

2. Cytokines and chemokines

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Cytokines and chemokines are redundant secreted proteins with growth, differentiation, and activation functions that regulate and determine the nature of immune responses and control immune cell trafficking and the cellular arrangement of immune organs. Which cytokines are produced in response to an immune insult determines initially whether an immune response develops and subsequently whether that response is cytotoxic, humoral, cell-mediated, or allergic. A cascade of responses can be seen in response to cytokines, and often several cytokines are required to synergize to express optimal function. An additional confounding variable in dissecting cytokine function is that each cytokine may have a completely different function, depending on the cellular source, target, and, most important, specific phase of the immune response during which it is presented. Numerous cytokines have both proinflammatory and anti-inflammatory potential; which activity is observed depends on the immune cells present and their state of responsiveness to the cytokine. For this chapter, cytokines are grouped according to those that are mononuclear phagocytic-derived or T-lymphocytic-derived; that mediate cytotoxic (antiviral and anticancer), humoral, cell-mediated, or allergic immunity; and that are immunosuppressive. The biology of chemokines are then reviewed, grouped by family. (*J Allergy Clin Immunol* 2003;111:S460-75.)

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Cytokines are involved in virtually every facet of immunity and inflammation, including innate immunity, antigen presentation, bone marrow differentiation, cellular recruitment and activation, and adhesion molecule expression (Fig 1). Which cytokines are produced in response to an immune insult determines initially whether an immune response develops and subsequently whether that response is cytotoxic, humoral, cell-mediated, or allergic. For presentation in this review, cytokines are grouped according to those that are predominantly mononuclear phagocytic-derived or T-lymphocyte-derived; those that predominantly mediate cytotoxic (antiviral and anticancer), humoral, cell-mediated, or allergic immunity; and those that are immunosuppressive.

CYTOKINE PRODUCTION BY ANTIGEN-PRESENTING CELLS

Cytokines primarily derived from mononuclear phagocytic cells and other antigen-presenting cells (APCs) are

Abbreviations used

ADCC: Antibody-dependent cellular cytotoxicity
AHR: Airway hyperreactivity
APC: Antigen-presenting cells
GCSF: Granulocyte-colony stimulating factor
ICAM: Intercellular adhesion molecule
ICE: Interleukin-1 converting enzyme
IFN: Interferon
IL: Interleukin
LPS: Lipopolysaccharide
MAPK: Mitogen-activated protein kinase
NK: Natural killer
SCF: Stem cell factor
TGF- β : Transforming growth factor- β
TNF: Tumor necrosis factor
Tr: T repressor
VCAM: Vascular cell adhesion molecule

particularly effective in promoting the cellular infiltrate and damage to resident tissue characteristic of inflammation. The processing of antigens as they are taken up by APCs, processed, and presented to T-helper lymphocytes provides one pathway for this class of cytokine production. Alternatively, monocytes are potentially triggered to produce cytokines through the innate immune system, using pattern recognition receptors that recognize stereotypic components of pathogens that do not occur on mammalian cells. These receptors, such as the lipopolysaccharide (LPS) receptor, contribute to the ability of the immune system to distinguish pathogens from nonpathogenic proteins to which the immune system may become exposed. The cytokines predominantly produced by monocytes include tumor necrosis factor (TNF), and several interleukin (IL) molecules known as IL-1, IL-6, IL-8, IL-12, IL-15, IL-18, and IL-23. IL-8 is also classified as a chemokine known as CXCL8; it and other chemokines are also secreted by APCs.

TUMOR NECROSIS FACTOR

TNF represents two homologous proteins primarily derived from mononuclear phagocytes (TNF- α) and lymphocytes (TNF- β).¹ The active form of both cytokines is a homotrimer. In addition to mononuclear phagocytes, TNF- α may be produced by neutrophils, activated lymphocytes, natural killer (NK) cells, endothelial cells, and mast cells. The most potent inducer of TNF by monocytes is LPS, acting through toll-like receptor 2 (TLR2) and TLR4. The toll-like receptors (Table I) represent a family of pattern recognition receptors that recognize motifs used by pathogens but not mammalian cells and are capable of potentially activating

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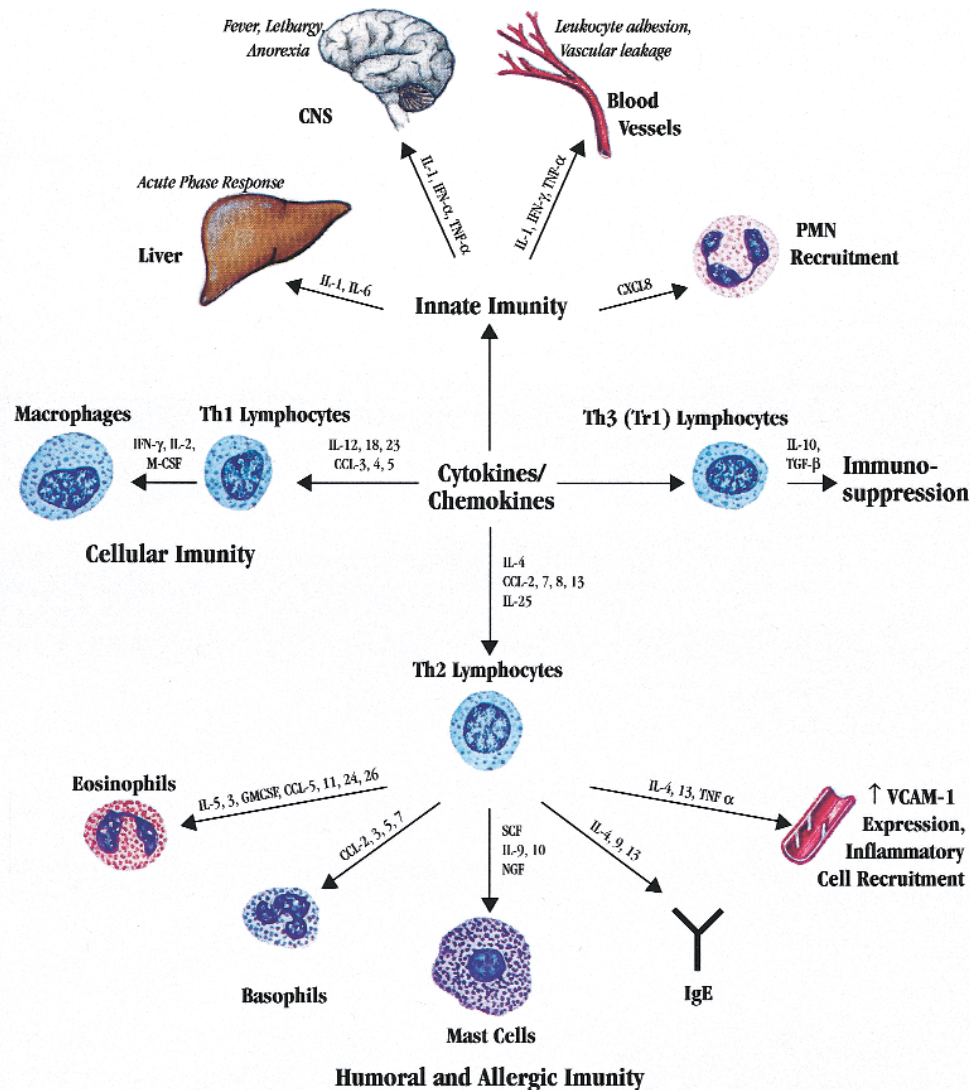


FIG 1. Summary of actions of cytokines and chemokines. Cytokines derived predominantly from mononuclear phagocytic cells are uniquely important in innate immunity and both initiate immune responses and generate symptoms associated with infections and inflammatory disorders. Phenotype of the subsequent immune response is a function of the repertoire of cytokines produced by the responding T-helper lymphocytes. T_H1 -like lymphocytes are characterized by their production of IFN- γ and primarily contribute to cellular immunity. T_H2 -like lymphocytes are characterized by their production of IL-4, IL-5, IL-9, and IL-13 and contribute to humoral and allergic responses. T_H3 -like lymphocytes have immunosuppressive tendencies and are characterized by their production of IL-10 and TGF- β .

innate immune responses, including mononuclear phagocytic cell cytokine production. TNF- α is processed as a membrane-bound protein from which the soluble active factor is derived by cleavage using the TNF- α converting enzyme.² TNF- β (also known as lymphotoxin- α) can be synthesized and processed as a typical secretory protein but is usually linked to the cell surface by forming heterotrimers with a third, membrane-associated, member of this family, LT- β . TNF- α and TNF- β bind to the same two distinct cell surface receptors—TNFR I (p55) and TNFR II (p75)—with similar affinities, and produce similar although not identical effects.³ TNFs induce antitu-

mor immunity through direct cytotoxic effects on cancerous cells and by stimulating antitumor immune responses. TNF interacts with endothelial cells to induce adhesion molecules known as intracellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, and E-selectin, thus permitting the egress of granulocytes into inflammatory loci. TNF is a potent activator of neutrophils, mediating adherence, chemotaxis, degranulation, and the respiratory burst. Enthusiasm for the potential therapeutic value of TNF to treat malignancies has been tempered by its severe side effects. TNF is responsible for the severe cachexia that

