Chemokines and Their Receptors

Roles in Specific Clinical Conditions and Measurement in the Clinical Laboratory

Massimo Locati, MD,[1] Raffaella Bonecchi, PhD,[1] and Massimiliano M. Corsi, MD, PhD,[2]

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Abstract

Considerable progress has been achieved in our knowledge of the function of the chemokine system and in understanding its role in the pathophysiology of human diseases. This complex system, presently including approximately 50 cytokines and 20 receptors, coordinates leukocyte recruitment in a variety of human diseases, ranging from infectious and inflammatory diseases to cancer. A large body of literature has been published describing various assays for the measurement of chemokines in biologic fluids and tissues. We review information available on the role of chemokines in selected human diseases and provide examples of clinical situations in which chemokine determination might be of practical value, and we describe the currently available assays for their measurement.

In recent years, a number of cytokines have been recognized as important intercellular signals with a relevant role in the pathogenesis of several human diseases, which is mirrored by their growing relevance in clinical pathology as biomarkers of disease onset, progression, and remission.[1] Leukocyte recruitment and activation are key steps in the pathogenesis of several human diseases. This process is coordinated by chemokines, a large subfamily of cytokines with chemotactic activity for different leukocyte subsets and an emerging role in major human diseases. After an overview of the chemokine system, this review briefly summarizes information available on clinical situations in which chemokine determination might be of diagnostic value with the aim to evaluate chemokines as new biomarkers of interest in clinical pathology.

The Chemokine System

The completion of the human genome project has led to the final identification in humans of about 50 structurally and functionally related molecules now recognized as a family of small secreted proteins and named *chemokine* because of their leukocyte chemotactic and cytokine-like activities. Chemokines bear a significant sequence identity to each other, and their protein structure is strictly dependent on 2 conserved disulfide bonds connecting conserved cysteine residues.[2-4]

According to cysteine number and spacing, 4 chemokine subfamilies have been defined **Figure 1**. The largest group of chemokines has the first 2 cysteines in an adjacent position (CC chemokines). Most of these molecules, products of a large multigenic cluster on chromosome 17q11.2, act on monocytes, whereas other CC chemokines, products of different chromosomal loci, are active on different cell types (Figure 1).

The other large group of chemokines has the first 2 of 4 total cysteines separated by an intervening amino acid (CXC chemokines). Most of these molecules are coded by 2 large multigenic clusters. The first, located on chromosome 4q12-q13, includes CXC chemokines containing an ELR-conserved amino acid sequence on the N-terminus (ELR+ CXC chemokines) that act on neutrophils. The second, located on 4q21.21, includes CXC chemokines lacking the ELR sequence (ELR– CXC chemokines) that act mainly on T lymphocytes (Figure 1).

