Ra has been thought by some to play a role in regulating the intensity of the inflammatory response. It has been cloned and is currently being investigated as a potential treatment for chronic inflammatory diseases.

Cytokine inhibitors are found in the bloodstream and extracellular fluid. These soluble antagonists arise from enzymatic cleavage of the extracellular domain of cytokine receptors. Among the soluble cytokine receptors that have been detected are those for IL-2, -4, -6, and -7, IFN-γ and -α, TNF-β, and LIF. Of these, the soluble IL-2 receptor (sIL-2R), which is released in chronic T-cell activation, is the best characterized. A segment containing the amino-terminal 192 amino acids of the α subunit is released by proteolytic cleavage, forming a 45-kDa soluble IL-2 receptor. The shed receptor can bind IL-2 and prevent its interaction with the membrane-bound IL-2 receptor. The presence of sIL-2R has been used as a clinical marker of chronic T-cell activation and is observed in a number of diseases, including autoimmunity, transplant rejection, and AIDS.

Some viruses also produce cytokine-binding proteins or cytokine mimics. The evolution of such anti-cytokine strategies by microbial pathogens is good biological evidence of the importance of cytokines in organizing and promoting effective anti-microbial immune responses. The poxviruses, for example, have been shown to encode a soluble TNF-binding protein and a soluble IL-1–binding protein. Since both TNF and IL-1 exhibit a broad spectrum of activities in the inflammatory response, these soluble cytokine-binding proteins may prohibit or diminish the inflammatory effects of the cytokines, thereby conferring upon the virus a selective advantage. Epstein-Barr virus produces an IL-10–like molecule (viral IL-10 or vIL-10) that binds to the IL-10 receptor and, like cellular IL-10, suppresses T_H1-type cell-mediated responses (see the next section), which are effective against many intracellular parasites such as viruses. Molecules produced by viruses that mimic cytokines allow the virus to manipulate the immune response in ways that aid the survival of the pathogen. This is an interesting and powerful modification some viruses have undergone in their continuing struggle to overcome the formidable barrier of host immunity. Table 12-3 lists a number of viral products that mimic cytokines or their receptors.

TABLE 12-3 Viral mimics of cytokine and cytokine receptors

Virus	Product
Leporipoxvirus (a myxoma virus)	Soluble IFN-γ receptor
Several poxviruses	Soluble IFN-γ receptor
Vaccinia, smallpox virus	Soluble IL-1 β receptor
Epstein-Barr	IL-10 homolog
Human herpesvirus-8	IL-6 homolog; also homologs of the chemokines MIP-I and MIP-II
Cytomegalovirus	Three different chemokine receptor homologs, one of which binds three different soluble chemokines (RANTES, MCP-1, and MIP-1α)

Cytokine Secretion by T_H1 and T_H2 Subsets

The immune response to a particular pathogen must induce an appropriate set of effector functions that can eliminate the disease agent or its toxic products from the host. For example, the neutralization of a soluble bacterial toxin requires antibodies, whereas the response to an intracellular virus or to a bacterial cell requires cell-mediated cytotoxicity or delayed-type hypersensitivity. A large body of evidence implicates differences in cytokine-secretion patterns among T_H-cell subsets as determinants of the type of immune response made to a particular antigenic challenge.

$CD4^+$ T_H cells exert most of their helper functions through secreted cytokines, which either act on the cells that produce them in an autocrine fashion or modulate the responses of other cells through paracrine pathways. Although $CD8^+$ CTLs also secrete cytokines, their array of cytokines generally is more restricted than that of $CD4^+$ T_H cells. As briefly discussed in Chapter 10, two $CD4^+$ T_H-cell subpopulations designated T_H1 and T_H2, can be distinguished in vitro by the cytokines they secrete. Both subsets secrete IL-3 and GM-CSF but differ in the other cytokines they produce (Table 12-4). T_H1 and T_H2 cells are characterized by the following functional differences:

- The T_H1 subset is responsible for many cell-mediated functions (e.g., delayed-type hypersensitivity and activation of T_C cells) and for the production of opsonization-promoting IgG antibodies (i.e. antibodies that bind to the high-affinity Fc receptors of phagocytes and interact with the complement system). This subset is also associated with the promotion of excessive inflammation and tissue injury.
- The T_H2 subset stimulates eosinophil activation and differentiation, provides help to B cells, and promotes the production of relatively large amounts of IgM, IgE, and noncomplement-activating IgG isotypes. The T_H2 subset also supports allergic reactions.

The differences in the cytokines secreted by T_H1 and T_H2 cells determine the different biological functions of these two subsets. A defining cytokine of the T_H1 subset, IFN-γ, activates macrophages, stimulating these cells to increase microbicidal activity, up-regulate the level of class II MHC, and secrete cytokines such as IL-12, which induces T_H cells to differentiate into the T_H1 subset. IFN-γ secretion by T_H1 cells also induces antibody-class switching to IgG classes (such as IgG2a in the mouse) that support phagocytosis and fixation of complement. TNF-β and IFN-γ are cytokines that mediate inflammation, and it is their secretion that accounts for the association of T_H1 cells with inflammatory phenomena such as delayed hypersensitivity (Chapter 16). T_H1 cells produce IL-2 and IFN-γ cytokines that promote the differentiation of fully cytotoxic T_C cells from $CD8^+$ precursors. This pattern of cytokine production makes the T_H1 subset particularly suited to respond to viral infections and intracellular pathogens. Finally, IFN-γ inhibits the expansion of the T_H2 population.