TABLE I. Innate immune receptors

Receptor	Ligand
TLR2	Gram-negative bacterial LPS (endotoxin) through CD14-dependent pathway Mycobacterial glycolipids lipoarabinomannan (AraLAM) and mannosylated phosphatidylinositol (PIM), Peptidoglycan (PGN)
TLR3	Double-stranded RNA (viral-derived RNA)
TLR4	Gram-negative bacterial LPS (lipid A; endotoxin), Heat shock protein 6 Plant product Taxol RSV protein F Lipoteichoic acid (LTA)
TLR5	Flagellin, Salmonella, Microbial lipoproteins
TLR6	Proteoglycans (bacterial) with TLR2
TLR9	CpG

occurs in chronic infections and cancer.¹ Furthermore, TNF induces vascular leakage, has negative inotropic effects, and is the primary endogenous mediator of toxic shock and sepsis.⁴

Interleukin-1

The IL-1 family represents four peptides (IL-1 α , IL-1 β , the IL-1 receptor antagonist [IL-1ra], and IL-18).⁵ IL-1 α and IL-1 β have similar biological activities, and both of these proteins along with IL-1ra interact with similar affinities to the two IL-1 receptors (IL-1Rs). Type I receptors transduce the biological effects attributed to IL-1.⁶ These are in contrast to type II receptors, which are expressed on B cells, neutrophils, and bone marrow cells and have a minimal intracellular domain. The "capture" and sequestration of IL-1 by these inactive type II receptors serves an anti-inflammatory function and hence are sometimes referred to as decoy receptors. The capacity of IL-1ra to bind to the type I (proinflammatory) IL-1R without transducing biological activities is the basis for its capacity to function as a cytokine antagonist.⁷ IL-1 is primarily produced by cells of the mononuclear phagocytic lineage but is also produced by endothelial cells, keratinocytes, synovial cells, osteoblasts, neutrophils, glial cells, and numerous other cells. IL-1 production may be stimulated by a variety of agents, including endotoxin, other cytokines, microorganisms, and antigens (Table I). Both IL-1 α and IL-1 β , as well as the related protein IL-18, are synthesized without a secretory leader sequence as a less active precursor. The mechanism for IL-1 and IL-18 secretion depends on their cleavage by a specific converting enzyme, termed IL-1 converting enzyme (ICE) or caspase 1, which cleaves the procytokines into their active secreted forms.⁸

One of the most important biological activities of IL-1 is its ability to activate T lymphocytes by enhancing the production of IL-2 and expression of IL-2 receptors. In the absence of IL-1, a diminished immune response or a state of tolerance develops. IL-1 augments B-cell proliferation and increases immunoglobulin synthesis. The production of IL-1 during the immune response produces a

spectrum of changes associated with being ill. IL-1 interacts with the central nervous system to produce fever, lethargy, sleep, and anorexia. An IL-1–hepatocyte interaction inhibits production of "housekeeping" proteins (eg, albumin) and stimulates the synthesis of acute phase response peptides (eg, amyloid peptide, C-reactive peptide, complement). IL-1 stimulates endothelial cell adherence of leukocytes through the upregulation of ICAM-1, VCAM-1, and E-selectin. IL-1 contributes to the hypotension of septic shock. TNF and IL-1 share numerous biological activities, the major distinction being that TNF has no direct effect on lymphocyte proliferation.

IL-1ra is secreted naturally in inflammatory processes. Its production is upregulated by many cytokines, including IL-4, IL-6, IL-13, and transforming growth factor- β (TGF- β). Production of IL-1ra is thought to modulate the potentially deleterious effects of IL-1 in the natural course of inflammation.

Interleukin-6

Mononuclear phagocytic cells are the most important source of IL-6⁹; however, IL-6 is also produced by T and B lymphocytes, fibroblasts, endothelial cells, keratinocytes, hepatocytes, and bone marrow cells. Under the influence of IL-6, B lymphocytes differentiate into mature plasma cells and secrete immunoglobulins. IL-6 mediates T-cell activation, growth, and differentiation. In addition to lymphocyte activation, IL-6 shares several activities with IL-1, including the induction of pyrexia and the production of acute phase proteins. IL-6 is considered the most important inducer of hepatocyte synthesis of acute-phase proteins. In contrast to these proinflammatory effects, IL-6 mediates several anti-inflammatory effects. Whereas both IL-1 and TNF induce synthesis of each other, as well as IL-6, IL-6 terminates this upregulatory inflammatory cascade and inhibits IL-1 and TNF synthesis. Furthermore, IL-6 stimulates synthesis of IL-1ra.

Interleukins-12, -18, and -23

IL-12 is derived from monocytes and macrophages but also B cells, dendritic cells, Langerhans cells, polymorphonuclear neutrophils (PMNs), and mast cells.¹⁰ The biologically active form is a heterodimer. The larger subunit (p40) is homologous to the soluble receptor for IL-6, whereas the smaller subunit (p35) is homologous to IL-6. Homodimers and monomers of the p40 peptide act as competitive antagonists by binding to IL-12Rs without transducing activating signals. IL-12 activates and induces proliferation, cytotoxicity, and cytokine production of NK cells. Other activities attributed to IL-12 include proliferation of T-helper and cytotoxic lymphocytes. Its counterregulatory role in allergic inflammation is discussed later.

IL-18 was originally derived from the liver and is also produced by lung tissue, pancreas, kidney, and skeletal muscle but not lymphocytes or NK cells.¹¹ Similar to IL-1, IL-18 requires a specific converting enzyme (ICE or caspase-1) to permit secretion and activation. In contrast to most cytokines, IL-18 is constitutively expressed, and

release of its active form is regulated through activation of this converting enzyme. However, its major biological activity is more similar to that of IL-12 than IL-1. IL-18 has an important role in cellular adhesion, being the final common pathway used by IL-1 and TNF that leads to ICAM-1 expression. IL-18 binds to a unique heterodimer receptor. IL-18R expression is upregulated by IL-12, and thereby these two cytokines synergize to stimulate interferon- γ (IFN- γ) release. Soluble IL-18 receptors are derived from a unique gene that has lost its signaling domain and thereby functions as a natural decoy receptor with potent anti-inflammatory functions.¹²

IL-23 is a recently described cytokine having homology to the p35 subunit of IL-12.¹³ It is a heterodimer consisting of the unique IL-23 α chain and the p40 chain of IL-12. IL-23 is secreted by activated dendritic cells. As with IL-12 and IL-18, it is a potent inducer of IFN- γ and is presumed to contribute to T_H1-like lymphocyte differentiation. Its receptor includes the IL-12R β 1 chain.

Interleukin-15

IL-15 has activity similar to that of IL-2 and is primarily distinguished from IL-2 through its use of a unique α chain as part of its receptor signaling complex.¹⁴ Both receptors share the use of the β and γ chain. Mononuclear phagocytic cells, epithelium, fibroblasts, and placenta are other sources of IL-15, whereas activated T lymphocytes, the most important source of IL-2, do not express IL-15. As discussed later, like IL-2, IL-15 is a T-cell growth factor, is chemotactic for T lymphocytes, differentiates NK cells, and stimulates B-cell growth and differentiation. IL-15 provides a mechanism by which mononuclear phagocytic cells can regulate T- and NK-cell proliferation and function in a fashion similar to T-cell-derived IL-2.

CYTOTOXIC IMMUNITY

Immune responses directed against virus-infected and neoplastic cells are primarily mediated by CD8⁺ cytotoxic lymphocytes and NK cells. Cytokines that activate cytotoxic immunity include IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12, and IL-15—which are discussed elsewhere—as well as IL-11 and, most importantly, TNF- α , TNF- β , and the interferons.

Interleukin-11

IL-11 was originally described as a stimulatory factor for hematopoietic precursor cells. It contributes to lymphoid production in the bone marrow and synergizes with other growth factors to produce erythrocytes, platelets, and mast cells. IL-11 stimulates the production of acute phase proteins and induces lymphoid cell differentiation. IL-11 is an important stimulatory factor for connective tissue cells such as fibroblasts. A role for IL-11 in asthma remodeling is suggested by studies demonstrating expression of IL-11 in severe asthma,¹⁵ and the capacity of this cytokine to stimulate fibroblast proliferation and collagen deposition.

Interferons

There are three members of the IFN family (α , β , and γ), and their nomenclature is based on their ability to “interfere” with viral growth. IFN- α is primarily derived from monocytes, macrophages, B lymphocytes, and NK cells. It has significant antiviral activity mediated through its ability to inhibit viral replication within virus-infected cells, protect uninfected cells from infection, and stimulate antiviral immunity by cytotoxic lymphocytes and NK cells. IFN- α has other important biological actions, including upregulation of class I MHC molecules and mediation of antitumor activity. IFN- β mirrors many of the biological effects of IFN- α .