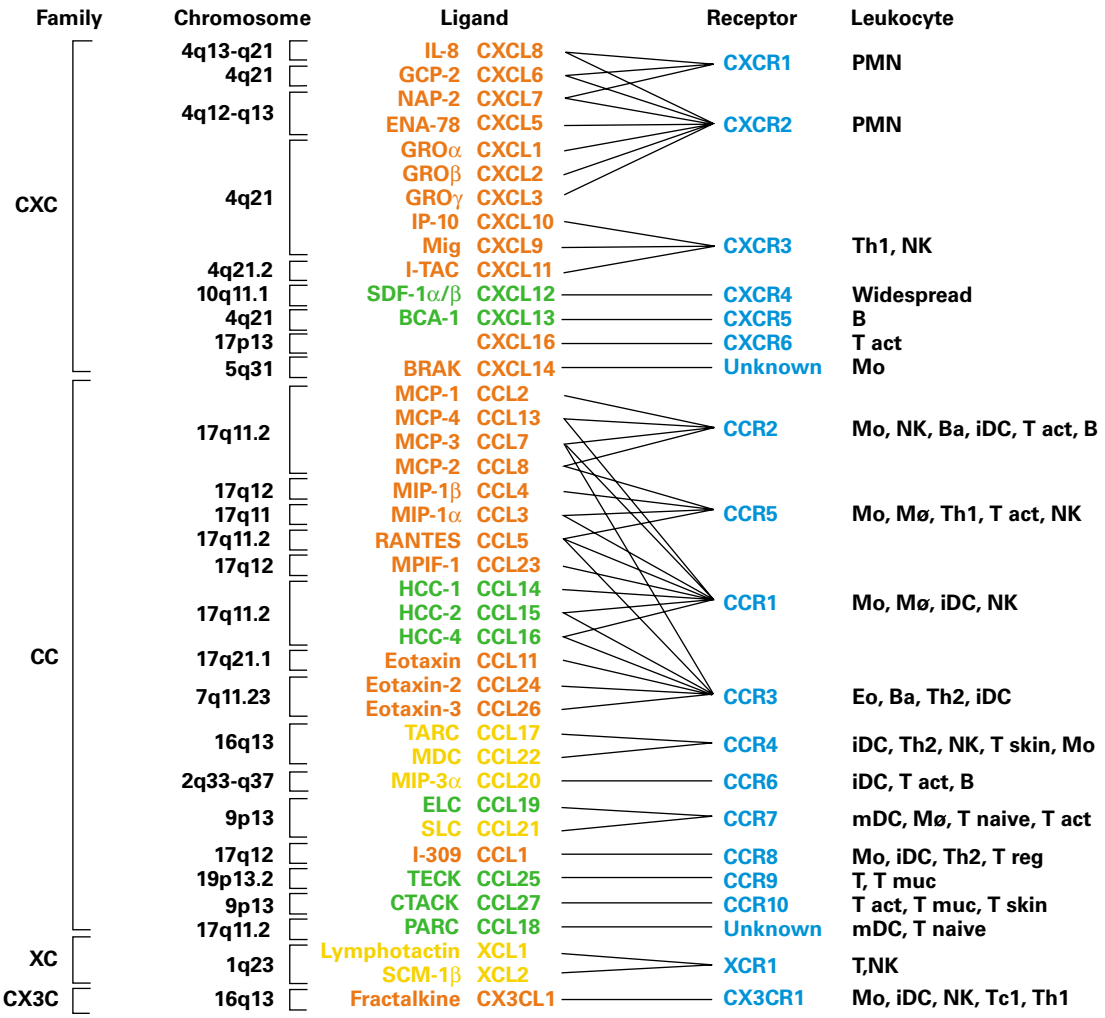

Figure 1 The chemokine system: an overview. Chemokines, their receptors, and predominant receptor repertoires in different leukocyte populations are listed. The selected ligands are identified with one old acronym and the new nomenclature, in which the first part of the name identifies the family and L stands for "ligand," followed by a progressive number. Red identifies predominantly "inflammatory" or "inducible" chemokines; green, "homeostatic" agonists; yellow molecules belong to both realms. Chemokine acronyms are as follows: BCA, B-cell activating chemokine; BRAK, breast and kidney chemokine; CTACK, cutaneous T-cell–attracting chemokine; ELC, Epstein-Barr virus–induced receptor ligand chemokine; ENA-78, epithelial cell–derived neutrophil-activating factor (78 amino acids); GCP, granulocyte chemoattractant protein; GRO, growth-related oncogene; HCC, hemofiltrate CC chemokine; IP, interferon-inducible protein; I-TAC, interferon-inducible T-cell A chemoattractant; MCP, monocyte chemoattractant protein; MDC, macrophage-derived chemokine; Mig, monokine induced by γ interferon; MIP, macrophage inflammatory protein; MPIF, myeloid progenitor inhibitory factor; NAP, neutrophil-activating protein; PARC, pulmonary and activation-regulated chemokine; RANTES, regulated upon activation normal T cell–expressed and secreted; SCM, single C motif; SDF, stromal cell–derived factor; SLC, secondary lymphoid tissue chemokine; TARC, thymus and activation-related chemokine; TECK, thymus-expressed chemokine. Ba, basophils; CC, chemokine with the first 2 cysteines in adjacent positions; Eo, eosinophils; iDC, immature dendritic cells; MC, mast cells; mDCs, mature dendritic cells; Mo, monocytes; Mø, macrophages; NK, natural killer cells; PMN, neutrophils; T act, activated T cells; T naive, naive T cells; T muc, mucosal-homing T cells; Treg, regulatory T cells; T skin, skin-homing T cells.

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The third chemokine subfamily includes 2 highly related molecules with only 2 cysteine residues (C chemokines), encoded by a single cluster on chromosome 1q23, selectively active on T lymphocytes. The fourth family (CX3C chemokines) include a single molecule with 3 intervening amino acids between the first 2 cysteine residues. This chemokine is coded by a gene located on 16q13 and acts on monocytes and T lymphocytes. Differently from all other chemokines, it has a transmembrane domain that allows it to be tethered to the cell surface.

The cysteine residue positions also dictate the new nomenclature for chemokines, based on the subclass followed by a number provided by the position of the corresponding coding gene in the cluster.[5] Thus, chemokines now are identified by a name providing information on the respective structural subfamily, corresponding also to the type of receptor they engage, followed by a number provided by and referring to the respective coding gene (for the relationship between old and new nomenclatures, refer to Figure 1).

Chemokine receptors define a distinct subfamily in the rhodopsin-like G protein–coupled receptor family.[6,7] At present, 18 receptors have been defined molecularly, 10 for CC chemokines (CCR1 to 10), 6 for CXC chemokines (CXCR1 to 6), and 1 for C chemokines and CX3C chemokines (XCR1 and CX3CR1, respectively). All chemokine receptors are single polypeptide chains spanning the membrane 7 times, with an acidic N-terminal extracellular domain and a serine-threonine–rich intracellular C-terminal domain. Two disulfide bonds between the N-terminal domain and the second extracellular loop and between the first and third extracellular loops normally are required for the definition of the molecular structure. In most cases, each individual receptor binds multiple chemokines, but subclass restriction is strictly respected. Thus, a major functional correlate of chemokine subclassification is represented by the use of different receptors whose names include the chemokine subclass specificity followed by a number.

Although distinct chemokines also exert several biologic functions, including regulation of hematopoiesis, fibrosis, and angiogenesis, they share a major (and eponymous) function represented by their ability to induce directional cell migration, thus coordinating leukocyte recruitment in physiologic and pathologic conditions **Figure 2**. Leukocyte contact with endothelium might be transient, reversible, and activation-independent. In this phase, cells roll across the endothelial surface by chemokine-independent interactions of selectins with counteradhesins. At inflamed sites, the leukocyte enters a second phase involving chemokine receptor engagement by specific chemokines that are immobilized on the endothelial surface by proteoglycans. Chemokine receptor activation in turn activates β_2 integrins, allowing leukocyte high-affinity binding to endothelial cell counterreceptors and subsequent extravasation[8] (Figure 2). The simultaneous action of chemokines and integrins also is needed for full activation of leukocytes and, in synergy with primary cytokines, enhances phagocytosis, superoxide production, granule release, and bactericidal activity.

Chemokines act as intercellular signals, being produced under appropriate conditions by virtually every cell type and acting on several target cells, leukocytes in particular. Some chemokines are produced constitutively, but most

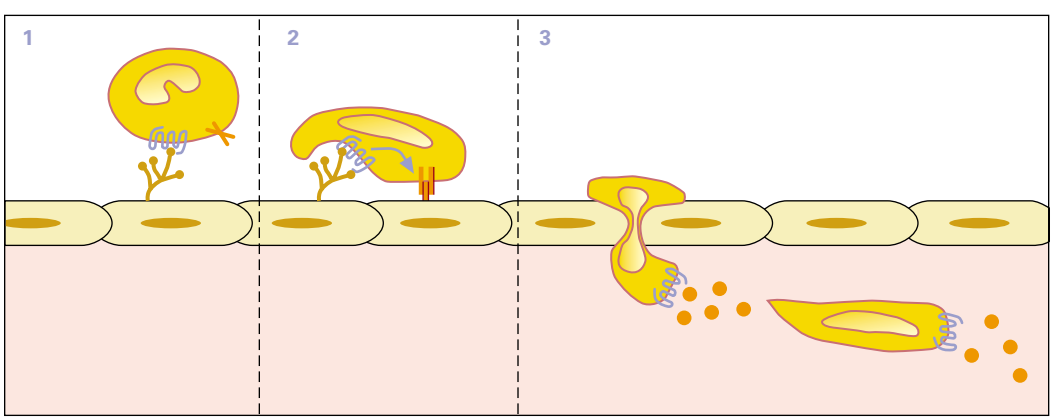

Figure 2 Chemokine biologic functions. All chemokines share a common biologic property represented by leukocyte chemoattraction and recruitment during immune responses. Selected chemokines also exert other chemotaxis-independent biologic functions acting on nonleukocyte populations. Chemotaxis-dependent functions include the following: (1) leukocyte recruitment, including cell adhesion, integrin activation, and cell migration and (2) application of immune responses (innate and specific). Chemotaxis-independent functions include the following: (1) modulation of angiogenesis, (2) regulation of fibrosis, (3) proliferation and differentiation of hematopoietic precursors, (4) role in ontogeny (central nervous system and vasculature), (5) control of cell survival, and (6) gene expression regulation.

