Chapter 2
Role of Cytokines

Introduction

Cytokines are small glycoproteins produced by a number of cell types, predominantly leukocytes, that regulate immunity, inflammation and hematopoiesis. They regulate a number of physiological and pathological functions including innate immunity, acquired immunity and a plethora of inflammatory responses. The discovery of cytokines was initiated in the 1950s, but the precise identification of their structure and function took many years. The original discoveries were those of IL-I, IFN and nerve growth factors (NGFs); however, these cytokines were purified and given their names years later. Elucidation of the precise physiological, pathological and pharmacological effects of some of the cytokines is still in progress. The modern techniques of molecular biology were principally responsible for their complete identification and as a consequence, several hundred cytokine proteins and genes have been identified, and the process still continues.

Cytokines are produced from various sources during the effector phases of natural and acquired immune responses and regulate immune and inflammatory responses. They are also secreted during nonimmune events and play a role unrelated to the immune response in many tissues. Generally, their secretion is a brief, self-limited event. They not only are produced by multiple diverse cell types, but also act upon many different cell types and tissues. Cytokines often have multiple effects on the same target cell and may induce or inhibit the synthesis and effects of other cytokines. After binding to specific receptors on the cell surface of the target cells, cytokines produce their specific effects. Multiple signals regulate the expression of cytokine receptors. The target cells respond to cytokines by new mRNA and protein synthesis, which results in a specific biological response.

Interleukin-1

Interleukin-1 was originally discovered as a factor that induced fever, caused damage to joints and regulated bone marrow cells and lymphocytes, it was given several different names by various investigators. Later, the presence of two distinct proteins, IL-1α and IL-1β, was confirmed, which belong to a family of cytokines, the

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IL-1 superfamily. Ten ligands of IL-1 have been identified, termed IL-1F1 to IL-1F10. With the exception of IL-1F4, all of their genes map to the region of chromosome 2. IL-1 plays an important role in both innate and adaptive immunity and is a crucial mediator of the host inflammatory response in natural immunity. The major cell source of IL-1 is the activated mononuclear phagocyte. Other sources include dendritic cells, epithelial cells, endothelial cells, B cells, astrocytes, fibroblasts and Large granular lymphocytes (LGL). Endotoxins, macrophage-derived cytokines such as TNF or IL-1 itself, and contact with $CD4^+$ cells trigger IL-1 production. IL-1 can be found in circulation following Gram-negative bacterial sepsis. It produces the acute-phase response in response to infection. IL-1 induces fever as a result of bacterial and viral infections. It suppresses the appetite and induces muscle proteolysis, which may cause severe muscle "wasting" in patients with chronic infection. IL-1β causes the destruction of β cells leading to type 1 diabetes mellitus. It inhibits the function and promotes the apoptosis of pancreatic β cells. Activation of T-helper cells, resulting in IL-2 secretion, and B-cell activation are mediated by IL-1. It is a stimulator of fibroblast proliferation, which causes wound healing. Autoimmune diseases exhibit increased IL-1 concentrations. It suppresses further IL-1 production via an increase in the synthesis of PGE_2.

IL-1s exert their effects via specific cell surface receptors that include a family of about nine members characterized as IL-1R1 to IL-1R9. All family members with the exception of IL-1R2 have an intracellular TLR domain. Each type of receptor in the family has some common and some unique features. The ligands (Table 2.1) for all of these receptors have not yet been identified.

Kineret (Anakinra)

Kineret is a human IL-1 receptor antagonist and is produced by recombinant DNA technology. It is nonglycosylated and is made up of 153 amino acids. With the exception of an additional methionine residue, it is similar to native human IL-1Ra. Human IL-1Ra is a naturally occurring IL-1 receptor antagonist, a 17-kDa protein, which competes with IL-1 for receptor binding and blocks the activity of IL-1.

Table 2.1 IL-1 Ligands and Their Receptors

Ligand Name	Receptor Name
IL-1F1	IL-1R1, IL-1R2
IL-1F2	IL-1R1, IL-1R2
IL-1F3	IL-1R1, IL-1R2
IL-1F4	IL-1R5
IL-1F5	IL-1R6
IL-1F6	Unknown
IL-1F7	IL-1R5
IL-1F8	Unknown
IL-1F9	IL-1R6
IL-1F10	IL-1R1

Kineret is recommended for the treatment of severely active rheumatoid arthritis for patients 18 years of age or older. It is recommended for patients who have not responded well previously to the disease-modifying antirheumatic drugs. It reduces inflammation, decreases bone and cartilage damage and attacks active rheumatoid arthritis. The drug can be used alone or in combination with other antirheumatic drugs. However, it is not administered in combination with TNF-α antagonists. Kineret also improves glycemia and β-cell secretory function in type 2 diabetes mellitus. It is administered daily at a dose of 100 mg/day by subcutaneous injection.

The most serious side effects of Kineret are infections and neutropenia. Injection site reactions are also common. Other side effects may include headache, nausea, diarrhea, flu-like symptoms and abdominal pain. The increased risk of malignancies has also been observed.

Interleukin-2

IL-2, a single polypeptide chain of 133 amino acid residues, is produced by immune regulatory cells that are principally T cells. When a helper T cell binds to an APC using CD28 and B7, $CD4^+$ cells produce IL-2. IL-2 supports the proliferation and differentiation of any cell that has high-affinity IL-2 receptors. It is necessary for the activation of T cells. Resting T lymphocytes (unstimulated) belonging to either the $CD4^+$ or the $CD8^+$ subsets possess few high-affinity IL-2 receptors, but following stimulation with specific antigen, there is a substantial increase in their numbers. The binding of IL-2 with its receptors on T cells induces their proliferation and differentiation.

IL-2 is the major growth factor for T lymphocytes, and the binding of IL-2 to its specific receptors on TH cells stimulates the proliferation of these cells and the release of a number of cytokines from these cells. IL-2 is required for the generation of $CD8^+$ cytolytic T cells, which are important in antiviral responses. It increases the effector function of NK cells. When peripheral blood lymphocytes are treated with IL-2 for 48–72 h, lymphokine-activated killer (LAK) cells are generated, which can kill a much wider range of targets including the tumor cells. IL-2 enhances the ability of the immune system to kill tumor cells and may also interfere with the blood flow to the tumors. It not only induces lymphoid growth but also maintains peripheral tolerance by generation of regulatory T cells. IL-2 knockout mice produce a wide range of autoantibodies and many die of autoimmune hemolytic anemia, which suggests that it plays a role in immune tolerance.

Interleukin-2 Receptors

The IL-2 receptor occurs in three forms with different affinities for IL-2; the three distinct subunits are the α, β and γ chains. The monomeric IL-2Rα possesses low affinity, the dimeric IL-2Rβγ has intermediate affinity and the trimeric IL-2Rαβγ has high affinity (Table 2.2). The α chain is not expressed on resting T cells but

Table 2.2 IL-2 Receptors

	Low Affinity	Intermediate Affinity	High Affinity
Affinity constant (M)	10^{-8}	10^{-7}	10^{-11}
Dissociation constant (M)	10^{-8}	10^{-9}	10^{-11}
Subunits	IL-2Rα	IL-2Rβγ	IL-2Rαβγ

only on activated T cells and is also called TAC (T cell activation) receptor. Both β and γ chains are required for the signal transduction mediated via IL-2 receptors. The low-affinity and high-affinity IL-2 receptors are expressed by activated $CD4^+$ and $CD8^+$ T cells and in low numbers on activated B cells. The intermediate-affinity IL-2 receptors are expressed on NK cells and in low numbers on resting T cells.