IFN- γ is primarily made by T cells and NK cells and to a lesser degree by macrophages. The biological activities of IFN- γ include only modest antiviral activity and its derivation primarily from T lymphocytes suggests that it may be more of an interleukin than an interferon. IFN- γ and its roles in cellular and allergic immunity are discussed later.

HUMORAL IMMUNITY

At least two cytokines contribute to B-lymphocyte maturation in the bone marrow, the lymphoid stem cell growth factors IL-7 and IL-11. IL-7 is critically important to the development of B and T lymphocytes through its production by stromal tissue of the bone marrow and thymus, from which it interacts with lymphoid precursors. In addition, IL-7 stimulates the proliferation and differentiation of cytotoxic T and NK cells and stimulates the tumoricidal activity of monocytes and macrophages.

After B cells egress from the bone marrow, isotype switching, the activation of mature B cells into immunoglobulin-secreting B cells, and their final differentiation into plasma cells are processes that are under T-cell control.¹⁶ Cytokines that trigger isotype switching include IL-4 and IL-13, which induces the IgE isotype, TGF- β , which triggers IgA, and IL-10, which contributes to the generation of IgG4. Other cytokines that influence B-cell maturation include IFN- γ , IL-1, IL-2, IL-5, IL-6, IL-12, IL-15, and IL-21.

CELLULAR IMMUNITY

Interleukin-2

Stimulation of T cells by antigen in the presence of accessory signals provided by the cytokines IL-1 and IL-6 and the cognate interaction of the B7 molecules (CD80 or CD88) with CD28 induces the simultaneous secretion of IL-2 and the expression of high-affinity IL-2R. Subsequently, the binding of secreted IL-2 to these IL-2R-positive T cells induces clonal T-cell proliferation. The requirement for both IL-2 production and IL-2R expression for T-cell proliferation ensures that only T cells specific for the antigen inciting the immune response become activated. In addition to its role as a T-cell growth factor, IL-2 is also involved in activation of NK cells, B cells, cytotoxic T cells, and macrophages.

Interleukin-21

IL-21 is a newly described cytokine having homology to both IL-2 and IL-15, which is predominantly produced by activated T lymphocytes.¹⁷ IL-21 receptors are expressed on activated B, T, and NK cells. It shares numerous biological activities with IL-2 and IL-15, including the capacity to activate NK cells and promote the proliferation of B and T lymphocytes.

Interferon- γ

The most important cytokine responsible for cell-mediated immunity is IFN- γ .¹⁸ It is primarily produced by T-helper lymphocytes but is also derived from cytotoxic T cells and NK cells. IFN- γ mediates increased MHC class I and II molecule expression. IFN- γ stimulates antigen presentation and cytokine production by monocytes and also stimulates monocyte effector functions, including adherence, phagocytosis, secretion, the respiratory burst, and nitric oxide production. The net result is the accumulation of macrophages at the site of cellular immune responses, with their activation into macrophages capable of killing intracellular pathogens. In addition to its effects on mononuclear phagocytes, IFN- γ stimulates killing by NK cells and neutrophils. It stimulates adherence of granulocytes to endothelial cells through the induction of ICAM-1, an activity shared with IL-1 and TNF. As with other interferons, IFN- γ inhibits viral replication. As discussed later, IFN- γ is an inhibitor of allergic responses through its capacity to inhibit IL-4-mediated effects.

Interleukins-16 and -17

Additional cytokines that are secreted by T-helper lymphocytes and contribute to cell-mediated immunity are TNF- β , IL-16, and IL-17. IL-16 is a T-cell-derived product that is chemotactic for CD4⁺ lymphocytes, eosinophils, and monocytes and uses the CD4 molecule as its receptor.¹⁹ Its production is upregulated by TNF- α , TGF- β , IL-4, IL-9, and IL-13, as well as by histamine. IL-17 represents a family of cytokines that are expressed by activated T cells predominantly of the memory phenotype (CD4⁺CD45RO⁺) and also by eosinophils. IL-17 activates macrophages, fibroblasts, and stromal cells, including their expression of ICAM-1 and secretion of cytokines (IL-6, IL-8, IL-11, granulocyte-colony stimulating factor [G-CSF]), prostaglandin E₂, and nitric oxide. IL-17 expression is increased in asthma, in which its ability to activate fibroblasts suggests a role in airway remodeling.

ALLERGIC IMMUNITY

A final possible outcome of T-cell activation is the development of allergic immunity. Several features specifically associated with the asthmatic state are regulated by cytokines. These include the regulation of IgE, eosinophilia, and mast cell proliferation.

Regulation of IgE

The inappropriate production of IgE in response to aller-

gen defines atopy. The regulation of IgE is primarily a function of the relative activities of IL-4, IL-13, and IFN- γ .

Interleukin-4. In addition to T-helper lymphocytes, IL-4²⁰ is derived from eosinophils, basophils, and possibly mast cells. In both eosinophils and mast cells, IL-4 exists as a preformed, granule-associated peptide and can be rapidly released in allergic inflammatory responses. IL-4 stimulates MHC class II molecules, B7, CD40, surface IgM, and low-affinity IgE receptor (CD23) expression by B cells, thereby enhancing the antigen-presenting capacity of B cells. IL-4 induces the immunoglobulin isotype switch from IgM to IgE.^{21,22} Other B-cell-activating cytokines, such as IL-2, IL-5, IL-6, and IL-9, synergize with IL-4 to increase the secretion of IgE. IL-4 has been identified in the serum, bronchoalveolar lavage fluid, and lung tissue of asthmatic subjects, in nasal polyp tissue, and in the nasal mucosa of subjects with allergic rhinitis.

In addition to these effects on B cells, IL-4 has important influences on T-lymphocyte growth, differentiation, and survival, producing important influences on allergic inflammation. As will be discussed later, IL-4 drives the initial differentiation of naïve T-helper type 0 (T_H0) lymphocytes toward a T_H2 phenotype. IL-4 is also important in maintaining allergic immune responses by preventing apoptosis of T lymphocytes.²³ The production of IL-4 by T_H2 lymphocytes renders these cells refractory to the anti-inflammatory influences of corticosteroids.

Other activities of IL-4 include enhancing the expression of MHC molecules and low-affinity IgE receptors (CD23) on macrophages. In contrast to these proinflammatory effects on monocytes, IL-4 downregulates antibody-dependent cellular cytotoxicity (ADCC), inhibits expression of Fc receptors, inhibits their differentiation into macrophages, and downregulates production of nitric oxide, IL-1, IL-6, and TNF- α while stimulating production of IL-1ra. Another important activity of IL-4 in allergic inflammation is its ability to induce expression of VCAM-1 on endothelial cells. This produces enhanced adhesiveness of endothelium for T cells, eosinophils, basophils, and monocytes but not neutrophils, as is characteristic of allergic reactions.²⁴ IL-4 but not IL-13 receptors are present on mast cells, where they function to stimulate IgE receptor expression. An additional important influence of IL-4 on allergic inflammation is its ability to induce mast cell expression of the enzyme leukotriene C4 (LTC₄) synthase, thereby determining the capacity of mast cells to produce cysteinyl leukotrienes.²⁵ IL-4 stimulates mucin production and contributes to the excessive mucous production in the asthmatic airway. Functional IL-4 receptors are heterodimers consisting of the IL-4R α chain interacting with the shared γ chain or the IL-13R α 1 chain.²⁶ The shared use of the IL-4R α chain by IL-13 and IL-4 and the activation by this chain of the signaling protein Stat6 explains many of the common biological activities of these two cytokines.

Interleukin-13. IL-13 is homologous to IL-4 and shares much of its biological activities on mononuclear

phagocytic cells, endothelial cells, epithelial cells, and B cells. Thus IL-13 induces the IgE isotype switch and VCAM-1 expression.²⁷ Functional IL-13 receptors are a heterodimer containing the IL-4R α chain and a unique IL-13R α 1 chain. The two IL-13R α chains that have been described include the active form of the receptor IL-13R α 1 and a putative inhibitory receptor, IL-13R α 2, which lacks the motif required for the binding of Janus kinases.²⁸ IL-13R α 1 expression is more limited than IL-4 receptors and includes endothelial cells, B cells, mononuclear phagocytes, and basophils but not mast cells or T cells. This more limited distribution of IL-13R α 1 explains the unique ability of IL-4 to induce T_H2 lymphocyte differentiation and mast cell activation. However, IL-13 is more widely produced than IL-4, including by T_H1-like lymphocytes and is more readily identified in allergic inflammatory tissue.²⁹ IL-13-overexpressing mice have eosinophilic inflammation, mucus hypersecretion, airway fibrosis, and nonspecific airway hyperreactivity (AHR).

Interleukin-9. IL-9 was originally described as a mast cell growth factor³⁰ and contributes to mast cell-mediated allergic responses through its ability to stimulate the production of mast cell proteases and the IgE high-affinity receptor α chain. IL-9 supports the growth and survival of antigen-specific T lymphocytes. IL-9 is derived from eosinophils and T_H2-like lymphocytes. Its selective production by T_H2 cells suggests a role in allergic inflammation and, in human T lymphocytes, this is a feature shared only with IL-4, IL-5, and IL-25. IL-9 has other important activities in allergic inflammation including inducing expression of CCL11 (eotaxin), IL-5 receptors, and chemokine receptor 4. It synergizes with IL-4 to enhance the production of IgE and with IL-5 to enhance the production of eosinophils.