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chemokines must be induced (Figure 1). In general, proinflammatory cytokines such as tumor necrosis factor α, interleukin 1β, or interferon (IFN)-γ up-regulate inflammatory chemokines, whereas anti-inflammatory mediators, such as interleukin 10 and glucocorticoids, have an opposite effect.[9] Most inducible chemokines are regulated at the transcriptional level, but some are stored for immediate release, as in the case of CXCL4 and CCL5 in platelet α granules. Chemokine receptors also are subjected to expression control. It is interesting that receptors for inflammatory chemokines usually are regulated opposite to the ligands, and several receptors are detected (or are functional) exclusively in specific cell states (eg CXCR3 on activated T cells).

Although narrow- and broad-spectrum chemokines exist, the spectra of action of different chemokines usually overlap widely, and collectively they span the entire leukocyte spectrum, presumably to provide flexibility and specificity in leukocyte trafficking. Neutrophil-targeted chemokines are found mainly in the CXC subfamily, whereas monocyte/macrophages, eosinophils, and basophils are attracted mainly by CC chemokines (Figure 1). Both CC and CXC subfamilies also contain T lymphocyte–specific members, and specific chemokine receptors mark T_H1 (CXCR3 and CCR5) vs T_H2 (CCR3 and CCR4) subsets **Figure 3**. The chemokine system is characterized by a high level of pleiotropism, with a given chemokine acting on different leukocyte populations. The sensitivity to the same chemoattractant allows the coordinated recruitment of different but functionally related leukocyte populations in specific immune responses. The chemokine system also is characterized by considerable redundancy. Some monogamous signaling units exist, such as CXCL12 and CXCR4, but in general a given receptor, in particular inflammatory chemokine receptors, recognizes more than a single chemokine, and a given chemokine usually binds to more than one receptor. Redundancy provides the molecular basis for robustness of the system, with alternative recruitment circuits being available to guarantee the final outcome.[10]

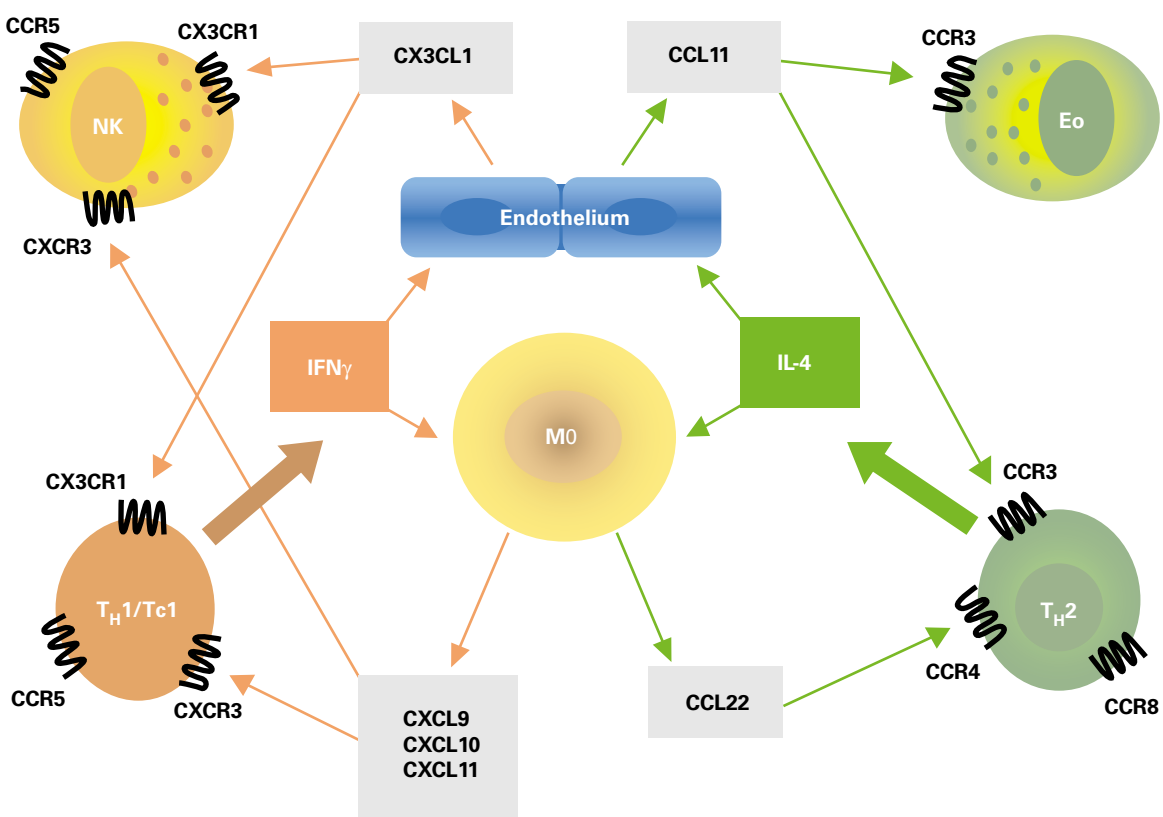

Figure 3 Role of chemokine in polarized immune responses. During type I and type II immune responses, master cytokines represented by interferon (IFN)-γ and interleukin (IL)-4, respectively, regulate chemokine production by stromal and inflammatory cells. Chemokines then support selective recruitment of polarized T cells and specific type I and type II effector cells expressing distinct panels of chemokine receptors. CC, chemokine with the first 2 cysteines in adjacent positions; CCL, CC ligand; CCR, CC receptor; CXC, chemokine with the first 2 of 4 total cysteines separated by an intervening amino acid; CXCL, CXC ligand; CXCR, CXC receptor; Ba, basophils; Eo, eosinophils; MC, mast cells; Mø, macrophages; NK, natural killer cells.