The secretion of IL-4 and IL-5 by cells of the T_H2 subset induces production of IgE and supports eosinophil-mediated attack on helminth (roundworm) infections. IL-4 promotes a pattern of class switching that produces IgG that does not activate the complement pathway (IgG1 in mice, for example). IL-4 also increases the extent to which B cells switch from IgM to IgE. This effect on IgE production meshes with eosinophil differentiation and activation by IL-5, because eosinophils are richly endowed with Fcε receptors, which bind IgE. Typically, roundworm infections induce T_H2 responses and evoke anti-roundworm IgE antibody. The antibody bound to the worm binds to the Fc receptors of eosinophils, thus forming an antigen-specific bridge between the worm and the eosinophils. The attack of the eosinophil on the worm is triggered by crosslinking of the Fcε-bound IgE. Despite these beneficial actions of IgE, it is also the Ig

TABLE 12-4 Cytokine secretion and principal functions of mouse T_H1 and T_H2 subsets

Cytokine/function	T_H1	T_H2
CYTOKINE SECRETION		
IL-2	+	–
IFN-γ	++	–
TNF-β	++	–
GM-CSF	++	+
IL-3	++	++
IL-4	–	++
IL-5	–	++
IL-10	–	++
IL-13	–	++
FUNCTIONS		
Help for total antibody production	+	++
Help for IgE production	–	++
Help for IgG2a production	++	+
Eosinophil and mast-cell production	–	++
Macrophage activation	++	–
Delayed-type hypersensitivity	++	–
T_C-cell activation	++	–

SOURCE: Adapted from F. Powrie and R. L. Coffman, 1993, *Immunol. Today* **14**:270.

class responsible for allergy. Finally, IL-4 and IL-10 suppress the expansion of T_H1 cell populations.

Because the T_H1 and T_H2 subsets were originally identified in long-term in vitro cultures of cloned T-cell lines, some researchers doubted that they represented true in vivo subpopulations. They suggested instead that these subsets might represent different maturational stages of a single lineage. Also, the initial failure to locate either subset in humans led some to believe that T_H1, T_H2, and other subsets of T helper cells did not occur in this species. Further research corrected these views. In many in vivo systems, the full commitment of populations of T cells to either the T_H1 or T_H2 phenotype often signals the endpoint of a chronic infection or allergy. Hence it was difficult to find clear T_H1 or T_H2 subsets in studies employing healthy human subjects, who would not be at this stage of a response. Experiments with transgenic mice demonstrated conclusively that T_H1 and T_H2 cells arise independently. Furthermore, it was possible to demonstrate T_H1 or T_H2 populations in T cells isolated from humans during chronic infectious disease or chronic episodes of allergy. It is also important to emphasize that many helper T cells do not show either a T_H1 or a T_H2 profile; individual cells have shown striking heterogeneity in the T_H-cell population. One of the best described of these is the T_H0 subset, which secretes IL-2, IL-4, IL-5, IFN-γ, and IL-10, as well as IL-3 and GM-CSF.

Numerous reports of studies in both mice and humans now document that the in vivo outcome of the immune response can be critically influenced by the relative levels of T_H1-like or T_H2-like activity. Typically, the T_H1 profile of cytokines is higher in response to intracellular pathogens, and the T_H2 profile is higher in allergic diseases and helminthic infections.

The Development of T_H1 and T_H2 Subsets Is Determined by the Cytokine Environment

The cytokine environment in which antigen-primed T_H cells differentiate determines the subset that develops (Figure 12-12). In particular, IL-4 is essential for the development of a T_H2 response, and IFN-γ, IL-12, and IL-18 all are important in the physiology of the development of T_H1 cells. The source of IL-12, one of the key mediators of T_H1 differentiation, is typically macrophages or dendritic cells activated by an encounter with intracellular bacteria, with bacterial products such as LPS, or with a number of other intracellular parasites. T_H1 development is also critically dependent on IFN-γ, which induces a number of changes, including the up-regulation of IL-12 production by macrophages and dendritic cells, and the activation of the IL-12 receptor on activated T cells, which it accomplishes by up-regulating expression of the β chain of the IL-12 receptor. At the beginning of an immune response, IFN-γ is generated by stimulation of T cells and can also come from activated NK cells. Yet another cytokine, IL-18, promotes proliferation and IFN-γ production by both developing and fully differentiated T_H1 cells and by NK cells. So a regulatory network of cytokines positively controls the generation of T_H1 cells. The critical role played by each of these cytokines and their receptors has been demonstrated in a series of experiments in which either the cytokine or its receptor has been knocked out. Mice in which the genes for any of these critical components have been knocked out fail to generate populations of T_H1 cells.

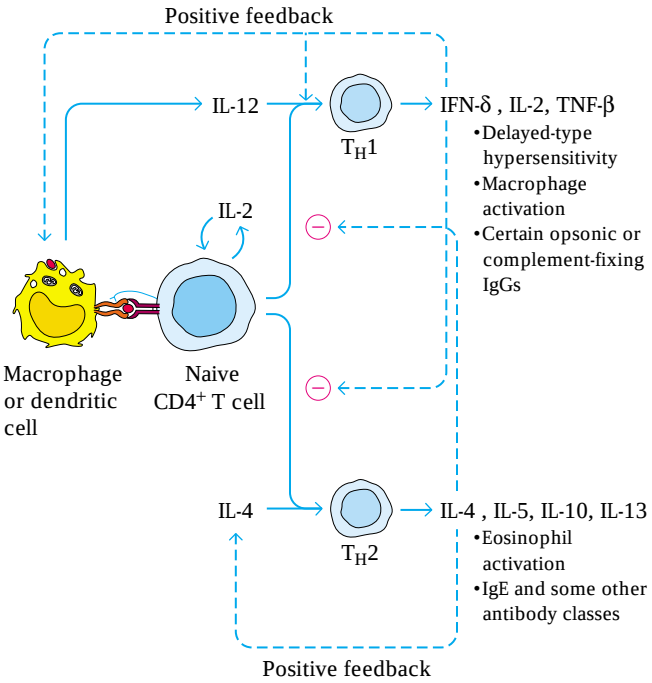


FIGURE 12-12 Cytokine-mediated generation and cross regulation of T_H subsets. Antigen-activated naive $CD4^+$ T cell produces IL-2 and proliferates. If it proliferates in an IL-12 dominated environment, it generates a population of T_H1 cells that secretes a characteristic profile of cytokines including interferon γ. A positive feedback loop is established when IFN-γ secreted by the expanding T_H1 population stimulates dendritic cells or macrophages to produce more IL-12. If the environment is dominated by IL-4, a T_H2 population emerges and secretes a profile of cytokines that promotes eosinophil activation and the synthesis of certain antibody classes. Key cytokines produced by each subset positively regulate the subset that produces it and negatively regulate the other subset. *[Adapted from J. Rengarajan, S. Szabo, and L. Glimcher, 2000,* Immunology Today ***21****:479.]*