Interferon- γ . The third cytokine critically important in the regulation of IgE synthesis is IFN- γ . IFN- γ functions as an inhibitor of allergic responses through its capacity to inhibit IL-4-mediated expression of low-affinity IgE receptors and the isotype switch to IgE. The downregulation of IL-4- and IL-13-dependent IgE production is therefore a product of IFN- γ , but physiologically this results as a consequence of the biological activity of the IFN- γ inducers IL-12, IL-18, and IL-23.

Interleukin-25. A recently described cytokine, IL-25 contributes to IgE secretion through its ability to stimulate IL-4 and IL-13 production.³¹ Similar to IL-4, IL-5, and IL-9, it is derived from T_H2-like lymphocytes. It stimulates release of IL-4, IL-5, and IL-13 from nonlymphoid accessory cells. Intraperitoneal injection of mice with IL-25 leads to enhanced IL-4 and IL-13 production, which is associated with increased IgE production. IL-25 stimulation of IL-5 production promotes blood eosinophilia, and mice treated with IL-25 have eosinophilic inflammation.

Eosinophilia

Another characteristic feature of allergic diseases is the presence of increased numbers of activated circulating eosinophils.

Interleukin-5. IL-5 is the most important eosinophilopoietin, and mice transgenic for constitutive IL-5 expression have eosinophilia.³² In addition to stimulating eosinophil production, IL-5 is chemotactic for eosinophils and activates mature eosinophils, inducing eosinophil secretion and enhanced cytotoxicity. Another mechanism by which IL-5 promotes accumulation of eosinophils is through its ability to upregulate responses to chemokines and α β 2 integrins on eosinophils, thereby promoting their adherence to VCAM-1-expressing endothelial cells. IL-5 prolongs eosinophil survival by blocking apoptosis.³³ Administration of IL-5 to human beings causes mucosal eosinophilia and an increase in bronchial hyperreactivity. Other activities of IL-5 include maturation of cytotoxic T lymphocytes and basophilic differentiation. In addition to T-helper lymphocytes, other sources for IL-5 include mast cells, natural T cells, and perhaps eosinophils themselves. IL-5 interacts with specific IL-5Rs that consist of a heterodimer containing IL-5R α and a β chain (CD131) shared with GM-CSFR and IL-3R.³⁴

Interleukin-3 and GM-CSF. In addition to IL-5, two CSFs, IL-3³⁵ and GM-CSF,³⁶ contribute to the activity of eosinophils in allergic inflammation through their capacities to prolong eosinophil survival and to generate activated eosinophils. IL-3 is an important factor that supports the growth of precursors for a variety of hematopoietic cells, including dendritic cells, erythrocytes, granulocytes (especially basophils), macrophages, mast cells, and lymphoid cells. The major source of IL-3 is T lymphocytes, but in allergic inflammation it is also derived from eosinophils and mast cells.

Like IL-3, GM-CSF is an important CSF that primarily supports the maturation of dendritic cells, neutrophils, and macrophages. GM-CSF also synergizes with other CSFs to support the production of platelets and erythrocytes. GM-CSF is an activating factor for mature neutrophils and mononuclear phagocytic cells. The role of GM-CSF in allergic immunity is derived from its shared ability with IL-3 and IL-5 to inhibit apoptosis of eosinophils and thereby prolong the survival of eosinophils at sites of allergic inflammation. GM-CSF activates mature eosinophils, increasing their degranulation, cytotoxicity, and response to chemoattractants. As noted, all three of these eosinophil-activating cytokines—IL-5, IL-3, and GM-CSF—bind to α β heterodimer receptors, which have unique α chains but share the common β chain.

Mast cell proliferation and activation

Increased numbers of mast cells characterize allergic diseases, and, as with elevated IgE concentrations and eosinophilia, this is a T-cell-dependent process. The most important cytokine responsible for mast cell growth and proliferation is stem cell factor (SCF, or *c-kit* ligand).³⁷ SCF is derived from bone marrow stromal cells, endothelial cells, and fibroblasts. SCF induces histamine release from human mast cells but not basophils and remains the only cytokine with this property. The importance of this factor in human beings is supported by clinical observa-

tions that the local administration of SCF is associated with mast cell histamine release³⁸ and when administered systemically is associated with cutaneous mast cell proliferation and chronic urticaria. In addition to being essential for mast cell differentiation, SCF interacts with other hematopoietic growth factors to stimulate myeloid, lymphoid, and erythroid progenitor cells. Several cytokines, especially including IL-3, IL-5, IL-6, IL-9, IL-10, IL-11, and nerve growth factor, may also contribute to mast cell proliferation.³⁹ In addition to the factors that stimulate mast cell proliferation, several cytokines have been demonstrated to induce histamine release from basophils. These histamine-releasing factors include several members of the chemokine family.

ANTI-INFLAMMATORY CYTOKINES

In addition to cytokines that stimulate cytotoxic, cellular, humoral, and allergic inflammation, several cytokines have predominantly anti-inflammatory effects including, as previously discussed, IL-1ra but also TGF- β and members of the IL-10 family.

Transforming growth factor- β

TGF- β represents a family of peptides that regulate cell growth, having both stimulatory and inhibitory effects on different cell types.⁴⁰ It is produced primarily by chondrocytes, osteocytes, fibroblasts, platelets, monocytes, and some T cells. The TGF- β -producing T-helper lymphocyte has been proposed to represent a distinct phenotype termed the T repressor (Tr1) or T-helper type 3 (T_H3) cell. TGF- β is synthesized as an inactive precursor that requires proteolytic cleavage to become active. TGF- β is an important stimulant of fibrosis, inducing formation of the extracellular matrix, and it promotes wound healing and scar formation. In immunity, it is inhibitory for B lymphocytes and T-helper and cytotoxic lymphocytes. It inhibits immunoglobulin secretion by B lymphocytes and cytotoxicity of mononuclear phagocytes and NK cells. In general, it inhibits the proliferation of many different cell types. Production of TGF- β by apoptotic T cells creates an immunosuppressive milieu and is one explanation for the absence of inflammation and autoimmunity as a consequence of apoptotic cell death.⁴¹ In contrast to these anti-inflammatory effects, TGF- β is a chemoattractant for macrophages and supports the α isotype switch to IgA by B cells.⁴² Production of TGF- β in gut lymphoid tissue is responsible for secretory IgA production and is critical for the maintenance of immune nonresponsiveness to otherwise benign gut pathogens and food allergens. TGF- β is also constitutively produced in the healthy lung. In allergic inflammation, the expression of TGF- β may be associated with the fibrosis observed in asthma. TGF- β may lessen allergic inflammation through a capacity to inhibit IgE synthesis and mast cell proliferation.

Interleukins-10, -19, -20, -22, and -24

IL-10 is a product of numerous cells, including T_H1 and T_H2 lymphocytes,⁴³ cytotoxic T cells, B lympho-

cytes, mast cells, and mononuclear phagocytic cells. The primary T-cell source for IL-10 is the Tr1 lymphocyte. However, monocytes and B cells are the major sources of IL-10 in human beings. IL-10 inhibits production of IFN- γ and IL-2 by T_H1 lymphocytes; IL-4 and IL-5 by T_H2 lymphocytes⁴³; IL-1 β , IL-6, IL-8, IL-12, and TNF- α by mononuclear phagocytes; and IFN- γ and TNF- α by NK cells. In addition, IL-10 inhibits monocyte MHC class II molecule, CD23, ICAM-1, and B7 expression. Inhibition of B7 expression results in the inhibition of the ability of the APC to provide the accessory signal necessary for T-helper activation.⁴⁴ This inhibition of accessory function is primarily responsible for the inhibition of T_H1 and T_H2 cytokine production. Constitutive expression of IL-10 by APCs in the respiratory tract of normal subjects has a critical role in the induction and maintenance of tolerance to allergens and otherwise benign bioaerosols. In contrast, asthma and allergic rhinitis are associated with diminished IL-10 expression in the allergic airway, which will contribute to the development of an inflammatory milieu.⁴⁵ Support for a modulating role for IL-10 in human allergic disease is further derived from observations that IL-10 inhibits eosinophil survival and IL-4-induced IgE synthesis. These inhibitory effects of IL-10 are in contrast to its effect on B lymphocytes, in which it functions as an activating factor that stimulates cell proliferation and Ig secretion. IL-10 enhances isotype switching to IgG4 and functions as a growth cofactor for cytotoxic T cells. Thus IL-10 inhibits cytokines associated with cellular immunity and allergic inflammation while stimulating humoral and cytotoxic immune responses. TNF- α and other cytokines stimulate IL-10 secretion, suggesting a homeostatic mechanism whereby an inflammatory stimulus induces TNF- α secretion, which in turn stimulates IL-10 secretion, which feeds back to terminate TNF- α synthesis.