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The Chemokine System in Inflammatory and Infectious Diseases

Since the description of the first chemokine in 1977, an impressive amount of information has been accumulated, correlating the chemokine system with the pathogenesis of different human diseases ▮Table 1▮. One large group of disorders is represented by those with a prominent inflammatory component.[11,12] A nonredundant role of CXCL8 in neutrophil-mediated inflammatory disease has been demonstrated by CXCL8 neutralization that results in almost complete protection from multiple inflammatory challenges,[13] by genetic deletion of CXCR2 that causes defective neutrophil recruitment ▮Table 2▮,[14-50] and more recently by pharmacologic inhibition of CXCR1.[51]

Consistent with in vitro data, gene-targeted studies in mice also demonstrated the importance of CC inflammatory chemokines and respective receptors in monocyte recruitment. CCR1–/– and CCR2–/– mice present altered *Schistosoma* egg- or purified protein derivative–induced granulomatous inflammation that correlates with abnormal T1 and T_H2 cell responses (Table 2). CCR1–/– mice also have reduced pancreatitis-associated pulmonary infiltration, and CCR5–/– mice have enhanced delayed hypersensitivity reactions and increased humoral responses to T cell–dependent antigenic challenge (Table 2). Collectively, these results demonstrate a nonredundant role for inflammatory chemokines in leukocyte recruitment associated with acute and chronic inflammatory responses.

As predicted, studies with gene-targeted animals also have demonstrated a role for chemokines in host defense. For example, CXCR2–/– and CCR1–/– mice have increased susceptibility to inoculation with *Aspergillus fumigatus,* whereas CCR5–/– and CCR2–/– mice are more susceptible to infection with *Listeria monocytogenes* (Table 2). Similarly, CCL3–/– mice have markedly reduced coxsackie and influenza A virus–induced inflammation of the heart and lung, respectively (Table 2).

Although chemokines and chemokine receptors probably evolved to coordinate leukocyte recruitment that supports an antimicrobial response, many have been exploited by infectious agents to facilitate infection. Two models of exploitation have been identified. In a first scenario, pathogens interfere with the chemokine system by producing chemokine-binding molecules that inhibit their biologic activities, such as the M3 protein secreted by herpesvirus 68, or by pirating chemokines or chemokine receptors and modifying them to generate antagonists or chemokine scavengers.[52] Some pathogens produce multiple chemokine-related molecules affecting the host immune response in several ways. The Kaposi sarcoma–associated herpesvirus, also known as human herpesvirus 8, encodes a 7-transmembrane domain receptor known as open reading frame 74, highly related to the human CXCR2 but constitutively active (which exerts mitogenic and angiogenic activity in Kaposi sarcoma[53]), and 3 chemokine-related molecules (v-macrophage inflammatory protein [MIP]I, MIPII and MIPIII) that act on CCR3 and CCR4 and support T_H2 recruitment, allowing tumors to escape the immune response.[54]

A second mechanism used by intracellular pathogens is represented by exploitation of cellular receptors for cell entry. A first example of such a mechanism is represented by the malaria-causing protozoan *Plasmodium vivax,* which enters erythrocytes by using a chemokine promiscuous receptor called Duffy antigen receptor for chemokines.[55] A second example is HIV-1, which gains access to immune cells using CD4 as a primary cellular receptor and a chemokine receptor, CXCR4 or CCR5, as strain-specific coreceptors.[56] A variety of blocking agents, including agonists, antagonists, and antibodies, clearly have demonstrated a nonredundant role for CCR5 and CXCR4 in HIV infection. Moreover, a clear-cut role for CCR5 also has been demonstrated through the discovery that a mutant allele bearing a 32-base-pair deletion in the open reading frame (*CCR5-Δ32*), which encodes a truncated and inactive

▮Table 1▮
Chemokines and Chemokine Receptors Associated With Major Human Diseases*

Type	Diseases	Chemokines and Chemokine Receptors Involved
Inflammatory	Arthritis; colitis	Inflammatory CC and CXC
Infectious	Several acute and chronic bacterial and viral infections; sepsis	CXCR4 and CCR5; inflammatory CC and CXC
Autoimmune	Rheumatoid arthritis; systemic lupus erythematosus; multiple sclerosis	Inflammatory CC
Allergic	Asthma	CCL11 and CCR3, CCL22 and CCR4, CCL1 and CCR8
Neoplasia	Metastasis; angiogenesis	CXCL12 and CXCR4, CCL19 and CCR7
Graft rejection	Heart allograft	CCL5, CCL2, CCL3 and CCR5
	Kidney allograft	CXCL8 and CXCR1
	Lung allograft	CXCL9, CXCL10, CXCL11 and CXCR3
Vascular	Atherosclerosis; ischemia-reperfusion	CCL2 and CCR2, CX3CL1 and CX3CR1

* Principal human diseases associated with different chemokines and their receptors are listed. Correlations are supported by data referring to chemokine detection (see text), animal models (detailed in Table 2), and genetic evidence (detailed in Table 3).

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receptor not translocated to the membrane surface, is highly protective against initial infection in *CCR5-Δ32* homozygotes ▮**Table 3**▮.[57-64] The clinical relevance of chemokines and their receptors at the genetic level is best understood currently for the pathogenesis of HIV-1 infection, but it likely represents an important aspect of other clinical entities (Table 3).