Just as T_H1 cells require IL-12 and IFN-γ, the generation of T_H2 cells depends critically on IL-4. Exposing naive helper cells to IL-4 at the beginning of an immune response causes them to differentiate into T_H2 cells. In fact, this influence of IL-4 is predominant in directing T_H cells to the T_H2 route. Provided a threshold level of IL-4, T_H2 development is

greatly favored over T_H1 even if IL-12 is present. The critical role of signals from IL-4 in T_H2 development is shown by the observation that knocking out the gene that encodes IL-4 prevents the development of this T-cell subset. Additional evidence supporting the central role of IL-4 comes from an experiment that interrupted the IL-4 signal-transduction pathway. Like so many other cytokines, IL-4 uses a pathway that involves JAK and STAT proteins. The Stat6 transcription factor is the one activated in signaling by IL-4. Consequently, in mice in which the gene for Stat6 has been disrupted (Stat6 knockouts), IL-4 mediated processes are severely inhibited or absent. The observation that Stat6 knockout mice have very few T_H2 cells confirms the importance of IL-4 for the differentiation of this subset.

Cytokine Profiles Are Cross-Regulated

The critical cytokines produced by T_H1 and T_H2 subsets have two characteristic effects on subset development. First, they promote the growth of the subset that produces them; second, they inhibit the development and activity of the opposite subset, an effect known as *cross-regulation*, (see Figure 12-12). For instance, IFN-γ (secreted by the T_H1 subset) preferentially inhibits proliferation of the T_H2 subset, and IL-4 and IL-10 (secreted by the T_H2 subset) down-regulate secretion of IL-12, one of the critical cytokines for T_H1 differentiation, by both macrophages and dendritic cells. Similarly, these cytokines have opposing effects on target cells other than T_H subsets. IFN-γ secreted by the T_H1 subset promotes IgG2a production by B cells but inhibits IgG1 and IgE production. On the other hand, IL-4 secreted by the T_H2 subset promotes production of IgG1 and IgE and suppresses production of IgG2a. The phenomenon of cross-regulation explains the observation that there is often an inverse relationship between antibody production and cell-mediated immunity; that is, when antibody production is high, cell-mediated immunity is low, and vice versa. Furthermore, recent research has shown that IL-4 and IFN-γ make members of the T-cell subset that releases them less responsive to the cytokine that directs differentiation of the other T-cell subset. Thus, IL-4 enhances T_H2 cell development by making T_H cells less susceptible to the cytokine signals that cause these cells to enter a differentiation pathway that would lead to T_H1 development. On the other hand, as explained below, IFN-γ up-regulates the expression of a key regulatory molecule that favors the differentiation and activity of T_H1 cells.

Recent work has given insight into the molecular basis for the cytokine-mediated cross-regulation by which one subset promotes its own expansion and development while inhibiting the development of the opposite subset. Two transcription factors, T-Bet and GATA-3, are key elements in determining subset commitment and cross-regulation. The expression of T-Bet drives cells to differentiate into T_H1 cells and suppresses their differentiation along the T_H2 pathway. Expression of GATA-3 does the opposite, promoting the development of naive T cells into T_H2 cells while suppressing their differentiation into T_H1 cells. As shown in Figure 12-13, the expression of T-Bet versus GATA-3 is determined by the cytokines IFN-γ and IL-4. In the presence of IFN-γ, T cells up-regulate the expression of T-Bet and down-regulate GATA-3. This IFN-γ receptor/Stat1–dependent process shifts the cytokine profile to the production of IFN-γ, the signature cytokine of T_H1 cells, and other cytokines typical of the T_H1 set. On the other hand, in a process that involves the IL-4 receptor and Stat6, IL-4 induces the cell to produce IL-4 and other T_H2 cytokines. Further study has revealed that the up-regulation of T-Bet represses the expression of GATA-3. Similarly, expression of GATA-3 down-regulates T-Bet. Consequently, cytokine signals that induce one of these transcription factors set in motion a chain of events that repress the other. At the intracellular level, the differentiation of a T cell along the T_H1 pathway, prevents its development of T_H2 characteristics and vice versa.

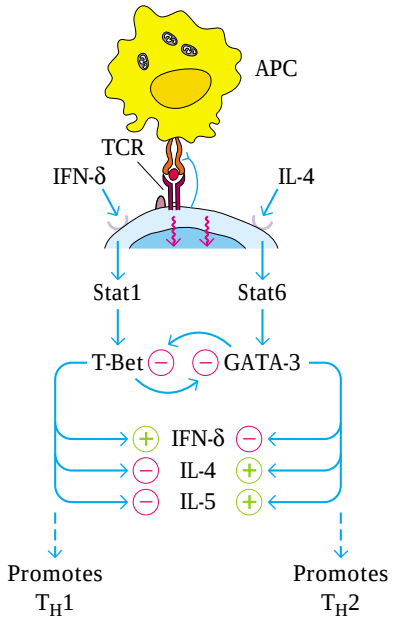


FIGURE 12-13 Cross-regulation at the intracellular level. Signals through the TCR and cytokine receptors determine whether the cell will produce the T_H1-promoting transcription factor, T-Bet, or the T_H2-promoting transcription factor, GATA-3. Experimental evidence supports a model in which exposure of cells bearing receptors for IFN-γ to IFN-γ induces the formation of T-Bet, which up-regulates the synthesis of IFN-γ and represses the expression of GATA-3. Exposure of IL-4 R–bearing cells to IL-4 induces the formation of GATA-3, which up-regulates the synthesis of IL-4 and IL-5 but represses the expression of T-Bet. *[Adapted from J. Rengarajan, S. Szabo, and L. Glimcher, 2000,* Immunology Today ***21****:479.]*

The cross-regulation of T_H1 cells by IL-10 secreted from T_H2 cells is not a direct inhibition of the T_H1 cells; instead, IL-10 acts on monocytes and macrophages, interfering with their ability to activate the T_H1 subset. This interference is thought to result from the demonstrated ability of IL-10 to down-regulate the expression of class II MHC molecules on these antigen-presenting cells. IL-10 has other potent immunosuppressant effects on the monocyte-macrophage lineage, such as suppressing the production of nitric oxide and other bactericidal metabolites involved in the destruction of pathogens, and also suppressing the production of various inflammatory mediators (e.g., IL-1, IL-6, IL-8, GM-CSF, G-CSF, and TNF-γ). These suppressive effects on the macrophage serve to further diminish the biologic consequences of T_H1 activation.