When IL-2 binds to high-affinity receptors, it becomes internalized following receptor-mediated endocytosis. After high-affinity binding, there is an increase in the stimulation of phosphoinositol turnover, redistribution of protein kinase C from the cytoplasm to the cell membrane, and an increased expression of IL-2 receptors, with low-affinity receptors being preferentially increased.

Clinical Uses of Interleukin-2

Immunotherapy for Cancer

Proleukin (Aldesleukin)

Proleukin is a recombinant human IL-2 that received approval for the treatment of renal cell carcinoma in 1992 and for the treatment of metastatic melanoma in 1998. It is also being evaluated for the treatment of non-Hodgkin's lymphoma (NHL). The therapy is restricted to patients with normal cardiac and pulmonary functions.

The treatment generally consists of two treatment cycles, each lasting for 5 days and separated by a rest period. Every 8 h a dose of 600,000 IU/kg (0.037 mg/kg) is administered. The IV infusion period is 15 min and a maximum of 14 doses are administered. After a rest period of 9 days, another 14 doses are administered. Additional treatment can be given following an evaluation after 4 weeks.

The most frequent adverse reactions associated with the administration of proleukin include fever, chills, fatigue, malaise, nausea and vomiting. It has also been associated with capillary leak syndrome (CLS). CLS is defined as a loss of vascular tone and effusion of plasma proteins and fluids into the extravascular space. This leads to hypotension and decreased organ perfusion, which may cause sudden death. Other side effects include anaphylaxis, injection site necrosis and possible autoimmune and inflammatory disorders.

Lymphokine-Activated Killer Cell Therapy

IL-2 has been tested for antitumor effects in cancer patients as part of LAK therapy. LAK cell therapy involves infusion into cancer patients of their own (autologous)

lymphocytes after they have been treated in vitro with IL-2 for a minimum of 48 h to generate LAK cells. IL-2 needs to be administered with LAK cells in doses ranging from 10^3 to 10^6 U/m^2 body area or from 10^4 to 10^5 U/kg body weight.

Interleukin-2 and AIDS

HIV is a retrovirus that infects $CD4^+$ cells. After HIV becomes integrated into the genome of the $CD4^+$ cells, activation of these cells results in the replication of virus, which causes lysis of the host cells. Patients infected with HIV, and with AIDS, generally have reduced numbers of helper T cells and the CD4:CD8 ratio may be as low as 0.5:1 instead of the normal 2:1. As a consequence, very little IL-2 is available to support the growth and proliferation of $CD4^+$ cells despite the presence of effector cells, B cells and cytolytic T cells.

Proleukin has not been approved for the treatment of HIV; however, studies show that proleukin in combination with antiretroviral therapy significantly increases the number of $CD4^+$ cells. Low-frequency doses of subcutaneous proleukin at maintained intervals increased $CD4^+$ cell levels. The CD4 count increased from 520 cells/μl to 1005 cells/μl, and the mean of $CD4^+$ cells present from 27 to 38%. The overall effects of proleukin administration in combination with other anti-HIV drugs are being studied to determine the regulation of immune response as well as a delay in the progression of HIV disease.

Interleukin-4

IL-4 is a pleiotropic cytokine produced by TH_2 cells, mast cells and NK cells. Other specialized subsets of T cells, basophils and eosinophils also produce IL-4. It regulates the differentiation of antigen-activated naïve T cells. These cells then develop to produce IL-4 and a number of other TH_2-type cytokines including IL-5, IL-10 and IL-13. IL-4 suppresses the production of TH_1 cells. It is required for the production of IgE and is the principal cytokine that causes isotype switching of B cells from IgG expression to IgE and IgG4. As a consequence, it regulates allergic disease. IL-4 leads to a protective immunity against helminths and other extracellular parasites. The expression of MHC class II molecules on B cells and the expression of IL-4 receptors are upregulated by IL-4. In combination with TNF, IL-4 increases the expression of VCAM-1 and decreases the expression of E-selectin, which results in eosinophil recruitment in lung inflammation.

IL-4 mediates its effects via specific IL-4 receptors that are expressed on a number of tissues including hematopoietic cells, endothelium, hepatocytes, epithelial cells, fibroblasts, neurons and muscles. The receptor is composed of an α chain, which is the high-affinity receptor, but its signaling requires a second chain, a γ chain (γC), which is also a component of IL-2 receptors. However, the presence of a γ chain does not significantly increase the affinity of the receptor complex for IL-4. IL-4 causes the heterodimerization of the α chain with the γ chain, resulting in IL-4 receptor-dependent signaling pathway. As is the case with other cytokines,

the signaling pathways activated after the binding of IL-4 to its receptors are insulin receptor substrate (IRS-1/2) and Janus family tyrosine kinases–signal transducers and activators of transcription (JAK–STAT) pathways. However, for IL-4, the specificity results from the activation of STAT-6.

The antibodies to IL-4 inhibit allergen-induced airway hyperresponsiveness (AHR), globlet cell metaplasia and pulmonary eosinophilia in animal models. Inhibition of IL-4 by soluble IL-4 receptor (SIL-4R, Nuvance) has proven to be very promising in treating asthma. Clinical trials with recombinant SIL-4R administered by a single weekly dose of 3 mg via nebulization have been effective in controlling the symptoms of moderate persistent asthma.

Interleukin-5

IL-5 is secreted predominantly by TH_2 lymphocytes. However, it can also be found in mast cells and eosinophils. It regulates the growth, differentiation, activation and survival of eosinophils. IL-5 contributes to eosinophil migration, tissue localization and function, and blocks their apoptosis. Eosinophils play a seminal role in the pathogenesis of allergic disease and asthma and in the defense against helminths and arthropods. The proliferation and differentiation of antigen-induced B lymphocytes and the production of IgA are also stimulated by IL-5. TH_2 cytokines IL-4 and IL-5 play a central role in the induction of airway eosinophilia and AHR. It is a main player in inducing and sustaining the eosinophilic airway inflammation.

IL-5 mediates its biological effects after binding to IL-5R, which is a membrane-bound receptor. The receptor is composed of two chains, a ligand-specific α receptor (IL-5Rα) and a shared β receptor (IL-5Rβ). The β chain is also shared by IL-3 and GM-CSF, resulting in overlapping biological activity for these cytokines. The signaling through IL-5R requires receptor-associated kinases. Two different signaling cascades associated with IL-5R include JAK/STAT and Ras/mitogen-activated protein kinase (MAPK) pathways.

IL-5 is usually not present in high levels in humans. However, in a number of disease states where the number of eosinophils is elevated, high levels of IL-5 and its mRNA can be found in the circulation, tissue and bone marrow. These conditions include the diseases of the respiratory tract, hematopoietic system, gut and skin. Some other examples include food and drug allergies, atopic dermatitis, aspirin sensitivity and allergic or nonallergic respiratory diseases.