Role of the Chemokine System in Autoimmune Diseases

Leukocyte recruitment, accumulation, and activation are common events in autoimmune disorders. The use of potent and cytotoxic immunosuppressive therapies for the treatment of these diseases reflects limited understanding of

▮**Table 2**▮
Phenotypic Abnormalities in Chemokine Receptor Gene-Targeted Animals*

Receptor	Phenotype	Insight
CXCR2	Defective recruitment of neutrophils[14]; increased sensitivity to *Toxoplasma*[15]; increased sensitivity to urinary tract infections[16]; abnormal wound healing[17]; reduced progression of atherosclerotic plaques[18]	Host defense; innate immunity; graft rejection (acute); atherogenesis
CXCR3	Reduction in acute allograft rejection[19]	Graft rejection (chronic)
CXCR4	Embryo, lethal defect[20]; defective development in cardiovascular, central nervous, and hematopoietic systems[20]	Embryogenesis
CXCR5	Abnormalities in lymph nodes and Peyer patch development[21]	Lymphoid organogenesis
CCR1	Imbalance of T_H1/T_H2 cytokines[22]; defective granuloma formation[22]; reduction in acute allograft rejection[23]; resistance to experimental autoimmune encephalitis[24]; reduced pancreatitis-associated lung injury[25]; increased nephritis-associated glomerular injury[26]; increased sensitivity to *Toxoplasma*[27] and *Aspergillus*[28]	Host defense; innate immunity; graft rejection (acute); adaptive immunity; autoimmunity; atherogenesis
CCR2	Defective recruitment of macrophages[29]; defective production of T_H1 cytokines[29]; increased sensitivity to *Listeria*,[30] *Aspergillus*[31]; normal susceptibility to *Leishmania*; reduction in acute allograft rejection[32]; resistance to experimental autoimmune encephalitis[33]; reduced progression of atherosclerotic plaques[34]	Host defense; innate immunity; graft rejection (acute); autoimmunity; atherogenesis
CCR3	Reduced eosinophil recruitment, but increased bronchoconstriction in airway allergic models[35]; reduced eosinophil recruitment but normal basophil and IL-4 concentrations in skin allergic diseases[36]	Type II immune response
CCR4	No effect in T_H2-dependent airway inflammation[37]; resistance to LPS-induced endotoxic shock[37]	No role in type II immune response
CCR5	Enhanced DTH reactions[38]; increased sensitivity to *Listeria*[38] and *Cryptococcus*[39]; reduced macrophage infiltration and demyelination[40]; normal sensitivity to experimental autoimmune encephalitis[41]; normal immune response to choriomeningitis virus[42]	Innate immunity; no role in antiviral immunity
CCR6	Defective homing of dendritic cells in Peyer patches[43]; impaired humoral response to orally administered antigens[43]; increased inflammation in contact hypersensitivity[44]; reduced DTH reactions[44]; reduction of eosinophil recruitment, airway resistance, IL-5 and IgE levels in airway allergic models[45]	Mucosal immune responses ; type II immune response
CCR7	Abnormalities in lymph nodes and Peyer patch development[46]; defective lymph node homing of dendritic cells[46]; reduced contact hypersensitivity[46]; reduced DTH reactions[46]	Lymphoid organogenesis; adaptive immunity
CCR8	T_H2 normal development, but aberrant cytokine production[47]; defective immune response to *Schistosoma* eggs[47]; normal immune response to *Mycobacterium*[47]; reduced eosinophil recruitment in airways allergic models[47]	Type II immune response
CCR9	Reduced intraepithelial homing of γδ+ T cells[48]	Mucosal immunity
CX3CR1	Defective NK cell recruitment[49]; reduced allograft rejection in immunosuppressed conditions[49]; normal susceptibility to experimental autoimmune encephalitis[49]; normal antibody-induced glomerulonephritis[49]; possible role in atherosclerosis[50]	Type I immune response

CC, chemokine with the first 2 cysteines in adjacent positions; CCR, CC receptor; CXC, chemokine with the first 2 of 4 total cysteines separated by an intervening amino acid; CXCR, CXC receptor; IL, interleukin; LPS, lipopolysaccharide; NK, natural killer.
* Phenotypic analysis in disease-free and selected pathologic conditions revealed insight into the possible roles of different molecules in distinct biologic processes.

▮**Table 3**▮
Allelic Variants of Chemokines or Chemokine Receptors Associated With Altered Disease Susceptibility or Progression in Humans

Molecule	Allelic Variant	Functional Effect	Clinical Correlation
CCR5	CCR5-Δ32	32-bp deletion in ORF (null allele)	Resistance to HIV infection[57]; improved kidney allograft survival[58]; reduced relapse rate in multiple sclerosis[59]
	M303	Protein truncation	Resistance to HIV infection[60]
	59029 A/G	Promoter SNP	Prolonged survival in patients with AIDS[61]
CCL5	–28G	Promoter SNP	Delayed AIDS progression[62]
CXCL12	–3A	3'-UTR SNP	Prolonged survival in patients with AIDS[63]
CCR2	V64I	Protein point mutation	Prolonged survival in patients with AIDS[64]

bp, base pairs; CC, chemokine with the first 2 cysteines in adjacent positions; CCL, CC ligand; CCR, CC receptor; CXC, chemokine with the first 2 of 4 total cysteines separated by an intervening amino acid; CXCL, CXC ligand; ORF, open reading frame; 3'-UTR, 3' untranslated region.

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the mechanisms that allow leukocytes to be recruited and sustain the chronic inflammatory reaction characteristic of autoimmune diseases. The importance of chemokines and chemokine receptors in the pathogenesis of autoimmunity was suggested initially by a number of animal models (Table 2) and has been confirmed by genetic evidence and clinical studies (Table 3).

In animal models of multiple sclerosis (MS), experimental autoimmune encephalomyelitis, and rheumatoid arthritis (RA), chemokine levels correlate with disease progression,[65,66] and the treatment of affected mice with chemokine antagonists or blocking antibodies has provided the first proof of the concept attesting the involvement of chemokines in autoimmune diseases.[67] The study of gene-targeted mice has revealed that the absence of a chemokine or its receptor might prevent or attenuate the insurgence of autoimmune diseases. For example, the absence of CCR1 and CCR2 is protective in experimental autoimmune encephalomyelitis,[67] and CCL3 deficiency in nonobese diabetic mice is protective for induced insulitis and spontaneous diabetes.[68]

Consistent with results obtained in animal models, clinical studies have demonstrated that chemokine and chemokine receptor expression is significantly altered during the evolution of certain autoimmune diseases. In patients with RA, clinical disease activity correlates with CCL2 and CCL5 levels,[69] which have been proposed as markers of disease activity. Moreover, methotrexate treatment of patients with RA correlates with a reduction of CCL5 levels.[70] Similarly, elevated serum levels of CXCL10 have been associated with clinical disease activity in systemic lupus erythematosus.[71] Elevated levels of inflammatory CC chemokines (CCL3, CCL4, and CCL5, in particular) and their receptors (CCR2, CCR3, and CCR5) also have been found in the central nervous system of patients with MS,[72,73] and T cells from patients with MS treated with IFN-β showed reduced CCR5 expression and migration in response to CCL5 and CCL3.[74] A relevant role of CCR5 in T-cell recruitment to brain lesions correlates with genetic evidence showing that patients with MS with the *CCR5-Δ32* allelic variant are not protected in the initial phase of the disease but have a lower risk of recurrent clinical disease[59] (Table 3).