The T_H1/T_H2 Balance Determines Disease Outcomes

The progression of some diseases may depend on the balance between the T_H1 and T_H2 subsets. In humans, a well-studied example of this phenomenon is leprosy, which is caused by *Mycobacterium leprae,* an intracellular pathogen that can survive within the phagosomes of macrophages. Leprosy is not a single clinical entity; rather, the disease presents as a spectrum of clinical responses, with two major forms of disease, tuberculoid and lepromatous, at each end of the spectrum. In **tuberculoid leprosy,** a cell-mediated immune response forms granulomas, resulting in the destruction of most of the mycobacteria, so that only a few organisms remain in the tissues. Although skin and peripheral nerves are damaged, tuberculoid leprosy progresses slowly and patients usually survive. In **lepromatous leprosy,** the cell-mediated response is depressed and, instead, humoral antibodies are formed, sometimes resulting in hypergammaglobulinemia. The mycobacteria are widely disseminated in macrophages, often reaching numbers as high as 10^{10} per gram of tissue. Lepromatous leprosy progresses into disseminated infection of the bone and cartilage with extensive nerve damage.

The development of lepromatous or tuberculoid leprosy depends on the balance of T_H1 and T_H2 cells (Figure 12-14). In tuberculoid leprosy, the immune response is characterized by a T_H1-type response with delayed-type hypersensitivity and a cytokine profile consisting of high levels of IL-2, IFN-γ, and TNF-β. In lepromatous leprosy, there is a T_H2-type immune response, with high levels of IL-4, IL-5, and IL-10. This cytokine profile explains the diminished cell-mediated immunity and increased production of serum antibody in lepromatous leprosy.

There is also evidence for changes in T_H-subset activity in AIDS. Early in the disease, T_H1 activity is high, but as AIDS progresses, some workers have suggested, there may be a shift from a T_H1-like to a T_H2-like response. In addition, some pathogens may influence the activity of the T_H subsets. The Epstein-Barr virus, for instance, produces vIL-10, which has IL-10–like activity and, like cellular IL-10, tends to suppress T_H1 activity by cross-regulation. Some researchers have speculated that vIL-10 may reduce the cell-mediated response to the Epstein-Barr virus, thus conferring a survival advantage on the virus.

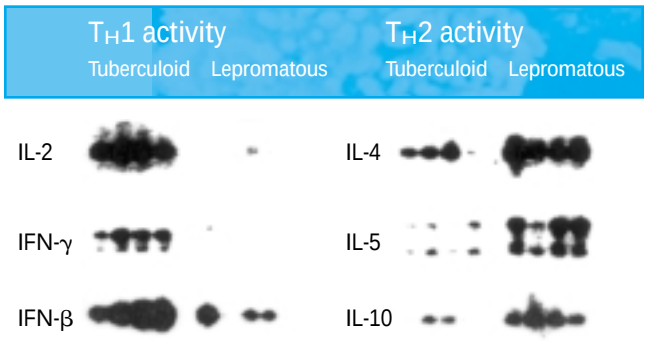


FIGURE 12-14 Correlation between type of leprosy and relative T_H1 or T_H2 activity. Messenger RNA isolated from lesions from tuberculoid and lepromatous leprosy patients was analyzed by Southern blotting using the cytokine probes indicated. Cytokines produced by T_H1 cells predominate in the tuberculoid patients, while cytokines produced by T_H2 cells predominate in the lepromatous patients. *[From P. A. Sieling and R. L. Modlin, 1994,* Immunobiology ***191:*** *378.]*

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. This section describes several diseases resulting from overexpression or underexpression of cytokines or cytokine receptors.

Bacterial Septic Shock Is Common and Potentially Lethal

The role of cytokine overproduction in pathogenesis can be illustrated by bacterial septic shock. This condition may develop a few hours after infection by certain gram-negative bacteria, including *E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterobacter aerogenes,* and *Neisseria meningitidis.* The symptoms of bacterial septic shock, which is often fatal, include a drop in blood pressure, fever, diarrhea, and widespread blood clotting in various organs. This condition afflicts about 500,000 Americans annually and causes more than 70,000 deaths. The annual cost for treating bacterial septic shock is estimated to be in excess of $5 billion.

Bacterial septic shock apparently develops because bacterial cell-wall **endotoxins** stimulate macrophages to overproduce IL-1 and TNF-α to levels that cause septic shock. In

one study, for example, higher levels of TNF-α were found in patients who died of meningitis than in those who recovered. Furthermore, a condition resembling bacterial septic shock can be produced by injecting mice with recombinant TNF-α in the absence of gram-negative bacterial infection. Several studies offer some hope that neutralization of TNF-α or IL-1 activity with monoclonal antibodies or antagonists may prevent this fatal shock from developing in these bacterial infections. In one study, monoclonal antibody to TNF-α protected animals from endotoxin-induced shock. Another study has shown that injection of a recombinant IL-1 receptor antagonist (IL-1Ra), which prevents binding of IL-1 to the IL-1 receptor, resulted in a three-fold reduction in mortality. It is hoped that these experimental results will lead to clinically useful products for the treatment of bacterial septic shock in humans.