IL-19 is a member of the IL-10 family, and its expression by monocytes can be induced by LPS and GM-CSF. IL-20, another recently described member of the IL-10 family, is predominantly expressed by keratinocytes in the skin and is overexpressed in psoriasis.⁴⁶ An additional new member of the IL-10 family IL-22 is derived from T lymphocytes and mast cells, and its expression is induced by IL-9 and LPS. The predominant biological activity described for IL-22 is induction of acute phase responses. Finally, IL-24 is the fourth new member of the IL-10 family and is produced by murine T_H2 lymphocytes in an IL-4-inducible fashion. Similar to IL-19, IL-20, and IL-22, it does not inhibit cytokine production by mononuclear cells, an activity that remains unique for IL-10.

T-HELPER LYMPHOCYTE FAMILIES

Subclasses of T-helper lymphocytes can be identified on the basis of their repertoire of cytokines⁴⁷ (Table II). Naive T_H0 lymphocytes produce primarily IL-2 but may also synthesize cytokines characteristic of both T_H1 and T_H2 lymphocytes. In human beings, T_H1 lymphocytes produce interferon- γ and TNF- β but not IL-4 and IL-5.

Type 2 helper lymphocytes produce IL-4, IL-5, IL-9, and IL-25 but not IFN- γ or TNF- β . Both classes produce GM-CSF, TNF- α , IL-2, IL-3, IL-10, and IL-13. Although distinct T_H1/T_H2 cytokine profiles may not be apparent in human cells, there remains an inverse relation between the tendency of T lymphocytes to produce IFN- γ as opposed to IL-4 or IL-5. Type 1 helper lymphocytes activate T cells and monocytes and promote cell-mediated immune responses and are important in antibody-dependent immunity. Type 2 T-helper lymphocytes produce IL-4, IL-5, and IL-13 and function in the relative absence of IFN- γ to induce allergic immune responses. T_H3 lymphocytes produce the immunosuppressive cytokines TGF- β and IL-10 and may be important in actively suppressing or terminating immune responses.⁴⁸

One of the more important questions in understanding the cause of allergic disorders is to determine the basis for T_H1/T_H2 lymphocyte differentiation in response to allergen. One of the most critical elements in determining T-helper differentiation is the cytokine milieu in which the T lymphocyte is activated. The major determinant of T_H2 lymphocyte differentiation is the cytokine IL-4.⁴⁹ The original source of the IL-4 responsible for T_H2 lymphocyte differentiation is unclear but is likely to be provided by the naïve T_H0 lymphocytes themselves. Mast cells and natural T lymphocytes may also have a role in occasional circumstances. The result, however, is that in a milieu in which allergic inflammation is present (eg, bronchial lymphatics), more and more extensive allergenic responses against bystander antigens are expected to develop. T_H1 lymphocyte differentiation is mediated by IL-12, IL-18, and IL-23.⁵⁰ Insofar as mononuclear phagocytes are the major source of IL-12, this suggests a mechanism whereby antigens more likely to be processed by macrophages, including bacterial antigens and intracellular parasites, produce T_H1 lymphocyte responses. Like IL-12, IL-18 also induces the differentiation of T_H1 lymphocytes, and IL-18 is a growth factor for these cells. IL-23 is a heterodimer that uses one component of IL-12 and, similarly, uses the IL-12R β 1 chain for its receptor. It is a potent inducer of IFN- γ and is therefore likely to also contribute to T_H1 lymphocyte differentiation.

SIGNAL TRANSDUCTION BY CYTOKINE RECEPTORS

Cytokine receptors generally do not have cytoplasmic domains with intrinsic tyrosine kinase activity; however, cytokine receptors do activate cytoplasmic tyrosine kinases. These processes are displayed in Figure 2, with IL-4 and IL-12 used as models. The first step in the activation of cytokine receptors is ligand-induced dimerization, which results in the juxtaposition of two contact surfaces, allowing their stable interaction with cytoplasm-derived tyrosine kinases. Although numerous biochemical cascades are involved in cytokine signaling, this discussion will primarily focus on two novel families of protein tyrosine kinases, termed Janus kinases (JAKs), and the signal

TABLE II. T-helper cell subtypes classified by cytokine production

T-helper lymphocyte family	Cytokines
T _H 0	IL-2
T _H 1	IFN- γ , TNF- β TNF- α , GM-CSF, IL-2, IL-3, IL-10, IL-13
T _H 2	IL-4, IL-5, IL-9, IL-25 TNF- α , GM-CSF, IL-2, IL-3, IL-10, IL-13
Tr1 (T _H 3)	TGF- β , IL-10

transducers and activators of transcription (STATs), which uniquely function in cytokine signaling.^{51,52}

The role for JAK family members in the pathway to gene activation was largely deduced from studies of signal transduction by the IFN receptors. The two chains of the IFN- α receptor bind JAK1 and TYK2, respectively, whereas the two chains of the IFN- γ receptor bind JAK1 and JAK2. The receptors and the JAKs themselves become phosphorylated, and this phosphorylated complex becomes the catalyst for the phosphorylation of cytoplasmic substrates. There are four JAKs: JAK1, JAK2, JAK3, and TYK2, and, as such, receptor signaling is mediated by a surprisingly limited number of highly redundant tyrosine kinases. For example, JAK2 is involved in GM-CSF, G-CSF, IL-6, and IL-3 signaling. JAK1 and JAK3 are tyrosine-phosphorylated in response to IL-2, IL-4, and all the other cytokines whose receptors are members of the γ c family.

Once engagement of a cytokine receptor has led to tyrosine phosphorylation of the receptor and of receptor-associated JAKs, the next step in signal transduction involves the tyrosine phosphorylation of the STATs.^{51,52} After their activation, these proteins migrate to the nucleus, where they bind to specific regulatory sequences in the promoters of cytokine-responsive genes, thereby initiating gene transcription (see Fig 2). As with the JAKs, the function of STATs was originally characterized with studies involving the biochemical events of IFN-induced gene transcription. Ligand binding of IFN- α/β induces the formation of a complex composed of three proteins: either Stat1 α (p91) or Stat1 β (p84), Stat2 (p113), and a non-STAT protein, p48. In contrast, the stimulation of cells with IFN- γ results in the tyrosine phosphorylation of Stat1 by JAK1 and JAK2 but not of Stat2. There are 4 additional members of the STAT family. Stat3, Stat4, and Stat6 were identified as IL-6-, IL-12-, and IL-4-inducible peptides, respectively. Although important in cytokine signaling, Stat5 was originally defined as a prolactin-inducible peptide. Engagement of the IL-4 receptor leads to the activation of JAK1, which in turn phosphorylates Stat6. Stat6 is necessary for IL-4-dependent expression of IL-4R α , ϵ heavy chain, MHC class II, CD23, and mucin⁵³ (Fig 2). An important endogenous inhibitor of Stat6 is referred to as the suppressor of