Taken together, these observations make the chemokine system a potential target for the clinical management of autoimmune diseases. Indeed, several pharmaceutical companies are actively involved in the identification of small molecule receptor antagonists acting on chemokine receptors **Table 4**. The complexity of the chemokine system, however, imposes a need to clarify the role of specific chemokines in specific autoimmune disorders to understand which molecules are the best targets for each disease.

Role of Chemokines in Allergic Diseases

Allergic inflammation is a T_H2 disease associated with the selective recruitment of eosinophils and allergen-specific T_H2 lymphocytes. Selective expression of chemokine receptors on these leukocytes has been postulated to be the mechanism by which they are recruited selectively to the allergic site.[75]

In vitro and in vivo studies have provided evidence that CCR3, CCR4 and CCR8 are involved in the recruitment of T_h2 lymphocytes.[76] Mouse models with blocking antibodies against CCR3 and CCR4 ligands (CCL11, CCL22, and CCL17) show decreased airway inflammation and airway hyperresponsiveness,[77-79] whereas neutralization of the CCR8 ligand (CCL1) has no effect on the recruitment of T_h2 cells in the lung.[80] However, genetically modified animals do not confirm in vivo data obtained with inhibitors. CCR3–/– mice have reduced eosinophil recruitment to the lung after allergen challenge but increased airway hyperresponsiveness.[35] CCR4–/– mice show no protection against the development of allergic inflammation,[37] and 2 of 3 studies reported no effect of CCR8 deficiency on the development of allergen-driven airway inflammation.[81] The number of CCR4+ and CCR8+ T cells in human lung biopsy specimens is increased after allergen challenge, whereas CCR3+ cells are not detected. Moreover, CCL22 and CCL17 levels are increased after allergen challenge, but CCL1 and CCL11 expression are not detected.[82,83] Thus, several indications suggest a role for chemokines in allergic diseases, but a clear definition of the specific role of different molecules is still missing.

Role of Chemokines in Neoplastic Diseases

Chemokines and chemokine receptors have been found in almost all tumors, and the composition of the tumor infiltrate is related to tumor and stromal cell production of

Table 4
Chemokine Receptor Inhibitors Under Pharmacologic Development

Receptor	Clinical Phase	Indications
CCR1	I/II	Multiple sclerosis, psoriasis, rheumatoid arthritis
CCR2	I/II	Multiple sclerosis, rheumatoid arthritis
CCR3	I	Asthma, allergic diseases
CCR5	II/III	HIV, transplant
CXCR1	I	Ischemia-reperfusion injury, transplant
CXCR3	I	Transplant, psoriasis
CXCR4	I	HIV, cancer

CC, chemokine with the first 2 cysteines in adjacent positions; CCR, CC receptor; CXC, chemokine with the first 2 of 4 total cysteines separated by an intervening amino acid; CXCR, CXC receptor.

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chemokines. In vitro and in vivo experiments suggest that the chemokine CCL2 can suppress tumor growth, inducing a dense mononuclear infiltrate.[84] Moreover, it has been demonstrated that the same chemokine is able to act as an adjuvant to enhance T cell–dependent host antitumor response.[85] However, most clinical and epidemiologic studies suggest that chemokine expression might be advantageous for the tumor. In patients with ovarian and breast cancer, chemokine levels (CCL2 and CCL5) correlate with macrophage infiltration, lymph node metastasis, and clinical aggressiveness.[86] In contrast, high serum levels of CCL2 in patients with pancreatic cancer correlate with macrophage infiltration and good prognosis.[87]

Chemokines also might help the tumor to subvert the immune system by the polarization of the immune response to a T_H2 type to suppress specific anticancer responses. Examples are provided by Hodgkin lymphoma in which there is a prominent production of T_H2 chemokines by Reed-Sternberg cells[88] and by human herpesvirus 8 that encodes 3 viral chemokines (v-MIPI, v-MIPII, and v-MIPIII) that selectively attract T_H2 lymphocytes,[89] as described in the preceding text.

Cancer cells not only produce high amount of chemokines, but also express some functional chemokine receptors. CXCR4 seems to be expressed by the majority of cancer cells, including breast, prostate, pancreatic, lung, and ovarian carcinomas.[90] CCR7 is expressed by gastric and esophageal carcinoma cells and by melanoma cells.[91] Experimental murine cancer models provide some proof that cancer cells might use chemokine receptors to migrate to metastatic sites where their ligands are overexpressed.[91] Moreover, data from 600 patients with prostate cancer revealed that CXCR4 protein expression was higher in localized and metastatic prostate cancer than in normal or benign prostate tissue.[92]

Chemokines also might act as growth and survival factors in an autocrine and/or a paracrine manner. For example, melanoma cells express high levels of the receptor CXCR2 and also constitutively produce the chemokines CXCL1 and CXCL8 that stimulate, in an autocrine way, proliferation and survival.[93] Similarly, prostate cancer cells and glioblastoma cells express CXCR4 and CXCL12 stimulates their proliferation.[92]

Chemokines might regulate angiogenesis within the primary and metastatic tumors. ELR+ CXC chemokines and some CC chemokines promote angiogenesis, whereas ELR– CXC chemokines are antiangiogenic. CXCL10 levels in lung cancers are inversely related to tumor progression,[94] and CXCL5 levels in no–small cell lung cancer are correlated with the vascularity of the tumor and angiogenesis.[76] Although no evidence exists about their involvement in cancer pathogenesis, it is likely that chemokines will have important effects in cancer pathobiology because they affect different activities that impact cancer, such as leukocyte infiltration, metastatic potential, tumor growth, and angiogenesis.[95-97]