Bacterial Toxic Shock Is Caused by Superantigens

A variety of microorganisms produce toxins that act as **superantigens.** As Chapter 10 described, superantigens bind simultaneously to a class II MHC molecule and to the V_β domain of the T-cell receptor, activating all T cells bearing a particular V_β domain (Figure 10-16). Because of their unique binding ability, superantigens can activate large numbers of T cells irrespective of their antigenic specificity.

Although less than 0.01% of T cells respond to a given conventional antigen, between 5% and 25% of T cells can respond to a given superantigen. The large proportion of T cells responsive to a particular superantigen results from the limited number of TCR V_β genes carried in the germ line. Mice, for example, have about 20 V_β genes. Assuming that each V_β gene is expressed with equal frequency, then each superantigen would be expected to interact with 1 in 20 T cells, or 5% of the total T-cell population.

A number of 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 toxic-shock syndrome toxin, for example, has been shown to induce extremely high levels of TNF and IL-1. As in bacterial septic shock, these elevated concentrations of cytokines can induce systemic reactions that include fever, widespread blood clotting, and shock.

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 cells (a benign heart tumor), myeloma and plasmacytoma cells, and cervical and bladder cancer cells. In myeloma 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 addition, transgenic mice that express high levels of IL-6 have been found to exhibit a massive, fatal plasma-cell proliferation, called plasmacytosis. Although these plasma cells are not malignant, the high rate of plasma-cell proliferation possibly contributes to the development of cancer.

Chagas' Disease Is Caused by a Parasite

The protozoan *Trypanosoma cruzi* is the causative agent of Chagas' disease, which is characterized by severe immune suppression. The ability of *T. cruzi* to mediate immune suppression can be observed by culturing peripheral-blood T cells in the presence and in the absence of *T. cruzi* and then evaluating their immune reactivity. Antigen, mitogen, or anti-CD3 monoclonal antibody normally can activate peripheral T cells, but in the presence of *T. cruzi,* T cells are not activated by any of these agents. The defect in these lymphocytes has been traced to a dramatic reduction in the expression of the 55-kDa α subunit of the IL-2 receptor. As noted earlier, the high-affinity IL-2 receptor contains α, β, and γ subunits. The α subunit is specific for cytokine binding (see Figure 12-9). Co-culturing of T cells with *T. cruzi* and subsequent staining with fluorescein-labeled anti-TAC, which binds to the α subunit, revealed a 90% decrease in the level of the α subunit.

Although the mechanism by which *T. cruzi* suppresses expression of the α subunit is still unknown, the suppression can be induced across a filter that prevents contact between the lymphocytes and protozoa. This finding suggests that a diffusible factor mediates suppression. Such a factor, once isolated, might have numerous clinical applications for regulating the level of activated T cells in leukemias and autoimmune diseases.

Therapeutic Uses of Cytokines and Their Receptors

The availability of purified cloned cytokines and soluble cytokine receptors offers the prospect of specific clinical therapies to modulate the immune response. A few cytokines—notably, interferons (see Clinical Focus)—and colony-stimulating factors, such as GM-CSF, have proven to be therapeutically useful. However, despite the promise of cytokines as powerful mediators of immune and other biological

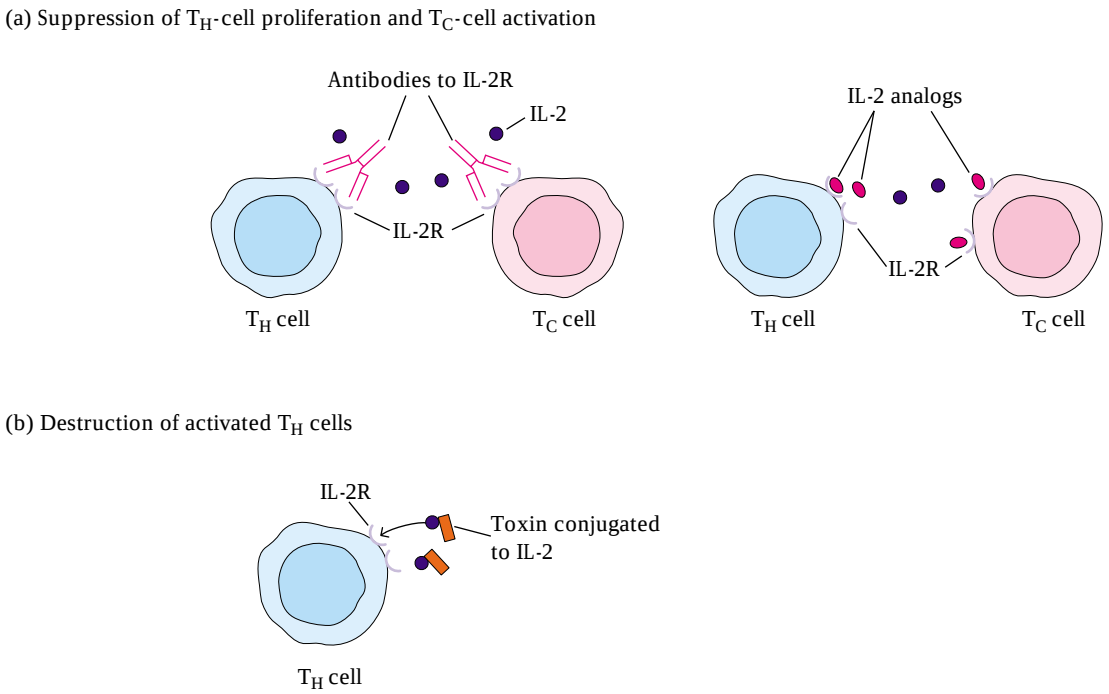


FIGURE 12-15 Experimental cytokine-related therapeutic agents offer the prospect of selectively modulating the immune response. (a) The anti-IL-2R monoclonal antibody binds to the cytokine receptor (IL-2R) on the cell surface, thereby preventing interaction of the cytokine with its receptor. (b) Conjugation of a toxin with a cytokine results in destruction of cells expressing the cytokine receptor.