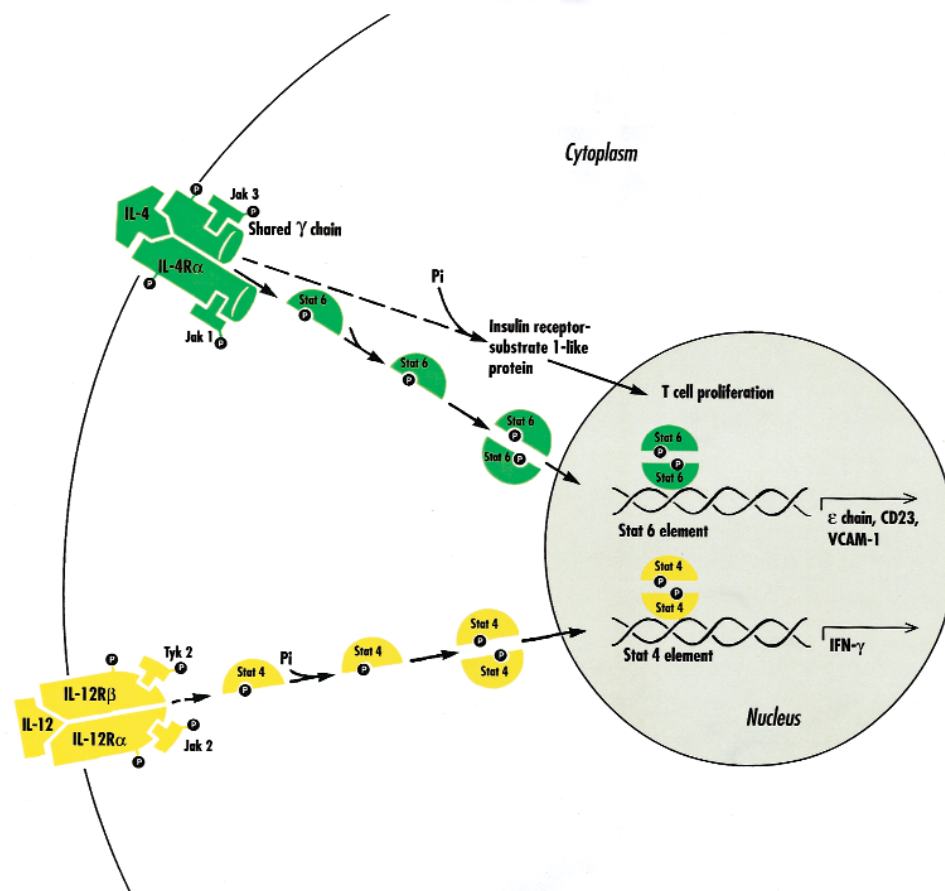


FIG 2. Model of intracellular signaling pathways leading to transcription modulation by IL-4 and IL-12. A novel family of four related cytoplasmic protein tyrosine kinases, termed Janus kinases (JAKs), function in cytokine signaling: JAK1, JAK2, JAK3, and TYK2. On binding of the ligand, the JAKs physically associate with the receptor and mediate tyrosine kinase activity. JAK1 and JAK3 are activated in response to IL-4. JAK1 binds to IL-4R, whereas JAK3 becomes physically linked to the shared γ c. Signaling mediated by IL-12 involves JAK2 and TYK2. Activation of the cytokine receptor leads to tyrosine phosphorylation of the receptor and of receptor-associated JAKs. The next step in signal transduction involves tyrosine phosphorylation of cytoplasmic factors termed signal transducers and activators of transcription (STATs). After activation, these proteins become phosphorylated, form homodimers, and migrate to the nucleus, where they bind to regulatory sequences in the promoters of cytokine-responsive genes. IL-4 signaling is mediated through Stat6. Phosphorylation allows Stat6 to dimerize, and it then undergoes nuclear translocation. In the nucleus, Stat6 mediates the major biological activities of IL-4: ϵ germline and VCAM-1 transcription and T_H2 differentiation. Other signaling pathways, including the activation of the insulin receptor substrates-1 and -2, mediate other biological activities including proliferation and inhibition of apoptosis. Homodimers of phosphorylated Stat4 mediate the biological activities of IL-12, including induction of IFN- γ transcription and differentiation of T_H1 lymphocytes.

cytokine signaling-1 (SOCS-1).⁵⁴ SOCS-1 inhibits IL-4-induced activation of JAK1 and Stat6 and thereby effectively inhibits IL-4 signaling.

Compared with the number of cytokines, relatively few STATs exist, so the signaling pathways of several distinct cytokines share common STAT proteins. For example, epidermal growth factor (EGF), PGDF, M-CSF, IL-6, IL-11, and the interferons all activate Stat1 α . Mechanisms must exist that lead to the distinct responses to different cytokines. In part, these reflect the activities of other signaling pathways stimulated by cytokine receptors. For example, the *Ras*-dependent pathway is also a major signaling pathway activated by members of

the cytokine receptor families. In this cascade, *Ras*, *Raf*-1, Map/Erk kinase kinase (MEKK), and mitogen-activated protein (MAP) kinases are sequentially phosphorylated and activated. The MAP kinase pathway is associated with induction of several transcription factors such as *c-myc*, *c-fos*, and nuclear factor-IL-6. This *ras* pathway is activated by several growth factors as well as by the cytokines IL-2, IL-3, IL-5, and EPO. An example of another distinct pathway used for cytokine signaling is provided by IL-4, which activates the signaling protein insulin response substrate-1 (IRS-1) and its homologue, IRS-2. IRS-1 and IRS-2 regulate cellular proliferation and protection from apoptosis.

CYTOKINES AND THE IMMUNE RESPONSES TO ALLERGENS

Bronchial biopsy specimens of patients with allergic asthma, skin test challenge sites from atopic patients, and the nasal mucosa in allergic rhinitis are all characterized by the presence of T-helper lymphocytes displaying a T_H2-like cytokine profile. However, although there may be a reduced presence of the T_H1 cytokines, allergic inflammatory tissue is also characterized by the presence of IFN- γ , and it is likely that IFN- γ exacerbates allergic inflammation through its ability to activate accessory cell function, stimulate cytokine secretion, induce adhesion molecule expression, and activate eosinophils. The concept that IFN- γ promotes allergic inflammation is supported by data that IFN- γ -producing T_H1 lymphocytes exacerbate murine asthma.⁵⁵

The pattern of cytokine response to allergens observed in nonallergic individuals is even more complex. Normal individuals are exposed to the same concentrations of allergens as their allergic counterparts living in the same environment. Remaining healthy requires active systems that prevent the development of inflammation. It is frequently stated that the immune response to allergens in nonallergic subjects is characterized by T_H1 lymphocyte responses. However, functional T_H1 lymphocyte responses stimulate the recruitment and activation of mononuclear phagocytes and are associated with cellular immunity and granuloma formation, features not present in healthy subjects. If present *in vivo*, these T_H1 lymphocytes must therefore be present in a milieu that prevents cellular inflammation from developing. The absence of inflammation in normal subjects is maintained by influences that promote the development of tolerance. Immune responses to allergens do develop in nonatopic subjects, but these responses are generally of a lower order of magnitude than those observed in allergic subjects. Thus, nonallergic subjects demonstrate decreased allergen-induced T-cell proliferation and lower allergen-specific IgG antibody responses compared with their allergic counterparts.⁵⁶ One influence contributing to immune nonresponsiveness is diminished accessory cell function. In contrast to asthmatic lungs, in the healthy lung, alveolar macrophages and dendritic cells have reduced or absent expression of B7, are unable to present allergen to T-helper lymphocytes, and cannot stimulate cellular activation and proliferation.⁵⁷ The cytokine milieu of the nonasthmatic respiratory tract is characterized by elevated concentrations of IL-10 and TGF- β , which may also help mitigate inflammatory responses.

CHEMOKINES

Chemokines are a group of small (8 to 12 kD) molecules able to induce chemotaxis in a variety of cells including neutrophils, monocytes, lymphocytes, eosinophils, fibroblasts, and keratinocytes. These molecules regulate activity through interactions with members of the 7-transmembrane, G-protein-coupled receptor

superfamily. This chapter will use the new systematic nomenclature with the common names listed in parentheses the first time the chemokine is described.⁵⁸ To date, 47 chemokines and 18 chemokine receptors have been described, which are listed in Table III, along with the known chromosomal location and physiological properties of each. Many of the chemokine receptors can bind more than one ligand, allowing extensive overlap and redundancy of chemokine function.

Although chemotaxis stands as the hallmark feature of chemokines, their physiological role is more complex than originally described, and new functions continue to be identified. Originally, chemokines were described as inflammatory, being produced at the site of infection or in response to a proinflammatory stimulus. The inflammatory chemokines recruit and activate leukocytes to mount an immune response and initiate wound healing. Other chemokines have now been shown to have a homeostatic or housekeeping function. These functions are involved in adaptive immune responses including lymphocyte trafficking, hematopoiesis, antigen sampling in secondary lymphoid tissue, and immune surveillance.⁵⁹ Homeostatic chemokines tend to be expressed in specific tissues or organs, whereas inflammatory chemokines can be produced by many cell types in multiple locations.

CLASSIFICATION OF CHEMOKINES

As a group, the chemokines exhibit between 20% and 50% homology and are characterized by the presence of three to four conserved cysteine residues. They can be subdivided into four families, based on the positioning of the N-terminal cysteine residues (Table III). The C-X-C subfamily is characterized by the separation of the first two cysteines by a variable amino acid. In the C-C subfamily, the cysteine residues are adjacent to each other. The majority of the known chemokines are contained in these two families. In addition, these groups may be distinguished by their primary target cell: the C-X-C subfamily primarily targeting neutrophils and the C-C family targeting monocytes and T cells. A new family of chemokines has been identified that lack the first and third cysteine, containing a single cysteine residue in the conserved position, and are referred to as the "C" subfamily. This subfamily includes the lymphocyte-specific chemotactic peptide XCL1 (lymphotactin). A fourth subfamily (CX3C) has been identified in which the two N-terminal cysteine residues are separated by three variable amino acids. To date, this family only has one member, CX3CL1 (fractalkine), and it is unique in that it is the only membrane-bound chemokine with a mucin-like glycosylated stalk.