Role of Chemokines in Transplant Rejection

Chemokine effects on inflammatory and immune responses not surprisingly influence several aspects of allograft biology.[98] Allograft analysis at different time points after transplantation indicates that 2 chemokine cascades are activated.[99] A first set of events takes place in the hours and days after allograft surgery, and the event s are associated with trauma and ischemia-reperfusion graft damage. This acute reaction involves local production of proinflammatory cytokines, vascular endothelium activation, and chemokine production. In an early phase, ELR+ CXC chemokines dominate and have a central role in neutrophil recruitment and subsequent ischemia-reperfusion–dependent tissue damage. Experimental approaches based on blocking antibodies,[100] gene-targeted mice (Table 2), and pharmacologic inhibition of chemokine receptors (Table 4) have demonstrated that attenuation of neutrophil infiltration by interfering with ELR+ CXC chemokines significantly reduces tissue damage and preserves organ function. Subsequently, inflammatory CC chemokines directed at mononuclear cells, such as CCL2, CCL3, and CCL4, also are produced, although at considerably lower levels.[100] Consistent with these observations, blocking antibodies to inflammatory CC chemokines[101] and targeted deletion of their respective receptors CCR1, CCR2, or CCR5 suppresses acute allograft rejection (Table 2).

After resolution of the initial inflammatory phase, a delayed reaction associated with the infiltration of alloantigen-specific T lymphocytes takes place. This second phase is controlled mainly by the T cell–specific ELR– CXC chemokines CXCL9, CXCL10, and CXCL11, induced by local production in the allograft of IFN-γ.[102] The importance of this group of chemokines has been demonstrated by the protective effect of blocking antibodies[102,103] and by the extended allograft survival in CXCR3–/– mice (Table 2). It is interesting to note that the infiltration of effector CD8+ T cells, responsible for the type I polarization of the immune response with IFN-γ production and consequent induction of CXCR3 agonists (Figure 3), requires the phagocyte-dependent acute phase.[99] This indicates that therapeutic intervention focusing not only on chemokines responsible for recruitment of final effectors (CXCR3-dependent), but also on chemoattractants involved in the acute reaction (mainly dependent on inflammatory CC and ELR+ CXC

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chemokines), could result in prolonged allograft survival, especially if used in conjunction with subtherapeutic doses of immunosuppressive agents.[19,49,104] Chemokine receptor antagonists targeting CXCR1, CXCR3, CCR1, and CCR5 are under development (Table 4).

The importance of chemokines and their receptors in allograft rejection has taken on greater importance because of new genetic (Table 3) and clinical studies. Expression levels of CXCL10 and its receptor CXCR3 in endomyocardial, lung, and liver biopsy specimens of transplanted organs correlates with ongoing acute allograft rejection.[105,106] Similarly, in renal transplants, inflammatory CC chemokines and ELR– CXC chemokines have been detected during rejection episodes,[107,108] and their levels correlate with the histologic grade of rejection,[109] suggesting a relationship between type and level of chemokines locally produced, leukocyte infiltration, and allograft evolution. Although the number of studies is limited, existing data indicate that the presence of specific chemokines and their receptors is predictive of increased risk for acute or chronic allograft rejection.

Chemokines also might have a role in 2 aspects of major relevance in the management of transplant recipients, ie, host responses to opportunistic infectious agents (see earlier text) and allograft-accelerated arteriosclerosis (see subsequent text).

Role of Chemokines in Vascular Diseases

Atherosclerotic plaques are thought to result from an inflammatory response to arterial damage.[110] Chemokines are involved in this process, at first because they can mediate monocyte adhesion to the vascular endothelium and migration to subendothelium (where they become the foam cells originating fatty streaks) and later by activating macrophages and promoting migration of smooth muscle cells into the intima. Chemokines also might influence thrombus formation over the plaque.[111,112] Animal models of atherosclerosis have revealed a role for many chemokines, including CXCL8, CXCL12, CXCL10, and CCL1.[18,113-115]

CCL2–/– and CCR2–/– mice have 65% to 85% less arterial lipid deposition than normal mice in hypercholesterolemia models. Disease reduction is correlated with a decrease of macrophages in the arterial wall, suggesting that CCL2 might attract CCR2-bearing monocytes in the vessel wall.[29,116] Moreover, proof of the concept of the role of CCL2 and CCR2 in this disease derives from the use of a CCL2 antagonist that prevents monocyte recruitment in a coronary artery remodeling system.[117]

CX3CR1–/– mice also are protected against diet-induced atherosclerosis.[118,119] It has been reported that a polymorphic variant of this receptor (V280) correlates with protection from coronary artery disease in humans.[50,120] The relevance of the chemokine system in the human disease also is supported by data from human lesions, as several chemokines, including CCL2, CCL5, CCL3, CCL11, and CX3CL1, have been detected within atherosclerotic plaques.[121,122] Moreover, clinical data reveal that members of the statin family inhibit expression of CCL2,[123] suggesting that these molecules reduce atherosclerotic risk in part by inhibiting expression of inflammatory chemokines and the consequent recruitment of macrophages in the arterial wall.

Chemokines and Their Clinical Uses

Although the role of chemokines and their receptors in the pathogenesis of different human diseases has been evaluated extensively in the scientific literature (and summarized briefly herein), the possible relevance of chemokines and their receptors as biomarkers has been evaluated in a limited number of diseases.[124] Significant alterations in chemokine profiles have been highlighted in a number of clinical studies, but a convincing indication that chemokine determination could be applied to the diagnosis or the monitoring of most diseases is still missing.

In general, determination of chemokines and their receptors has been proven useful for monitoring disease activity and predicting relapses,[125] monitoring the efficacy of surgical and pharmacological therapies,[126] and providing prognostic indications.[127] However, it also is important to realize that chemokines and their receptors have some significant theoretical limitations as potential biomarkers.