responses, not many have made their way into clinical practice. A number of factors are likely to raise difficulties in adapting cytokines for safe and effective routine medical use. One of these is the need to maintain effective dose levels over a clinically significant period of time. During an immune response, interacting cells produce sufficiently high concentrations of cytokines in the vicinity of target cells, but achieving such local concentrations when cytokines must be administered systemically for clinical treatment is difficult. In addition, cytokines often have a very short half-life, so that continuous administration may be required. For example, recombinant human IL-2 has a half-life of only 7–10 min when administered intravenously. 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 instance, range from mild (e.g., fever, chills, diarrhea, and weight gain) to serious, such as anemia, thrombocytopenia, shock, respiratory distress, and coma. Despite these difficulties, the promise of cytokines for clinical medicine is great and efforts to develop safe and effective cytokine-related strategies continue, particularly in areas such as inflammation, cancer therapy, and modification of the immune response during organ transplantation, infectious disease, and allergy.

Some specific examples of various approaches being explored include cytokine receptor blockade and the use of cytokine analogs and cytokine-toxin conjugates. For instance, proliferation of activated T_H cells and activation of T_C cells can be blocked by anti-TAC, a monoclonal antibody that binds to the α subunit of the high-affinity IL-2 receptor (Figure 12-15a, left panel). Administration of anti-TAC has prolonged the survival of heart transplants in rats. Similar results have been obtained with IL-2 analogs that retain their ability to bind the IL-2 receptor but have lost their biological activity (Figure 12-15a, right panel). Such analogs have been produced by site-directed mutagenesis of cloned IL-2 genes. Finally, cytokines conjugated to various toxins (e.g., the β chain of diphtheria toxin) have been shown to diminish rejection of kidney and heart transplants in animals. Such conjugates containing IL-2 selectively bind to and kill activated T_H cells (Figure 12-15b).

Cytokines in Hematopoiesis

Early work in Australia and Israel demonstrated that soluble factors could support the growth and differentiation of red and white blood cells. The first of these soluble factors to be

CLINICAL FOCUS

Therapy with Interferons

Interferons are an extraordinary group of proteins whose antiviral activity led to their discovery almost 50 years ago. Subsequent studies showed that interferons have other effects, including the capacity to induce cell differentiation, to inhibit proliferation by some cell types, to inhibit angiogenesis, and to function in various immunoregulatory roles. Their effects on the immune system are important and dramatic. Interferons induce increases in the expression of class I and class II MHC molecules, and augment NK-cell activity. Increased class I expression increases the display of antigen to $CD8^+$ cells, a class that includes most of the T_C population. This enhanced display of antigen not only makes the antigen-presenting cells more effective in inducing cytotoxic T-cell populations, it also makes them better targets for attack by T_C cells. In addition to up-regulating class I MHC expression of many cell types, IFN-γ increases the expression of class II MHC molecules on such antigen-presenting cells as macrophages and dendritic cells. This makes them better presenters of antigen to T_H cells. IFN-γ is also a potent inducer of macrophage activation and general promoter of inflammatory responses. Cloning of the genes that encode all three types of interferon, IFN-α, IFN-β, and IFN-γ, has made it possible for the biotechnology industry to produce large amounts of all of these interferons at costs that make their clinical use practical. Some clinical uses of each type of interferon are described here:

- 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 found 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 American AIDS patients, 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. IFN-γ has also 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. Most of the effects mentioned above have been obtained in clinical studies that used IFN-α alone. Future clinical trials in which IFN-α is used in combinations with other agents may improve the effectiveness of this interferon in cancer therapy.
- 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, which leads 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 studies of CNS damage in treated and untreated patients revealed that MS-induced damage was less in a group of IFN-β-treated patients than in untreated ones.
- IFN-γ has found application in the clinic as an agent for the treatment of chronic granulomatous disease (CGD). This disease is hereditary and quite rare. Its central feature is a serious impairment of the ability of phagocytic cells to kill ingested microbes. Patients with CGD are beset with 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 was attempts to avoid infection, aggressive administration of

characterized, erythropoietin, was isolated from the urine of anemic patients and shown to support the development of red blood cells. Subsequently, many cytokines have been shown to play essential roles in hematopoiesis (see Table 12-5). During hematopoiesis, cytokines act as developmental signals that direct commitment of progenitor cells into and through particular lineages. As shown in Figure 12-16, a myeloid progenitor in the presence erythropoietin

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 goes down.

- 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 which 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. Although, in common with other cytokines, interferons are powerful modifiers of biological responses, fortunately, the side effects accompanying their use are much milder. 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, serious manifestations such as anemia and depressed platelet and white-blood-cell counts have been seen.

Cytokine-Based Therapies In Clinical Use

Agent	Nature of agent	Clinical application
Enbrel	Chimeric TNF-receptor/IgG constant region	Rheumatoid arthritis
Remicade	Monoclonal antibody against TNF-α receptor	Rheumatoid arthritis
Interferon α-2a	Antiviral cytokine	Hepatitis B Hairy cell leukemia Kaposi's sarcoma
Interferon α-2b	Antiviral cytokine	Hepatitis C Melanoma
Interferon β	Antiviral cytokine	Multiple sclerosis
Actimmune	Interferon γ	Chronic granulomatous disease (CGD) Osteopetrosis
Neupogen	G-CSF (hematopoietic cytokine)	Stimulates production of neutrophils Reduction of infection in cancer patients treated with chemotherapy
Leukine	GM-CSF (hematopoietic cytokine)	Stimulates production of myeloid cells after bone-marrow transplantation
Neumega	Interleukin 11 (IL-11), a hematopoietic cytokine	Stimulates production of platelets
Epogen	Erythopoietin (hematopoietic cytokine)	Stimulates red-blood-cell production

would proceed down a pathway that leads to the production of erythrocytes; suitable concentrations of a group of cytokines including IL-3, GM-CSF, IL-1, and IL-6 will cause it to enter differentiation pathways that lead to the generation of monocytes, neutrophils, and other leukocytes of the myeloid group. The participation of leukocytes in immune responses often results in their death and removal. However, both adaptive and innate immune responses generate cytokines that