Another way of interfering with IL-5 or IL-5R synthesis is by the use of antisense oligonucleotides. Antisense oligonucleotides are short synthetic DNA sequences that can hybridize specifically to the mRNA of the cytokine or its receptors. This will result in the inhibition of the transcription and processing of mRNA. The administration of IL-5-specific antisense oligonucleotides results in reduced lung eosinophilia in animal models. However, there is no complete inhibition of antigen-specific late-phase AHR, suggesting that in addition to IL-5, other pathways may also be involved in airway hyperreactivity.

Interleukin-6

IL-6 is a proinflammatory cytokine, which is a member of the family of cytokines termed "the IL-6 type cytokines." The cytokine affects various processes including the immune response, reproduction, bone metabolism and aging. IL-6 is synthesized by mononuclear phagocytes, vascular endothelial cells, fibroblasts and other cells in response to trauma, burns, tissue damage, inflammation, IL-1 and, to a lesser extent, TNF-α. Pathogen-associated molecular patterns (PAMPs) binding to the TLRs present on macrophage result in the release of IL-6. This cytokine is synthesized by some activated T cells as well. It is also secreted by osteoblasts to stimulate osteoclast formation. Acute-phase response and fever are caused by IL-6, which is also the case for IL-1 and TNF-α. It affects differentiation of B cells and causes neutrophil mobilization. IL-6 is elevated in patients with retroviral infection, autoimmune diseases and certain types of benign or malignant tumors. It stimulates energy mobilization in the muscle and fatty tissue, resulting in an increase in body temperature. IL-6 acts as a myokine — a cytokine produced by muscles — and muscle contraction occurs as a result of elevated IL-6 concentrations. The expression of IL-6 is regulated by various factors, including steroidal hormones, which could be at both transcriptional and posttranscriptional levels. IL-6 mediates its effects via binding to cell surface receptors, IL-6R, which are active in both membrane-bound and soluble forms.

Interleukin-9

Originally described as a mast cell growth factor due to its ability to promote the survival of primary mast cells and as an inducer of IL-6 production, IL-9, which is secreted by TH_2 cells, stimulates the release of a number of mediators of mast cells and promotes the expression of the high-affinity IgE receptors (FcεR1α). IL-9 augments TH_2-induced inflammation and enhances mucus hypersecretion and the expression of its receptors is increased in asthmatic airways. It also promotes eosinophil maturation in synergy with IL-5. IL-9 activates airway epithelial cells by stimulating the production of several chemokines, proteases, mucin genes and ion channels. It is important to point out that, as opposed to the IL-4-induced isotype switching and production of IgE or the IL-5-mediated stimulation of eosinophil maturation, IL-9 induces actions of other cytokines. It is an essential cytokine for asthmatic disease as biopsies from asthmatic patients show an increase in the expression of IL-9 compared to healthy individuals, and therefore it is an important therapeutic target for clinical intervention.

Interleukin-10

First identified as an inhibitor of IFN-γ synthesis in TH_1 cells, IL-10 is an important immunoregulatory cytokine. It is an anti-inflammatory cytokine that was first called

human cytokine synthesis inhibitory factor. IL-10 is secreted by macrophages, TH_2 cells and mast cells. Cytotoxic T cells also release IL-10 to inhibit viral infection-stimulated NK cell activity. IL-10 is a 36-kDa dimer composed of two 160-amino-acid-residue-long chains. Its gene is located on chromosome 1 in humans and consists of five exons. IL-10 inhibits the synthesis of a number of cytokines involved in the inflammatory process including IL-2, IL-3, GM-CSF, TNF-α and IFN-γ. Based on its cytokine-suppressing profile, it also functions as an inhibitor of TH_1 cells and by virtue of inhibiting macrophages, it functions as an inhibitor of antigen presentation. Interestingly, IL-10 can promote the activity of mast cells, B cells and certain T cells.

There are several viral IL-10 homologs: Epstein–Barr virus (BCRF.1), cytomegalovirus, herpesvirus type 2, orf virus and Yaba-like disease virus. Now the IL-10 family of cytokines includes not only IL-10 but also its viral gene homologs and several other cytokines including IL-19, IL-20, IL-22, IL-24, IL-26, IFN-λ1, IFN-λ2 and IFN-λ3. IL-10 mediates its effects after binding to two receptor chains, IL-10R1 (α) and IL-10R2 (β). These receptors are members of the class II or IFN receptor family. The interaction of IL-10 with its receptors is highly complex and the IL-10R2 (β) chain is essential for the production of its effects. Several hundred genes are activated after interaction of IL-10 with its receptors. The tyrosine kinases JAK1 and Tyk2 are activated by the interaction of IL-10 with its receptors, which results in the induction of transcription factors STAT1, STAT3 and STAT5, and eventual gene activation.

The major immunobiological effect of IL-10 is the regulation of the TH_1/TH_2 balance. TH_1 cells are involved in cytotoxic T-cell responses whereas TH_2 cells regulate B-cell activity and function. IL-10 is a promoter of TH_2 response by inhibiting IFN-γ production from TH_1 cells. This effect is mediated via the suppression of IL-12 synthesis in accessory cells. IL-10 is involved in assisting against intestinal parasitic infection, local mucosal infection by costimulating the proliferation and differentiation of B cells. Its indirect effects also include the neutralization of bacterial toxins.

IL-10 is a potent inhibitor of IL-1, IL-6, IL-10 itself, IL-12, IL-18, CSF and TNF. It not only inhibits the production of proinflammatory mediators but also augments the production of anti-inflammatory factors including soluble TNF-α receptors and IL-1RA. IL-10 downregulates the expression of MHC class II molecules (both constitutive and IFN-γ-induced), as well as that of costimulatory molecule, CD86, and adhesion molecule, CD58. It is an inhibitor of IL-12 production from monocytes, which is required for the production of specific cellular defense response. IL-10 enhances the expression of CD16, CD32 and CD64 and augments the phagocytic activity of macrophages. The scavenger receptors, CD14 and CD163, are also upregulated on macrophages by IL-10. It is a stimulator of NK cells, enhances their cytotoxic activity, and also augments the ability of IL-18 to stimulate NK cells. Based on its immunoregulatory function, IL-10 and ligands for its receptors are tempting candidates for therapeutic intervention in a wide variety of disease states, including autoimmune disorders, acute and chronic inflammatory diseases, cancer, infectious disease, psoriasis and allergic disease.

Modest but significant improvement has been observed in patients with chronic hepatitis C, Crohn's disease, psoriasis and rheumatoid arthritis after subcutaneous administration of IL-10 in human clinical trials. The systemic administration of IL-10 produces general immune suppression, inhibition of macrophage and T-cell infiltration, less secretion of IL-12 and TNF-α by monocytes and suppression of nuclear factor (NF)-κB induction. In patients with acute myelogenous leukemia, IL-10 increases the serum levels of TNF-α and IL-1β. The use of IL-10 for human cancer therapy is under investigation and despite its immunosuppressive effects it may serve a role as a facilitator in preconditioning tumors to be recognized by immune effector cells.