Chemokines are rarely organ-specific and almost never specific to a particular disease, and their pleiotropic and redundant activity translates to a very similar pattern of changes in different clinical circumstances. Therefore, chemokines and their receptors are not likely to be ideal biomarkers for the diagnosis of or screening for diseases. In this context, it is of interest to note that none of the chemokine–chemokine receptor gene-targeted mice demonstrated increased susceptibility to spontaneous infections, consistent with the idea that sufficient redundancy exists within the system for baseline host defense. On the other hand, in vivo results in gene-targeted animals exposed to specific pathogens surprisingly indicated a nonredundant role of individual chemokines in specific diseases. Therefore, spatial and temporal regulation might be more functionally significant than would be expected by extrapolating from in vitro results and suggest a role for chemokines in modulating other aspects of the inflammatory response beyond simple leukocyte recruitment (Figure 2), including cross-talk with primary inflammatory and immune cytokines[10,128] (Figure 3).

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Thus, emerging data indicate that the high level of redundancy suggested by in vitro studies might not be so relevant in vivo and should not diminish the interest for the evaluation of distinct chemokine–chemokine receptor systems in specific clinical conditions. The specificity that certain chemokines seem to demonstrate in the amplification of specific inflammatory responses is providing the rational for new therapeutic antichemokine approaches (Table 4).

Measurement of Chemokines in Body Fluids and Tissues

Among the numerous chemokines identified, only a limited number of chemokines is measured in clinical laboratory practice.[1,129] Nevertheless, technological progress during the last 20 years has generated a large series of analytic approaches that are being implemented in routine clinical laboratories **Table 5**. Immunoassays have been introduced rapidly in clinical practice for chemokine measurement in biologic fluids. Their specificity is high owing to the use of monoclonal antibodies. The detection limit is highly dependent on the quality of the capture monoclonal antibody, but in general these assays have high sensitivity, with the evaluation limit of around 10 pg/mL for most chemokine test kits.

The advantages of immunoassays are their excellent reproducibility (coefficient of variation, 5%-10%) and their ease of use.[130,131] Special attention must be given to the sampling and storage of biologic fluids, the major risks being the degradation of chemokines during storage and the cellular production or release of chemokines after sampling. Plasma or serum can both be used, and the samples are to be stored deep-frozen.

ELISPOT represents an interesting approach to determine the number of chemokine-producing cells in a heterogeneous population. In this assay, a stimulated cell suspension is laid on a microplate well coated with antibodies directed against the chemokine of interest.[131] ELISPOT is more sensitive than classic enzyme-linked immunosorbent assays, allowing for the detection of as few as 3 chemokine-secreting cells per 10^5 total cells. Moreover, because of its sensitivity, ELISPOT can be used to detect circulating chemokine-producing cells without in vitro stimulation. A biochip array technology based on a sandwich chemiluminescent immunoassay also has been developed, allowing for the simultaneous quantitative detection of multiple cytokines and chemokines in a single patient sample.

The local and paracrine activity of chemokines explains their low circulating levels and constitutes a strong limitation in the interpretation of their concentrations in body fluids. The tissue distribution can be studied at the protein or messenger RNA level (Table 5). Immunohistochemical analysis allows detection of chemokines or their receptors within tissues.[132] Three successive steps (cell fixation, cell

Table 5
Principal Methods for Chemokine Detection and Measurement

Method	Target Molecule	Sample	Advantages	Limitations
ELISA	Protein	Cell culture supernatant, cell lysate, serum, other biologic fluids	Highly specific and sensitive; rapid; adequate for high-throughput screening	Requires specific MoAb, high costs
IRMA	Protein	Cell culture supernatant, cell lysate, serum, other biologic fluids	Highly sensitive	Time-consuming, high cost, not adequate for high-throughput screening
Biochip array	Protein	Cell culture supernatant, cell lysate, serum	Simultaneous quantitative detection from a single sample	High costs
ELISPOT	Protein	Cell suspension	Highly specific and sensitive; provide indication of the cell source	Time-consuming; limited panel of molecules detectable
Immunohistochemical analysis	Protein	Tissues and cells	Provide indication of the cell source; allow for multiple labeling	Requires specific MoAb; time-consuming; not adequate for high-throughput screening
Flow cytometry	Protein	Cells	Adequate for high-throughput screening	Requires specific MoAb; high costs
In situ hybridization	mRNA	Tissues and cells	Does not require specific MoAb (complementary to immunohistochemical analysis)	Time-consuming; high costs
RT-PCR	mRNA	Tissues and cells	Possible for simultaneous detection of multiple compounds; adequate for high-throughput screening	Semiquantitative
DNA/oligo array	mRNA	Tissues and cells	Innovative application; simultaneous quantitative detection from a single sample	High costs

ELISA, enzyme-linked immunosorbent assay; IRMA, immunoradiometric assay; MoAb, monoclonal antibody; mRNA, messenger RNA; RT-PCR, reverse transcriptase–polymerase chain reaction.

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permeabilization, cytokine visualization using a specific antibody) are followed by a final visualization step, based on immunofluorescent or immunoenzymatic techniques. The possibility to combine double or triple labeling allows the identification of the phenotype of chemokine-producing cells. Recently, the detection of intracellular chemokines by flow cytometry also has been reported.[131,133] Detection in tissue specimens or cell suspensions of chemokine messenger RNA by in situ hybridization also allows the identification of cells directly involved in chemokine synthesis among a heterogeneous population. Although it is time-consuming and expensive, this method does not require specific antibodies and, therefore, can be considered complementary to immunohistochemical analysis.

Conclusion

An impressive amount of information has linked the most common human diseases to chemokines. Although in vitro this complex system is characterized by an important degree of redundancy, data obtained with gene-targeted animals indicate that in vivo specificity for selected disorders might exist. The chemokine system also is characterized by a significant and, at present, only marginally defined degree of genetic variance in the general population, with significant effects on disease susceptibility and prognosis. A number of methodological approaches are available for specific and sensitive determination of chemokines, in biologic fluids and in tissues, and some of these methods are adequate for high-throughput applications. Thus, although clinical laboratory use of chemokines as biomarkers is limited, the time is ready to design clinical trials to specifically assess the impact of individual chemokine determinations on clinical decisions in specific diseases.

From the [1]Laboratory of Leukocyte Biology and [2]Laboratory of Clinical Pathology, Institute of General Pathology, Faculty of Medicine, University of Milan, Milan, Italy.

Address reprint requests to Dr Corsi: Laboratory of Clinical Pathology, Institute of General Pathology, Medical Faculty, University of Milan, Via Mangiagalli 31, I-20133, Milan, Italy.

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