RECEPTORS AND SIGNAL TRANSDUCTION

Cell surface receptor number varies from 3000/cell for CCR1 and CCR2 on monocytes and lymphocytes to 40,000 to 50,000 per cell for CCR3 on eosinophils. A given cell can express multiple chemokine receptors; each of which can induce specific signals, suggesting

TABLE III. CC, C, CXC, and CX₃C chemokine/receptor families

Systematic name	Chromosome	Ligand	Chemokine receptor(s)	Physiological features
CC chemokine/receptor family				
CCL1	17q11.2	I-309	CCR8	Inflamm
CCL2	17q11.2	MCP-1/MCAF	CCR2	Inflamm
CCL3	17q11.2	MIP-1 α /LD78 α	CCR1, CCR5	Inflamm
CCL4	17q11.2	MIP-1 β	CCR5	Inflamm
CCL5	17q11.2	RANTES	CCR1, CCR3, CCR5	Inflamm
CCL6	Unknown	Unknown	Unknown	Unknown
CCL7	17q11.2	MCP-3	CCR1, CCR2, CCR3	Inflamm
CCL8	17q11.2	MCP-2	CCR3	Inflamm
CCL9	Unknown	Unknown	Unknown	Unknown
CCL10	Unknown	Unknown	Unknown	Unknown
CCL11	17q11.2	Eotaxin	CCR3	Inflamm
CCL12	Unknown	Unknown	CCR2	Unknown
CCL13	17q11.2	MCP-4	CCR2, CCR3	Inflamm
CCL14	17q11.2	HCC-1	CCR1	Unknown
CCL15	17q11.2	HCC-2/Lkn-1	CCR1, CCR3	Unknown
CCL16	17q11.2	HCC-4/LEC	CCR1	Unknown
CCL17	16q13	TARC	CCR4	Inflamm, Homeo
CCL18	17q11.2	DC-CK1/PARC	Unknown	Homeo
CCL19	9p13	MIP-3 β /ELC	CCR7	Homeo
CCL20	2q33-q37	MIP-3 α /LARC	CCR6	Inflamm, Homeo
CCL21	9p13	6Ckine.SLC	CCR7	Homeo
CCL22	16q13	MDC/STCP-1	CCR4	Inflamm, Homeo
CCL23	17q11.2	MPIF-1	CCR1	Unknown
CCL24	7q11.23	MPIF-2/Eotaxin-2	CCR3	Inflamm
CCL25	19p13.2	TECK	CCR9	Homeo
CCL26	7q11.23	Eotaxin-3	CCR3	Inflamm
CCL27	9p13	CTACK/ILC	CCR10	Homeo
CCL28	5(?)	MEC	CCR10	Inflamm, Homeo
C chemokine/receptor family				
XCL1	1q23	Lymphotactin	XCR1	Unknown
XCL2	1q23	SCM1- α	XCR1	Unknown
CXC chemokine/receptor family				
CXCL1	4q12-q13	GRO α /MGS α - α	CXCR2 > CXCR1	Inflamm
CXCL2	4q12-q13	GRO β /MGS α - β	CXCR2	Inflamm
CXCL3	4q12-q13	GRO γ /MGS α - γ	CXCR2	Inflamm
CXCL4	4q12-q13	PF4	Unknown	Unknown
CXCL5	4q12-q13	ENA-78	CXCR2	Unknown
CXCL6	4q12-q13	GCP-2	CXCR1, CXCR2	Unknown
CXCL7	4q12-q13	NAP-2	CXCR2	Unknown
CXCL8	4q12-q13	IL-8	CXCR1, CXCR2	Inflamm
CXCL9	4q21.21	Mig	CXCR3	Inflamm
CXCL10	4q21.21	IP-10	CXCR3	Inflamm
CXCL11	4q21.21	I-TAC	CXCR3	Inflamm
CXCL12	10q11.1	SDF-1 α / β	CXCR4	Unknown
CXCL13	4q21	BLC/BCA-1	CXCR5	Homeo
CXCL14	5q31	BRAK/bolekine	Unknown	Homeo
CXCL15	Unknown	Unknown	Unknown	Unknown
CXCL16	17p13	Unknown	CXCR6	Inflamm
CX ₃ C chemokine/receptor family				
CX3CL1	16q13	Fractalkine	CX3CR1	Inflamm

This table is an adaptation of the tables presented by Zlotnik et al.⁵⁴ and Moser et al.⁵⁵ The terms *Inflamm* and *Homeo* under the physiological features heading refer to inflammatory chemokines and homeostatic chemokines, respectively. The most common names for the human ligands are listed, but is not all inclusive of ligand names found in the literature.

that each receptor signals through different pathways. The ability to signal through different pathways is due in part to the heptahelical transmembrane property of the receptors. A large surface area, allowing interactions

with the α and $\beta\gamma$ subunits of the heterotrimeric G proteins and other effector molecules, is created by looping of the receptor along the inner plasma membrane and the lateral orientation of the carboxy terminus.⁶⁰

Signaling is initiated after binding of the chemokine to the receptor, which allows the association of guanine triphosphate (GTP) to the $G\alpha$ subunit. This results in dissociation of the heterotrimeric G-protein complex from the receptor and separation into the $G\alpha$ and $G\beta\gamma$ subunits. The $G\alpha$ subunit is able to directly activate the Src family kinases, which then leads to activation of the mitogen-activated protein kinases (MAPKs) and protein kinase B (PKB).⁹ Signaling through the $G\beta\gamma$ subunit is more complex and involves at least three separate pathways. $G\beta\gamma$ can activate PKB and the MAPKs through phosphatidylinositol 3 kinase γ (PI3K γ), PKC through phospholipase C (PLC) and Pyk-2.⁶¹ Activation of PLC induces calcium influx, which activates many cellular processes, including degranulation of neutrophils, eosinophils, and basophils (extensively reviewed by Thelen⁶⁰).

LOCOMOTION

Chemokines were originally described as having a primary role in directing lymphocytes to sites of inflammation. In a process mediated by the selectins, lymphocytes interact transiently with the vascular endothelium while searching for activating signals from chemokines. The selectins mediate low-affinity interactions, which, in combination with the shearing stress of blood flow, produce the process of "rolling adhesion." Upon binding of the chemokine to its receptor on the lymphocyte, integrins are upregulated and firmly adhere the leukocyte to the vessel wall. An example of this has been demonstrated for the chemokines CCL19 (ELC), CCL21 (SLC), and CXCL12 (SDF-1), which can rapidly induce a high-affinity state for the β_2 -integrin LFA-1.⁶² Once the cell has ceased rolling, it will traverse the endothelium. A protrusion known as a lamellipod is formed, which is composed of a dense array of actin fibers. Movement of the body of the cell results from contraction of myosin, which pulls actin filaments from around the cell body. The end result is movement of the main cell body toward the lamellipod.⁶³ As the cell moves forward, the affinity of the adhesion molecules at the rear of the cell decreases. The lymphocyte will continue this process as it migrates along a concentration gradient until it reaches the source of the chemokine. The expression of specific chemokines, receptors, and adhesion molecules contributes to the selective migration and tissue specificity of lymphocytes.

The most important chemoattractant for PMNs, CXCL8 (IL-8), is derived primarily from mononuclear phagocytes, endothelial, and epithelial cells but also from T cells, eosinophils, neutrophils, fibroblasts, keratinocytes, and hepatocytes. CXCL8 synthesis may be induced by lipopolysaccharide, IL-1, TNF, and viruses.⁶⁴ On a molar basis, CXCL8 is one of the most potent chemoattractants for neutrophils. It also stimulates neutrophil degranulation, the respiratory burst, and adherence to endothelial cells by CD11b/CD18. During the inflammatory response, CXCL8 appears relatively late in comparison with other chemoattractants. For example,

leukotriene B₄ (LTB₄) appears within minutes of cellular activation and its concentration peaks at 3 hours. As LTB₄ concentrations decline, the newly synthesized CXCL8 begins to be secreted and persists for at least 24 hours. Other members of the chemokine family, including CCL3 (MIP-1 α), share this capacity to activate PMNs.

Chemokines perform a variety of functions aside from chemotaxis. Chemokines can have direct effects on T-cell differentiation through ligand-receptor interactions on the developing cell or indirectly by altering APC trafficking or cytokine secretion. Functioning through the CCR5 receptor, CCL3 (MIP-1 α), CCL4 (MIP-1 β), and CCL5 (RANTES) can directly promote development of IFN- γ -producing T_H1 lymphocytes or indirectly by increasing IL-12 production from APCs. In contrast, CCL2 (MCP-1), CCL7 (MCP-3), CCL8 (MCP-2), and CCL13 (MCP-4) can inhibit IL-12 production from APCs and enhance IL-4 production from activated T cells, leading to a T_H2 lymphocyte phenotype.⁶⁵ Chemokine receptor expression can serve as a marker for maturation and differentiation of lymphocytes. When monocytes and immature dendritic cells migrate from the vasculature into tissues and begin immune surveillance, they express the inflammatory receptors CCR1, CCR2, CCR5, CCR6, and CXCR2. As antigen is encountered and the dendritic cells mature, the inflammatory receptors are downregulated and replaced by expression of CCR7, which allows the dendritic cells to accumulate in the draining lymphatics and T-cell areas of the lymph nodes. CXCR5 is expressed on a distinct memory T-cell subset that displays B-helper cell function. These cells respond to CXCL13 (BLC) and are directed to the B-cell follicle to support production of antibodies.⁶⁶

CLINICAL RELEVANCE OF CHEMOKINES

This section will focus on the role of chemokines in allergic disorders. The role of chemokines in neoplasia has been covered in recent reviews,^{67,68} and the role of chemokines in HIV infection and development of AIDS will be covered in Chapter 13. Increased levels of the chemokines CCL2 (MCP-1), CCL3, CCL5 (RANTES), CCL7 (MCP-3), CCL11 (eotaxin-1), CCL13 (MCP-4), CCL24 (eotaxin-2), and CXCL8 (IL-8) and CXCL10 (IP-10) have been demonstrated in bronchoalveolar lavage and biopsy samples of asthmatic patients compared with control patients.⁶⁹ In murine models of asthma, CCL2, CCL5, CCL11, and CXCL10 and CXCL12 (SDF-1) contribute to AHR and cellular emigration in a nonredundant fashion.