Interleukin-11

IL-11, a member of the IL-6 superfamily, is produced by bone marrow stroma and activates B cells, plasmacytomas, hepatocytes and megakaryocytes. The gene for IL-11 is located on chromosome 19. IL-11 induces acute-phase proteins, plays a role in bone cell proliferation and differentiation, increases platelet levels after chemotherapy and modulates antigen–antibody response. It promotes differentiation of progenitor B cells and megakaryocytes. The recovery of neutrophils is accelerated by IL-11 after myelosuppressive therapy. IL-11 also possesses potent anti-inflammatory effects due to its ability to inhibit nuclear translocation of NF-κB. Additional biological effects of this cytokine include epithelial cell growth, osteoclastogenesis and inhibition of adipogenesis. The effects of IL-11 are mainly mediated via the IL-11 receptor α chain. IL-11 forms a high-affinity complex in association with its receptor and associated proteins and induces gp130-dependent signaling.

Oprelvekin (Neumega)

Recombinant human IL-11 (oprelvekin) is a polypeptide of 177 amino acids. It differs from natural IL-11 due to lack of glycosylation and the amino-terminal proline residue. Oprelvekin is administered by subcutaneous injection, usually 6–24 h after chemotherapy, at a dose of 25–50 μg/kg per day. The drug has a half-life of about 7 h. It is used to stimulate bone marrow to induce platelet production in nonmyeloid malignancies in patients undergoing chemotherapy. The common side effects of oprelvekin include fluid retention, tachycardia, edema, nausea, vomiting, diarrhea, shortness of breath and mouth sores. Other side effects include rash at the injection site, blurred vision, paresthesias, headache, fever, cough and bone pain. Rarely, CLS may occur.

Interleukin-13

IL-13 belongs to the same α-helix superfamily as IL-4, and their genes are located 12 kb apart on chromosome 5q31. It was originally identified for its effects on B

cells and monocytes, which included isotype switching from IgG to IgE, inhibition of inflammatory cytokines and enhancement of MHC class II expression. Initially, IL-13 appeared similar to IL-4 until its unique effector functions were recognized. Nevertheless, IL-13 and IL-4 have a number of overlapping effects. IL-13 also plays an essential role in resistance to most GI nematodes.

It regulates mucus production, inflammation, fibrosis and tissue remodeling. IL-13 is a therapeutic target for a number of disease states including asthma, idiopathic pulmonary fibrosis, ulcerative colitis, cancer and others. Its signaling is mediated via IL-4 type 2 receptor. The receptor consists of IL-4Rα and IL-13Rα1 and IL-13Rα2 chains.

IL-13 induces physiological changes in organs infected with parasites that are essential for eliminating the invading pathogen. In the gut, it induces a number of changes that make the surrounding environment of the parasite less hospitable, such as increasing contractions and hypersecretion of glycoproteins from gut epithelial cells. This results in the detachment of the parasites from the wall of the gut and their subsequent removal. IL-13 response in some instances may not resolve infection and may even be deleterious. For example, IL-13 may induce the formation of granulomas after organs such as the gut wall, lungs, liver and central nervous system are infected with the eggs of *Schistosoma mansoni*, which may lead to organ damage and could even be life threatening.

IL-13 is believed to inhibit TH_1 responses, which will inhibit the ability of the host to eliminate the invading pathogens. The role of IL-13 in the etiology/pathogenesis of allergic disease/asthma has drawn broad attention. It induces AHR and goblet cell metaplasia, which result in airway obstruction and cause allergic lung disease. IL-13/chemokine interactions play a key role in the development of AHR and mucus production. IL-13 induces the expression of eotaxins. These chemokines recruit eosinophils into the site of inflammation in synergy with IL-5. Eosinophils release IL-13 and induce the production of IL-13 from TH_2 cells, which is mediated via IL-18. IL-13 then, through its effects on epithelial and smooth muscle cells, aids in the development of AHR and mucus production. In addition to its potent activation of chemokines, IL-13 is also an inducer of adhesion molecules involved in asthma.

Interleukin-18

IL-18 is a member of the IL-1 family that promotes the production of various proinflammatory mediators and plays a role in cancer and various infectious diseases. It was originally identified as IFN-γ-inducing factor and is produced by cells of both hematopoietic and nonhematopoietic lineages, including macrophages, dendritic cells, intestinal epithelial cells, synovial fibroblasts, keratinocytes, Kupffer cells, microglial cells and osteoblasts. The production of IL-18 is structurally homologous to that of IL-1β; it is produced as an inactive precursor of 24 kDa, which lacks a signal peptide. Endoprotease IL-1β-converting enzyme activates it after cleaving pro-IL-18, resulting in a biologically active cytokine. Caspase-1 plays an important role in the processing of IL-18, but is not exclusive since proteinase 3 can also perform the same function.

IL-18 augments T- and NK-cell maturation, cytotoxicity and cytokine production. It stimulates TH differentiation, promotes secretion of TNF-α, IFN-γ and GM-CSF and enhances NK cell cytotoxicity by increasing FasL expression. IL-8-mediated neutrophil chemotaxis is promoted by IL-18 via its effects on TNF-α and IFN-γ, which are stimulatory in action. It plays an important role in maintaining synovial inflammation and inducing joint destruction in rheumatoid arthritis. In synovium of patients with rheumatoid arthritis, enhanced levels of TNF-α and IL-1 are associated with augmented expression of IL-18.

IL-18 also induces IL-4, IL-10 and IL-13 production, increases IgE expression on B cells and in association with IL-2, it enhances stimulus-induced IL-4 production from TH_2 cells. Bone marrow-derived basophils produce IL-4 and IL-13 in response to a stimulus from IL-18 and IL-3. IL-18 in combination with IL-12 induces IFN-γ from dendritic cells and bone marrow-derived macrophages. Adhesion molecules, ICAM-1 and VCAM-1, are induced by this cytokine on synovial fibroblasts and endothelial cells. It inhibits osteoclast formation via its induction of GM-CSF from T cells. The receptors of IL-18, IL-18Rα and IL-18Rβ, share their signaling mechanisms via the IL-1R family. Toll-like receptors also share the downstream signaling pathway of IL-18 and are known to regulate IL-18 expression.

IL-18 plays a critical role in host defense against bacterial, viral, fungal and protozoan infections. One predominant mechanism is the induction of host IFN-γ production, which activates several effector pathways including nitric oxide production, resulting in the clearance of the invading pathogens. A role of IL-18 in robust TH_1 responses against *Mycobacterium tuberculosis* and *Mycobacterium avium* has been suggested. For viral infections, the effects of IL-18 are mediated not only via IFN-γ but also by activation of $CD8^+$ T cells. IL-12 and IL-15 also play a role in its effects in host defense and as mediator of inflammation where IL-18 works in concert with other cytokines and their signaling pathways. Its modulation of inflammation is at multiple checkpoints. IL-18 binding protein (IL-18 BP) is the naturally occurring antagonist that may serve as a negative feedback mechanism for IL-18 as several isoforms of this antagonist have been identified.

Interferons

Although originally identified as proteins with antiviral activity, these inducible cytokines play an important role in regulating innate and acquired immunity. Initially characterized by the secreting cell type, IFNs are now divided into two groups, type I and type II IFNs. Type I IFNs, which are also called IFN-α/β family, are the product of numerous genes and include IFN-α, IFN-β, IFN-ω, IFN-κ, IFN-ε and IFN-λ. Almost all cell types secrete type I IFN; however, hematopoietic cells are the major source of IFN-α and IFN-ω, and fibroblasts are the major producers of IFN-β. Macrophages under appropriate induction also secrete IFN-β. Their structural genes are located on chromosome 9 in humans. The type II IFN IFN-γ is the product of a single gene on chromosome 12 in humans. The stimuli for the production of type I IFNs are viral and microbial infections and double-stranded RNA.