TABLE 12-5 Haematopoietic cytokines

Haematopoietic growth factor	Sites of production	Main functions
Erythropoietin	Kidney, liver	Erythrocyte production
G-CSF	Endothelial cells, fibroblasts, macrophages	Neutrophil production
Thrombopoietin	Liver, kidney	Platelet production
M-CSF	Fibroblasts, endothelial cells, macrophages	Macrophage and osteoclast production
SCF/c-*kit* ligand	Bone marrow stromal cells, constitutively	Stem cell, progenitor cells survival/division; mast cell differentiation
Flt-3 ligand	Fibroblasts, endothelial cells	Early progenitor cell expansion; pre-B cells
GM-CSF	T cells (T_H1 and T_H2), macrophages, mast cells	Macrophage, granulocyte production; dendritic cell maturation and activation
IL-3	T cells (T_H1 and T_H2), macrophages	Stem cells and myeloid progenitor cell growth; mast cells
IL-5	Activated helper T cells –T_H2 response only	Eosinophil production murine B-cell growth
IL-6	Activated T cells monocytes, fibroblasts, endothelial cells	Progenitor cell stimulation; platelet production; immunoglobulin production in B cells
IL-11	As above	As LIF
IL-7		T-cell survival

G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; M-CSF, macrophage colony-stimulating factor; SCF, stem cell factor. Adapted from D. Thomas and A. Lopez, 2001. *Encyclopedia of Life Sciences:* Haematopoietic growth factors, Nature Publishing Group.

stimulate and support the production of leukocytes. The steps at which a number of cytokines participate in hematopoiesis is shown in Figure 12-16.

SUMMARY

- Cytokines are low-molecular-weight proteins that are produced and secreted by a variety of cell types. They play major roles in the induction and regulation of the cellular interactions involving cells of the immune, inflammatory and hematopoietic systems.
- The biological activities of cytokines exhibit pleiotropy, redundancy, synergy, antagonism, and, in some instances, cascade induction.
- There are over 200 different cytokines, most of which fall into one of the following families: hematopoietins, interferons, chemokines, and tumor necrosis factors.
- Cytokines act by binding to cytokine receptors, most of which can be classified as immunoglobulin superfamily receptors, class I cytokine receptors, class II cytokine receptors, members of the TNF receptor family, and chemokine receptors.
- A cytokine can only act on a cell that expresses a receptor for it. The activity of particular cytokines is directed to specific cells by regulation of the cell's profile of cytokine receptors.
- Cytokine-induced multimerization of class I and class I cytokine receptors activates a JAK/STAT signal-transduction pathway.
- Antigen stimulation of T_H cells in the presence of certain cytokines can lead to the generation of subpopulations of helper T cells known as T_H1 and T_H2. Each subset displays characterisic and different profiles of cytokine secretion.
- The cytokine profile of T_H1 cells supports immune responses that involve the marshalling of phagocytes, CTLs, and NK cells to eliminate intracellular pathogens. T_H2 cells produce cytokines that support production of particular immunoglobulin isotypes and IgE-mediated responses.
- Therapies based on cytokines and cytokine receptors have entered clinical practice.

References

Abbas, A., K. M. Murphy, and A. Sher. 1996. Functional diversity of helper T lymphocytes. *Nature* **383:**787.

Alcami, A., and U. H. Koszinowski. 2000. Viral mechanisms of immune evasion. *Immunol. Today* **9:**447–455.

Bach, E. A., M. Aguet, and R. D. Schreiber. 1998. The IFN-γ receptor: a paradigm for cytokine receptor signaling. *Ann. Rev. Immunol.* **15:**563.

Darnell, J. E. Jr. 1997. STATs and gene regulation. *Science* **5332:**1630–1635.