Because of their ability to recruit eosinophils, T cells, and monocytes to regions of inflammation, the C-C chemokine family has been extensively studied in allergic diseases. Unlike other eosinophil chemoattractants such as LTB₄, platelet-activating factor, and C5a, these chemokines are more selective for eosinophils. CCL5 (RANTES) and CCL11 (eotaxin) acting in synergy with IL-5 are the most important eosinophil chemoattractants in allergic inflammation.⁷⁰ Injection with CCL5 or

TABLE IV. Cytokines and chemokines in allergy and asthma

	Cytokine/chemokine	Activity
IgE regulation	IL-4, IL-13	ε Isotype switch
	IL-25	Enhanced production of IL-4 and IL-13
	IL-9	Synergizes with IL-4 and IL-13
	IFN-γ, TGF-β	Inhibition of IL-4 and IL-13
	IL-4	Generation of IL-4–producing (T _H 2-like) T lymphocytes
	CCL3, CCL4, CCL5	Recruitment of T _H 1-like cells
	IL-12, IL-18, IL-23	Stimulation of IFN-γ–producing (T _H 1-like) T lymphocytes
	CCL2, CCL7, CCL8, CCL13, CCL8, CCL13	Recruitment of T _H 2-like cells
IgA regulation	TGF-β	α Isotype switch
Eosinophilia	IL-5	Eosinophilopoietin
	IL-25	Enhanced production of IL-5
	IL-5, IL-3, GM-CSF, IL-4, TNF-α, IFN-γ	Inhibition of apoptosis
	IL-5, IL-3, GM-CSF, CCL3, CCL5, CCL7, CCL11, IL-1, TNF-α, IFN-γ	Eosinophil chemotaxis, degranulation, and activation
	CCL2, CCL3, CCL5, CCL7	Basophil chemotaxis and histamine release
Basophil activation	Stem cell factor	Mast cell growth and differentiation
Mast cell development	IL-3, IL-4, IL-9, IL-10, IL-11, nerve growth factor	Cofactors for mast cell growth
	Stem cell factor	Mast cell histamine release
	IL-1, IL-4, IL-13, TNF-α	Induction of VCAM-1
Adhesion molecule expression	IL-1, TNF-α, IFN-γ	Induction of ICAM-1
	IL-1, TNF-α	Induction of E-Selectin
	CCL19, CCL21, CXCL12	Upregulation of LFA-1
Airway hyperresponsiveness	IL-4, IL-5, IL-9, IL-13, CCL2, CCL5, CXCL10	
Airway fibrosis and remodeling	IL-4, IL-6, IL-9, IL-11, IL-13, IL-17, TGF-α, TGF-β, PDGF, β-FGF	Promote fibroblast proliferation and collagen deposition; subepithelial fibrosis
	IL-4, IL-9, IL-13	Smooth muscle hyperplasia

CCL11 results in an eosinophilic and mononuclear infiltrate in the absence of neutrophils. Aside from its production by eosinophils, macrophages, mast cells, and T cells, CCL11 production has been described in structural cells of the airway including airway smooth muscle and fibroblasts. CCL17 (TARC) is expressed by nasal epithelial cells, and that expression is higher in patients with allergic rhinitis compared with control patients. IL-4 and IL-13 stimulate CCL17 expression, thereby promoting a T_H2 response.⁷¹ It is tempting to speculate that CCL17 expression in the asthmatic lung could help drive T_H2 responses. CCL13 (MCP-4) may contribute to the allergic response by inducing histamine release from IL-3–primed basophils. These studies suggest that the priming of the lung for an inflammatory response may be beneficial in the clearance of viral or bacterial infections, but this is detrimental when dealing with nonspecific stimuli, as observed in the allergic response.

CHEMOKINE ANTAGONISTS AS A THERAPEUTIC OPTION

Several compounds have been developed that antagonize chemokine receptor function, some of which are currently undergoing clinical trials. A nonpeptide CCR1 antagonist has been identified (BX471) that is able to displace CCL3 (MIP-1α), CCL5 (RANTES), and CCL7

(MCP-3) from CCR1. Other properties include the ability to inhibit Ca²⁺ mobilization, CD11b expression, and leukocyte migration. In animal trials, BK471 was able to reduce inflammation in allergic encephalomyelitis.⁷² Instillation of CCL2 (MCP-1) in the lungs of mice induced prolonged AHR associated with mast cell degranulation. Neutralization of CCL2 blocked the development of AHR in response to antigen. Several potential antagonists to CCL2 or its receptor CCR2 are currently being developed. One of these is an indolopiperidine derivative that is able to selectively inhibit CCR2 without inhibiting the closely related CCR5 receptor.⁷³ Thiazolidinedione (TZD) is a compound currently being used to improve the insulin resistance of individuals with diabetes mellitus. Studies using human lung epithelial cell lines have demonstrated that TZD inhibited the IL-1β– and TNF-α–induced expression of CCL2. In addition, the CCL2-induced chemotaxis of monocytes was inhibited by TZD.⁷⁴ Many of the chemokines that have been implicated in the asthmatic response, including CCL5, CCL11 (eotaxin), CCL13 (MCP-4), CCL24 (eotaxin-2), and CCL26 (eotaxin-3), function through the CCR3 receptor. Through the use of a mouse model, a neutralizing antibody to CCL11 reduced eosinophil recruitment into the lung after allergen challenge and reduced associated AHR. Both a nonpeptide antagonist of CCR3, the CCL11 receptor (SB-328437), and an

amino piperidine derivative of CCL11 (UCB-35625) also block eosinophil recruitment in allergen models of asthma and are currently undergoing clinical trials.⁷⁵ Another compound that may prove beneficial is the CCR3 receptor antagonist F-1322. F-1322 also inhibits thromboxane A₂ synthase and 5-lipoxygenase and is a histamine antagonist. In vitro, F-1322 inhibited CCL11-induced chemotaxis and actin polymerization of eosinophils, and in vivo it displayed dose-dependent suppression of eosinophil migration into airways in response to IL-5 and CCL11 infusion in guinea pigs.⁷⁶ CCR4 is expressed on T_H2 cells and can be activated by CCL17 (TARC) and CCL22 (MDC). To date, no antagonists have been reported for CCR4, but it is tempting to speculate that inhibition of CCR4 would prevent recruitment of T_H2 cells to the lung. Finally, in human beings, the predominate chemokine receptor used for neutrophil chemotaxis is CXCR2. A nonpeptide antagonist (SB 225002) is capable of inhibiting CCL8-induced neutrophil migration.⁷⁷

Although the current focus has been to develop antagonists for specific receptors, the pleiotropy of the chemokines and their receptors may necessitate the use of multiple antagonists targeting multiple receptors, in order to achieve full inhibition of function.

SUMMARY

Cytokines and chemokines important in the pathophysiology of allergic disorders are summarized in Table IV. The IgE isotype switch results from the activities of IL-4 and IL-13 and is inhibited by IFN- γ and TGF- β . IL-2, IL-5, IL-6, and IL-9 synergize with IL-4 and IL-13 to enhance IgE secretion. IL-4 is responsible for the differentiation of IL-4-producing lymphocytes and recruitment of these T_H2-like cells is promoted by CCL2 (MCP-1). IL-12, IL-18, and IL-23 inhibit the differentiation of IL-4-producing T cells, and recruitment of these T_H1-like cells is mediated by CCL5 (RANTES). IL-5 is the most important eosinophilopoietin and with GM-CSF and IL-3 prolongs the survival of mature eosinophils and activates them. These three cytokines, along with TNF and the interferons, are responsible for generating the activated eosinophils that characterize the asthmatic state. Eosinophilia may also result from selective recruitment by the eosinophil chemotaxins CCL3 (MIP-1 α), CCL5, and CCL11 (eotaxin). Mast cell differentiation and proliferation results from the activity of SCF and other cytokines, including IL-3, IL-6, IL-9, IL-10, IL-11, and nerve growth factor to promote mast cell production. Stem cell factor is an important mast cell histamine-releasing factor; whereas CCL2, CCL3, CCL5, and CCL7 (MCP-3) are all basophil histamine-releasing factors. Many cytokines contribute to the inflammatory state of allergic inflammatory disorders. IL-1, TNF, and IFN- γ increase the expression of endothelial cell adhesion molecules such as ICAM-1 and support the egress of mononuclear cells, neutrophils, and eosinophils into the lungs. Induction of VCAM-1 by IL-4 and IL-13 may promote the selective recruitment of eosinophils,

basophils, and lymphocytes. Many cytokines and chemokines may then contribute to the activation of these leukocytes once they reach the airways. Cytokines important in promoting fibrosis and airway remodeling are IL-4, IL-6, IL-11, IL-13, IL-17, and TGF- β .

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