Type I Interferons

Type I IFNs are two distinct groups of proteins, IFN-α (approx. 18 kDa) and IFN-β (20 kDa). IFN-α is subdivided into two subgroups, IFN-α1 and IFN-α2/IFN-ω. Viral infection is the most potent natural signal for the synthesis of type I IFNs.

The principal biological actions of type I IFNs include inhibition of viral replication, inhibition of cell proliferation, increase in the lytic potential of NK cells and the modulation of MHC molecule expression. They increase the expression of MHC class I molecules and decrease the expression of MHC class II molecules.

Type I IFNs exerts their biological effects after binding to distinct heterodimeric cell surface receptors on the target cells. Binding of the agonist to the cell surface receptors results in activation of the Janus-activated kinase (JAK)–STAT signaling pathway. The JAK–STAT activation results in the induction of specific genes. These genes contain IFN-specific response elements or IFN-γ-stimulated sequence. The IFNs have both overlapping and distinct pharmacological activities because some genes overlap partially, whereas some IFNs are produced at different sites.

IFN-α/β mediate antiviral activity by multiple mechanisms. A series of antiviral proteins are produced after IFN-α/β bind to their specific cell surface receptors. The proteins induced by IFNs include a 2′, 5′-oligoadenylate synthetase and a protein kinase; both in the presence of double-stranded RNA can inhibit protein synthesis. A latent cellular endoribonuclease is activated by adenylate oligomers produced by an oligoadenylate synthetase, which breaks down viral as well as cellular single-stranded RNAs. The protein kinase inactivates eukaryotic initiation factor (EIF)-2 after phosphorylation, which is involved in protein synthesis and is also an effector for apoptosis. Furthermore, peptide elongation is prevented as a result of cleaving of transfer RNA by a phosphoesterase that is induced by IFN-α/β. Depending on the family of the virus, multiple steps may be inhibited by IFN to varying degrees.

Clinical Applications of Interferons

Interferon-α

IFN-α may be used for the treatment of condylomata acuminata (venereal or genital warts), malignant melanoma, hairy cell leukemia and hepatitis B and C, and other types of cancer including skin, kidney and bone cancers.

Interferon-α-2a (Roferon-A)

Produced by recombinant DNA technology, IFN-α-2a is used for the treatment of chronic myeloid leukemia, Kaposi sarcoma, lymphoma, hairy cell leukemia, hepatitis B and C and cancer of the skin and kidney. It can only be administered by injection or into the bloodstream, and the most common method is subcutaneous injection. This cytokine can be injected every day; however, commonly it is

administered three times a week. The antiviral or antitumor activity of IFN-α-2a is mediated via inhibition of viral replication and modulation of host immune response as well as its antiproliferative activity. It is filtered through the glomeruli, and its proteolytic degradation takes place during tubular reabsorption. The common side effects include flu-like symptoms of fever, fatigue, chills, dry mouth, GI disorders, changes in mood and temporary effects on the bone marrow. The occasional side effects may include skin rash, hair thinning, loss of appetite and loss of fertility.

Peginterferon-α-2a

Pegylated α-IFN is made by attaching polyethylene glycol (PEG) to the α-IFN. PEG is a large water-soluble molecule that decreases the clearance of α-IFN and also increases the duration of its activity. This modified cytokine is used to treat chronic hepatitis C. However, it is rarely used as a single therapeutic agent for hepatitis C because of its low response rate.

Interferon-α-2b

IFN-α-2b is a water-soluble α-IFN protein produced by recombinant DNA technology. Both IFN-α-2b and -α-2a are pure clones of single IFN subspecies, but they differ by virtue of two amino acids. The potencies of both α-2a and α-2b IFNs are similar. IFN-α-2b is also available in pegylated form. All IFN-α cytokines augment the killing of target cells by lymphocytes and inhibit the replication of virus in infected cells.

Interferon-β

Natural IFN-β is predominantly synthesized by fibroblasts. Its sequence is 30% homologous to that of IFN-α. The receptors for both IFN-α and -β are the same but the fit of the receptor is different for the two agonists. There are also differences between IFN-α and -β in structure (IFN-β is glycosylated on one site, pharmacokinetics and binding to tissues.

IFN-β-1a is used to treat patients with a relapsing form of MS. It is not a cure for MS; however, it may slow some disabling effects of the disease. IFN-β-1a may also decrease the number of relapses of MS. The possible mechanisms of action for the treatment of MS include the antagonism of IL-4 and IFN-γ. It also modifies the mechanics of blood barrier since it inhibits cell adhesion, cell migration and metalloproteinase activity. IFN-β induces IL-10 and TGF-β, which are anti-inflammatory cytokines. It is also used for the treatment of genital warts.

The available preparations for IFN-β-1a include Avonex and Rebif, both synthesized by recombinant DNA technology. They are similar but Rebif is administered more frequently and at a higher dose. A third preparation, Betaseron, is IFN-β-1b.

Type II Interferons

Interferon-γ

IFN-γ modulates a number of components of the immune response. This is the only type II IFN whereas there are more than 20 types of type I IFNs (IFN-α, IFN-β, IFN-ω and IFN-τ). It is not related to type I IFNs, has separate receptors and is encoded by a different chromosomal locus. IFN-γ is produced by activated T lymphocytes (TH_1 and $CD8^+$ cells), NK cells, B cells, NKT cells and professional APCs. It promotes the activity of cytolytic T lymphocytes, macrophages and NK cells. The cell self-activation and activation of nearby cells in part may result from IFN-γ production by professional APCs, which include monocyte/macrophage and dendritic cells. The early host defense against infection is likely to utilize IFN-γ secreted by NK and professional APCs. In acquired immune responses, T lymphocytes are the major source of IFN-γ.

IFN-γ production is regulated by IL-12 and IL-18, both cytokines secreted by APCs. In the innate immune response, a link is established between infection and IFN-γ by these cytokines. IL-12 and chemokines including macrophage inflammatory protein-1α (MIP-1α) are secreted as macrophages recognize pathogens, and NK cells are attracted to the site of inflammation by the chemokines. This is followed by the induction of IFN-γ production and secretion by IL-12. IL-12 and IL-18 further stimulate the production of IFN-γ from macrophages. The production of IFN-γ is inhibited by IL-4, IL-10 and TGF-β.