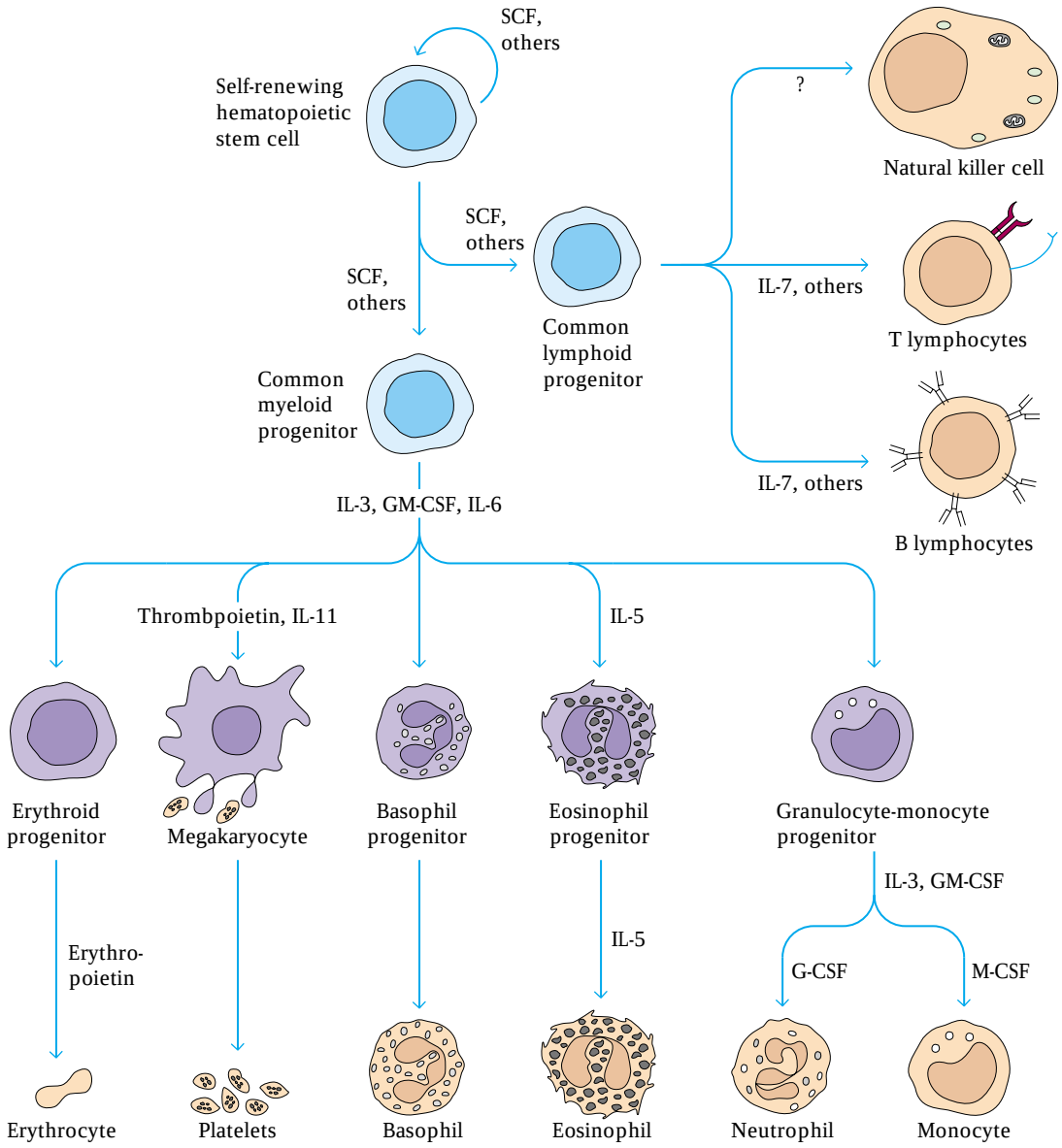


FIGURE 12-16 Hematopoietic cytokines and hematopoiesis. A variety of cytokines are involved in supporting the growth and directing the differentiation of hematopoietic cells. Note that additional factors may be required for some of the developmental pathways shown in the diagram. CFU = colony-forming unit, a cell capable of generating a colony of cells from which the fully differentiated cell type emerges.

Fitzgerald, K. A., et al. 2001. *The Cytokine Facts Book*, second edition. Academic Press.

Flynn, J. L., and J. Chan. 2001. Immunology of tuberculosis. *Annu. Rev. Immunol.* **19:**93–129.

Gadina, M., et al. 2001. Signaling by type I and II cytokine receptors: ten years after. *Curr. Opin. Immunol.* **3:**363–373.

Jaeckel, E., et al. 2001. Treatment of acute hepatitis C with interferon α-2b. *N. Engl. J .Med.* **345:**1452–1457.

Mossman, T. R., H. Cherwinski, M. W. Bond, M. A. Gledlin, and R. L. Coffman. 1986. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J. Immunology* **136:**2348.

Rengarajan, J., S. J. Szabo, and L. H. Glimcher. 2000. Transcriptional regulation of Th_H1/Th_H2 polarization. *Immunol. Today* **10:**479–483.

Szabo, S. J. et al. 2000. A novel transcription factor, T-bet, directs T_H1 lineage commitment. *Cell* **100:**655–669.

Walter, M. R., et al. 1995. Crystal structure of a complex between interferon-γ and its soluble high-affinity receptor. *Nature* **376:** 230.

USEFUL WEB SITES

http://www.rndsystems.com/

The cytokine minireviews found at R&D Systems Web site provide extensive, detailed, well-referenced, and often strikingly illustrated reviews of many cytokines and their receptors.

http://www.ncbi.nlm.nih.gov:80/LocusLink/index.html

LocusLink provides access to sequence and descriptive information about genetic loci of cytokines and other proteins. It also references papers discussing the basic biology (function and structure) of the gene or protein of interest.

Study Questions

CLINICAL FOCUS QUESTION Cytokines are proving to be powerful drugs, but their use is accompanied by side effects that can be harmful to patients. What are some of the side effects produced by Actimmune, Roferon, and interferon beta? (Hint: Manufacturer's Web sites often provide detailed information on the side effects of drugs they produce.)

1. Indicate whether each of the following statements is true or false. If you think a statement is false, explain why.
   a. The high-affinity IL-2 receptor consists of two transmembrane proteins.
   b. The anti-TAC monoclonal antibody recognizes the IL-1 receptor on T cells.
   c. All cytokine-binding receptors contain two or three subunits.
   d. Expression of the β subunit of the IL-2 receptor is indicative of T-cell activation.
   e. Some cytokine receptors possess domains with tyrosine kinase activity that function in signal transduction.
   f. All members of each subfamily of the class I cytokine (hematopoietin) receptors share a common signal-transducing subunit.
2. When IL-2 is secreted by one T cell in a peripheral lymphoid organ, do all the T cells in the vicinity proliferate in response to the IL-2 or only some of them? Explain.
3. Briefly describe the similarities and differences among cytokines, growth factors, and hormones.
4. Indicate which subunit(s) of the IL-2 receptor are expressed by the following types of cells:
   a. _____ Resting T cells
   b. _____ Activated T cells
   c. _____ Activated T cells + cyclosporin A
   d. _____ Resting T_C cells
   e. _____ CTLs
   f. _____ NK cells
5. Superantigens have been implicated in several diseases and have been useful as research tools.
   a. What properties of superantigens distinguish them from conventional antigens?
   b. By what mechanism are bacterial superantigens thought to cause symptoms associated with food poisoning and toxic-shock syndrome?
   c. Does the activity of superantigens exhibit MHC restriction?
6. IL-3, IL-5, and GM-CSF exhibit considerable redundancy in their effects. What structural feature of the receptors for these cytokines might explain this redundancy?
7. Considerable evidence indicates the existence of two T_H-cell subsets, differing in the pattern of cytokines they secrete.
   a. What type of immune response is mediated by the T_H1 subset? What type of antigen challenge is likely to induce a T_H1-mediated response?
   b. What type of immune response is mediated by the T_H2 subset? What type of antigen challenge is likely to induce aT_H1-mediated response?