IFN-γ is a potent activator of mononuclear phagocytes. The expression of both MHC class I and class II molecules is augmented by IFN-γ as IFN-γ-induced upregulation of MHC class I molecules is pivotal for host defense against intracellular pathogens, resulting in an increased susceptibility to cytolytic T cells for recognition and consequent promotion of cell-mediated immune response. The stimulation by IFN-γ results in the addition of "immunoproteosome subunits" and the removal of constitutive proteosome subunits. The unstimulated cells contain β_1, β_2 and β_5 proteosome enzymatic subunits, which are encoded outside the MHC locus. β_1 is replaced by LMP2, β_2 by MECL-1 and β_5 by LMP7, and the expression of the new subunit is stimulated by IFN-γ. This results in the formation of new subunits of proteosomes. This is a potential mechanism utilized by IFN-γ to enhance the characteristics of peptides for MHC class I loading. The ability of immunoproteosomes to cleave peptides enhances the ability of antigen fragments to bind to MHC class I molecules. The diversity of the antigenic fragments is increased, resulting in better immune surveillance. IFN-γ also augments the MHC class II antigen-presenting pathway and results in the activation of $CD4^+$ T cells via peptides. It not only stimulates the expression of MHC class II molecules on cells that constitutively express these antigens but also induces their expression on cells that do not constitutively express their genes. The expression of several other molecules including Ii chain, cathepsins B, H, L, lysosomal proteases and HLA-DM is upregulated by IFN-γ. These molecules are involved in various processes associated with antigen presentation, peptide accessibility and peptide loading.

Table 2.3 Characteristics of Selected Cytokines

Name	Source	Target	Biological Role
IL-1 (IL-1α and -β)	Macrophages, dendritic cells, endothelial cells, other cells	TH and B cells and various other tissues	Activation (other details provided in the text)
IL-2	TH_1 cells	TH, T_C and NK cells	T cell and NK proliferation and induction of activity
IL-3	TH_1 and TH_2 cells, mast cells, NK cells	Hematopoietic and mast cells	Progenitor cell proliferation and differentiation
IL-4	TH_2 cells, mast cells, NK cells	B cells, T cells, mast cells, macrophages	Proliferation, isotype switching, induction of MHC class II expression
IL-5	TH_2 cells, mast cells	Eosinophils	Proliferation and differentiation
IL-6	Macrophages, TH_2 cells	Plasma cells, B cells and others	Differentiation and antibody secretion
IL-8	Bone marrow, thymus (stromal cells)	Neutrophils	Chemoattractant
IL-9	TH_2 cells	TH cells, mast cells, eosinophils	Induces inflammatory responses
IL-10	TH_2 cells	Macrophages, APC	Anti-inflammatory cytokine inhibits cytokine production
IL-11	Bone marrow (stromal cells)	B-cell progenitors and others	Differentiation
IL-12	Macrophages, B cells	T_C, NK and LAK cells	Proliferation and differentiation in synergy with IL-2
IL-13	TH cells	Macrophages, B cells	Inhibition of inflammatory cytokines, regulation of inflammation. Parasitic infections
IL-16	T_C cells	TH cells	Chemotaxis
IL-18	Hematopoietic and nonhematopoietic lineage cells	T cells, NK cells	Proinflammatory cytokine; IFN-γ-inducing factor
IFN-α	Leukocytes		Inhibitor of viral replication
IFN-β	Fibroblasts		Inhibitor of viral replication
IFN-γ	TH_1, T_C, NK	Various cells including macrophages	Inhibitor of viral replication. Inhibitor of cell proliferation. Inhibitor of IL-4-induced isotype switching
TNF-α	Macrophages	Tumor cells, polymorphonuclear leukocytes, macrophages	Cytotoxicity, induction of cytokine secretion
TNF-β	T cells	Tumor cells, neutrophils, macrophages	Cytotoxicity, phagocytosis

IFN-γ is produced by TH_1 cells and shifts the response toward a TH_1 phenotype. This is accomplished by activation of NK cells that promotes innate immunity, augmenting specific cytolytic response and induction of macrophages. The induction of cytotoxic immunity can be direct or indirect via suppression of TH_2 response. Another direct effect of IFN-γ is the differentiation of naïve $CD4^+$ lymphocytes toward a TH_1 phenotype. The cytokines present are very important in this differentiation process. Furthermore, induction of IL-12 and suppression of IL-4 by IFN result in differentiation toward a TH_1 phenotype.

IFN-γ is an inhibitor of cell growth and proliferation. The proliferation is inhibited by augmenting the levels of Cip/Kip, CKIs and Ink4. It increases p21 and p27 CKIs, which inhibit the function of cyclin E:CDK2 and cyclin D:CDK4, respectively. This results in stopping the cell cycle at G1/S interphase. IFN-γ induces apoptosis via activation of STAT-1, which results in the production of large amounts of IRF-1 (IFN regulatory factors). Apoptosis may be needed to kill the invading pathogen-infected macrophages.

IFN-γ also induces the costimulatory molecules on the macrophages, which increases cell-mediated immunity. As a consequence, there is activation and increase in the tumoricidal and antimicrobial activity of mononuclear phagocytes, granulocytes and NK cells. The activation of neutrophils by IFN-γ includes an increase in their respiratory burst. IFN-γ stimulates the cytolytic activity of NK cells. It is an activator of vascular endothelial cells, promoting $CD4^+$ T lymphocyte adhesion and morphological alterations, which facilitates lymphocyte extravasation. IFN-γ promotes opsonization by stimulating the production of IgG subclasses that activate the complement pathway. A summary of the characteristics of selected cytokines is shown in Table 2.3.

Colony-Stimulating Factors

A major cause of morbidity and mortality in patients who receive cytotoxic treatment or radiotherapy for cancer is bacterial and fungal infections. Intensive chemotherapy is associated with fever and infection, and the development of neutropenia further increases this risk of infection. Consequently, maximum doses of some cytotoxic drugs are limited due to bone marrow toxicity. Higher doses of chemotherapy and radiation therapy have become possible due to a reduction in bone marrow damage with the availability of the CSFs for clinical use.

The CSFs are glycoproteins that support hematopoietic colony formation. They influence the survival, proliferation and maturation of hematopoietic progenitor cells and regulate the activities of the mature effector cells. There are three lineage-specific CSFs, granulocyte colony-stimulating factor (G-CSF), monocyte–macrophage colony-stimulating factor (M-CSF) and erythropoietin, and two multipotential CSFs, IL-3 and GM-CSF.

Clinical Uses of Colony-Stimulating Factors

The CSFs prevent chemotherapy-induced neutropenia. They stimulate hematopoiesis in marrow failure. The CSFs promote cell differentiation, assist in marrow transplantation, stimulate monocyte anticancer effects and augment effector cell function.

Granulocyte Colony-Stimulating Factor

G-CSF is a glycoprotein produced by macrophages, endothelium and various leukocytes. It stimulates the bone marrow to produce granulocytes and stem cells and then directs their migration from the bone marrow to the peripheral blood. G-CSF is a growth factor for the proliferation, differentiation, effector function and survival of neutrophils. The gene for G-CSF is located on chromosome 17, locus q11.2-q12.

G-CSF mobilizes bone marrow-derived cells into the bloodstream. These stem cells can migrate to ischemic myocardium and differentiate into cardiomyocytes, smooth muscle cells and endothelial cells. They may also induce metalloproteinases and vascular endothelial growth factor and thus play a role in tissue healing. Furthermore, G-CSF induces proliferation and enhanced survival of cardiomyocytes. This is accomplished via activation of G-CSF receptors in myocardium. G-CSF in association with TGF-β and collagen enhances ventricular expansion in the infarcted area.

G-CSF activates neutrophils, transforming them into cells capable of respiratory burst and release of secretory granules. It also modulates the expression of adhesion molecules on neutrophils as well as CD11b/CD18 and plasma elastase antigen levels. G-CSF induces proliferation of endothelial cells, phagocytic activity of neutrophils, reactive oxygen intermediate production by neutrophils and antibody-dependent cellular toxicity by neutrophils.

Filgrastim (Neupogen)

Recombinant human G-CSF (filgrastim) is a 175-amino-acid glycoprotein. It differs from natural G-CSF due to lack of glycosylation and has an extra N-terminal methionine. Pegylated recombinant human G-CSF (pegfilgrastim) is also available. Filgrastim administered to patients receiving cytotoxic chemotherapy for advanced cancer has resulted in a dose-dependent amelioration of neutropenia associated with cancer chemotherapy. It is well tolerated and may reduce the morbidity and mortality rate associated with chemotherapy, possibly permitting higher doses and a greater antitumor response. Filgrastim is also used after autologous stem cell transplantation to treat neutropenia. It reduces the duration of neutropenia and lessens morbidity secondary to bacterial and fungal infections. Additional use of this drug includes the treatment of severe congenital neutropenias, of neutropenia in patients with AIDS resulting from treatment with zidovudine and of patients donating peripheral blood stem cells for stem cell transplantation.

Filgrastim is administered by intravenous infusion or subcutaneous injection. The doses given are 1–20 μg/kg per day over at least a 30-min period. Generally a dose of 5 μg/kg is used in patients receiving chemotherapy for 14–21 days or longer. The half-life of the drug is 3.5 h. The side effects include bone pain, local skin reactions and rarely cutaneous vasculitis.

Granulocyte–Macrophage Colony-Stimulating Factor

GM-CSF is a glycoprotein produced by macrophages, T cells, mast cells, fibroblasts and endothelial cells. It stimulates stem cells to produce neutrophils, monocytes, eosinophils and basophils. Monocytes migrating into tissue from the circulating blood differentiate into macrophages and undergo maturation.

Sargramostim (Leukine)

Recombinant human GM-CSF (sargramostim) is a 127-amino-acid glycoprotein, which is similar to natural GM-CSF except for variation in glycosylation and presence of a leucine in position 23. It has beneficial effects on bone marrow function in patients receiving high-dose chemotherapy in the setting of autologous bone marrow transplantation as well as for the treatment of advanced cancers. Sargramostim is used in AIDS, myelodysplastic syndrome and aplastic anemia where it stimulates bone marrow function. It has not shown beneficial effects in graft-versus-host disease but may be of value in patients with early graft failure. It has been used in patients donating peripheral blood stem cells because it mobilizes $CD34^+$ progenitor cells. Sargramostim is administered either by slow intravenous infusion or by subcutaneous injection. The doses given are 125–500 $\mu g/m^2$ per day. Intravenous administration requires a period of at least 3–6 h. The half-life with subcutaneous injection is 2–3 h. The side effects with high doses include bone pain, flu-like symptoms, fever, diarrhea, nausea and vomiting. Prolonged administration has produced marked weight gain, generalized edema, capillary leak and hypotension. It also causes a dose-dependent, asymptomatic eosinophilia.

Tumor Necrosis Factor-α

This proinflammatory cytokine was first isolated in 1975, and its name is misleading in that it does not cause the necrosis of all tumors. As a matter of fact, it may stimulate the growth of some tumors. TNF-α is a 185-amino-acid glycoprotein, which is cleaved from a 212-amino-acid peptide, and the cleavage occurs on the cell surface of mononuclear phagocytes. In humans, the genes for TNF-α are present on chromosome 7p21. The major cell source of TNF-α is the macrophage, specifically the endotoxin-activated mononuclear phagocyte. Other sources include endothelium after tissue damage, antigen-stimulated T cells, activated NK cells and activated mast cells. IFN-γ augments TNF-α synthesis.

TNF-α is a mediator of both natural and acquired immunity. Local increasing concentrations of TNF-α cause heat, swelling, redness and pain. TNF-α causes vascular endothelial cells to express new adhesion molecules. It increases the mobilization and effector function of neutrophils and their adhesiveness for endothelial cells. TNF-α induces the production of IL-1, IL-6, TNF-α itself and chemokines via stimulation of macrophages. It exerts an IFN-like protective effect against viruses and augments expression of MHC class I molecules. TNF-α is an endogenous pyrogen that acts on cells in hypothalamic regulatory regions of the brain to induce fever. It suppresses appetite. The hypothalamic–pituitary–adrenal axis is stimulated via the release of corticotrophin-releasing hormone by TNF-α. TNF-α induces acute-phase responses by activating hepatocytes. Acute-phase proteins including C-reactive protein and mannose-binding protein (MBP) are detected in blood in response to an infection. TNF-α suppresses bone marrow stem cell division and reduces tissue perfusion by depressing myocardial contractility.

Tumor Necrosis Factor Receptors

There are two distinct types of TNF receptors, TNF-R1 (CD120a or P55) and TNF-R2 (CD120b or P75). They are implicated in inflammatory processes and both belong to the TNF receptor superfamily. TNF receptors are transmembrane proteins with intracellular domains that lack intrinsic enzymatic activity, and consequently, they require cytoplasmic proteins that help initiate the receptor-induced signaling pathways. TNF-R1 possesses an intracellular death domain and TNF-R2 interacts with molecules of the TNF receptor-associated factor 2 family (TRAF).

The receptors for TNF-α are widely distributed although TNF-R1 is more common in nonhematopoietic cells. Both groups of receptors interact with the ligand TNF-α (soluble form) with similar affinity. TNF-R1 recognizes both the membrane-bound and soluble TNF-α, whereas TNF-R2 binds to membrane-bound TNF-α with greater affinity. The signals initiated by the two receptors are different since there are structural differences between the intracellular domains of the two receptors. The activated TNF-R1 contains a death domain in its cytoplasmic region that recruits the adopter proteins. The downstream signaling involves different pathways that lead to cell death or survival.

After the binding of TNF-α to its receptors, there is induction of two major intracellular signaling pathways. One pathway leads to the transcription of other genes, and the other pathway leads to cell death or apoptosis. The two main transcription factors activated by TNF-α are AP-1 and NF-κB.

Etanercept (Enbrel)

Etanercept is a genetically engineered protein that is soluble TNF-α receptor. Its molecular weight is 75 kDa. It binds to TNF-α. It is used for the treatment of rheumatoid arthritis, juvenile rheumatoid arthritis, ankylosing spondylitis and psoriatic arthritis. Structurally, two TNF-α receptors are linked to an Fc portion of an

IgG1 molecule. Consequently, an artificial antibody is constituted with two Fab sites, which are soluble human 75-kDa TNF-α receptors. It competitively inhibits the binding of TNF molecules to the TNF receptor sites. The binding of etanercept to TNF renders the bound TNF biologically inactive, resulting in the reduction of the inflammatory activity. The most frequent adverse side effects are injection site reactions, infections and headache and malignancies are rare. Etanercept is not recommended for patients with serious infections or sepsis and does not appear to result in the reactivation of tuberculosis.

Chemokines

Chemokines are a large family of small heparin-binding chemotactic cytokines released by many cell types. They are composed of four groups called CXC, CC, C and CX3C. The designation and classification is based on the spacing of conserved cysteines and X is an amino acid. Many members constitute the CXC and CC groups, which is not the case for C and CX3C chemokines. Neutrophils and lymphocytes are the targets of CXC chemokines. The targets of CC chemokines are diverse, including basophils, dendritic cells, macrophages and eosinophils. The CXC family includes chemokines CXCL1–CXCL17, the CC family includes CCL1–CCL28, the C family includes XCL1–XCL2 and the CX3C family includes only CX3CL1.

The early signals produced during innate immune responses are the main stimuli for the secretion of chemokines. Various chemokines are secreted by a stimulus resulting from viral infection, bacterial products (e.g., LPS) and proinflammatory cytokines including IL-1 and TNF-α. Consequently, some chemokines are proinflammatory in nature and are produced during an immune response to direct leukocytes to the site of injury/infection, whereas others are homeostatic in nature and control the migration of cells during routine tissue maintenance or development.

CXC Chemokines

Also termed α-chemokines, CXC chemokines are composed of two N-terminal cysteines, separated by one amino acid designated with an “X” in the name. There are 17 different CXC chemokines and they are divided into two groups. One group has a specific motif of glutamic acid–leucine–arginine (ELR) right before the first cysteine of the CXC motif and is called ELR-positive. The other group does not have this motif and is called ELR-negative. Glutamic acid–leucine–arginine-positive CXC chemokines are specific for neutrophils and mediate their effects via CXCR1 and CXCR2 (CXC receptors 1 and 2). IL-8 is an example of ELR-positive chemokines that direct the migration of neutrophils to the infected tissue. Glutamic acid–leucine–arginine-negative CXC chemokines, for example, CXCL13, are chemoattractants for lymphocytes.

CC Chemokines

Also termed β-chemokines, CC Chemokines are composed of two adjacent cysteines near their amino-terminus. There are at least 27 different CC chemokines of which CCL9 and CCL10 are the same. Most of the members of this group possess four cysteines (C4-CC) but a small number have six cysteines (C6-CC). CC chemokines regulate the migration of monocytes, dendritic cells and NK cells. An important chemokine in this group is monocyte chemoattractant protein-1 (MCP-1, also called CCL2), which promotes the migration of monocytes from the bloodstream to the tissue where they differentiate to become macrophages. Other CC chemokines include MIPs, MIP-1α (CCL3) and MIP-1β (CCL4) and RANTES (CCL5). The effects of CC chemokines are mediated via specific cell surface receptors: 10 different types of these receptors (CCR1–CCR10) have been identified.

C Chemokines

Also termed γ-chemokines, C Chemokines are composed of only two cysteines: one on the N-terminus and the other a downstream cysteine. There are two chemokines in this group, lymphotactin-α (XCL1) and lymphotactin-β (XCL2). Their function is the attraction of T-cell precursors to the thymus.

CX3C Chemokines

Also termed δ-chemokines, CX3C Chemokines are composed of three amino acids between the two cysteines. This subgroup has only one member, fractalkine (CX3CL1). CX3C chemokines are secreted as well as present on the cell surface and serve both as a chemoattractant and as an adhesion molecule.

Biological Role of Chemokines

The primary function of chemokines is to induce the migration of leukocytes. A signal directs these cells toward the chemokines. During immunological surveillance, chemokines direct lymphocytes to the lymph nodes, which allows them to interact with the APCs and detect any invading pathogens. Such chemokines are called homeostatic chemokines and do not require a stimulus for their secretion. Some chemokines are proinflammatory in nature and require specific stimulus for their release. These stimuli include viral infection, bacterial products as well as other chemical agents. Proinflammatory cytokines including IL-1 and TNF-α promote their release. These chemokines are chemoattractants for neutrophils, leukocytes, monocytes and some effector cells, and they direct the migration of these leukocytes to the site of injury/infection. Some proinflammatory chemokines are also involved in wound healing similar to the proinflammatory cytokines. Chemokines are also

capable of activating leukocytes to initiate an immune response and are involved in both innate and acquired immunity. Other chemokines play a role in development and are involved in angiogenesis and cell maturation.

Chemokine Receptors

Chemokine receptors are a family of G protein-coupled receptors that contain seven transmembrane domains. Chemokine receptors are present on the cell surface membrane of leukocytes. As was the case for chemokines, these receptors are also divided into four subgroups: CCR is specific for CC chemokines, CXCR for CXC chemokines, XCR1 for C chemokines and CX3CR1 for CX3C chemokines. The CC chemokine receptor family has eleven members, the CXC chemokine receptor family has seven members, and both the C chemokine receptor family and the CX3C chemokine receptor family have one member each. The signal transduction is mediated via the standard G protein-dependent pathway.

Chemokines and Disease States

Human Immunodeficiency Virus Infection

Human immunodeficiency virus requires CD4 and either CXCR4 or CCR5 to enter target cells. This allows the entry of HIV into $CD4^+$ T cells or macrophages, which eventually leads to the destruction of $CD4^+$ T cells and almost total inhibition of antiviral activity. Individuals who possess a nonfunctional variant of CCR5 and are homozygous for this gene remain uninfected despite multiple exposures to HIV. Clinical trials are under way to develop antagonists of these chemokine receptors as potential therapeutic agents for HIV infection and AIDS.

Diabetes with Insulin Resistance

Cytokines and chemokines have been implicated in insulin resistance. The cytokines which may play a role include IL-6 and TNF-α. CCR2 are present on adipocytes, and activation of inflammatory genes by the interaction of CCR2 with the ligand CCL2 results in impaired uptake of insulin-dependent glucose. Adipocytes also synthesize CCL2, resulting in the recruitment of macrophages. CCL3 may also be involved in insulin resistance.

Atherosclerosis

CCL2 is present in lipid-laden macrophages and atherosclerotic plaques that are rich in these macrophages. The production of CCL2 in endothelial and smooth muscle cells is stimulated by minimally oxidized low-density lipoproteins (LDLs). As a consequence, CCL2 is involved in the recruitment of foam cells to the vessel

wall. Patients who are homozygous for the polymorphism in the promoter of CCL2 appear to have a high risk for developing coronary artery disease as opposed to patients who are heterozygous. CXCR2 and CX3CR1 are also implicated in cardiovascular disease.

Inflammatory Diseases

Chemokines are involved in various inflammatory diseases including asthma, arthritis, psoriasis and MS. Chemokine CC11 (eotaxin) and its receptors CCR3 are involved in the recruitment of eosinophils to the lungs, contributing to the etiology/pathogenesis of allergic disease/asthma. Elevated levels of CCL2, CCL3 and CCL5 are found in the joints of patients with rheumatoid arthritis, and are involved in the migration of monocytes and T cells to the inflamed joint. In psoriasis, CCR4 is expressed on infiltrating effector T cells, and cutaneous cells produce CCL17 and CCL22, which are ligands for CCR4. CXCR3 also plays a role in psoriasis. In MS, the levels of CXCL10 are elevated but there are lower levels of CCL2, and CXCR3 may also play a role. MS lesions contain many chemokines including CXCL10, CCL3, CCL4 and CCL8, where they may be predominantly involved in the migration of monocytes and macrophages from the peripheral blood into the tissue and lesions. The infiltrating monocytes express both CCR1 and CCR5.

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