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Updates on cells and cytokines

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Chemokines in allergic inflammation

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Cell motility plays a critical role in organogenesis during embryonic development, in immune surveillance, and in inflammatory responses. Chemokines are a group of chemotactic cytokines that induce cellular locomotion. Chemokines have additional functions, but chemotactic activity is their hallmark.^{1, 2} Their molecular weights are in the range of 8 to 10 kd. Like most cytokines, chemokines are pleiotropic. They also have significant structural homology. Many chemokine receptors bind more than one ligand. Consequently, chemokines have extensive overlapping functions.

CLASSIFICATION

The classification of chemokines (Fig. 1) is based on the position of the first two N-terminal-conserved cysteine residues. One family has two N-terminal cysteine (C) residues separated by one nonconserved residue. This family is called the CXC (X = any amino acid) or α chemokine family. The second family has two N-terminal-conserved cysteine residues in juxtaposition (the CC or β family). The third family has a single cysteine residue in the conserved position (the C or γ family). The genes for the CXC, CC, and C chemokines are clustered on chromosomes 4, 17, and 1, respectively. The exception is stromal cell-derived factor-1, which is

Abbreviations used

MCP:	Monocyte chemotactic peptide
MIP:	Macrophage inflammatory protein
PMNs:	Polymorphonuclear leukocytes

located on chromosome 10. There is 20% to 50% homology among the CXC chemokines and 28% to 45% homology among the CC chemokines at the amino acid level. There is 20% to 40% homology among the members of the three chemokine families. The nuclear magnetic resonance and crystallographic structures of some chemokines reveal a C-terminal α helix, followed by a β -pleated sheet and a loose N-terminal strand. The CC chemokines have an extended N-terminal strand as compared with the CXC chemokines. The similarity in the three-dimensional structure perhaps explains the functional overlap among the chemokines.

CELLULAR SOURCES

Chemokines are produced by a variety of cells. Virtually all nucleated blood and tissue cells that have been studied produce chemokines. Further, platelets secrete some chemokines (e.g., RANTES). In many chronic inflammatory conditions, epithelial cells and fibroblasts appear to be the major source of CC chemokines.

BIOLOGIC ACTIVITY OF THE CHEMOKINES

The important functions of CXC and CC chemokines and their target cells are presented in Fig. 2. The functions of the chemokines can be broadly classified as shown in Table I.

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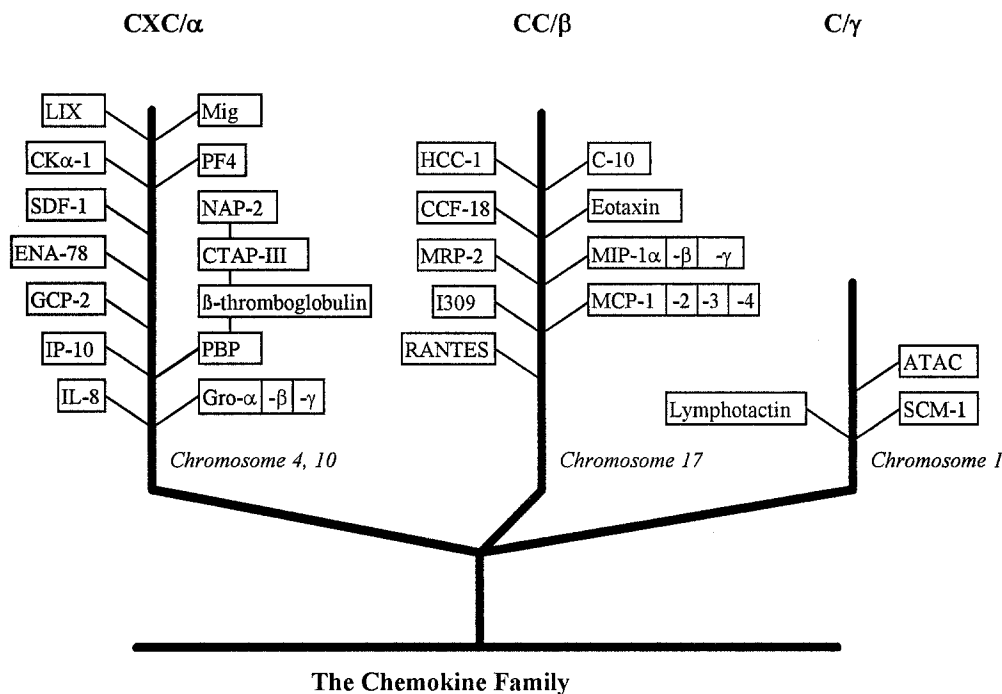


FIG. 1. Classification and members of the chemokine family. *CCF-18*, CC chemokine F-18; *CTAP*, connective tissue-activating peptide; *ENA*, epidermal cell-derived neutrophil chemotactic activity; *GCP*, granulocyte chemotactic protein; *Gro*, growth factor-related oncogene; *HCC*, hemofiltrate-derived CC chemokine; *IP-10*, inducible protein-10; *LIX*, lipopolysaccharide-induced CXC chemokine; *Mig*, monokine induced with γ -interferon; *MRP*, MIP-related protein; *NAP*, neutrophil-activating peptide; *PBP*, platelet basic protein; *SDF*, stromal cell-derived factor. Acronyms: C10: MRP-1(MIP-related protein-1); Gro: MIP-2, melanocyte growth-stimulating activity (MGSA), cytokine-induced neutrophil chemoattractant (CINC, rat homolog); I309: TCA-3 (T-cell activation gene-3, mouse homolog); IL-8: KC (mouse homolog); MCP-1: monocyte chemotactic and activating factor (MCAF), JE (mouse homolog); MCP-3: fibroblast-induced cytokine (FIC), MARC; SDF-1: pre-B cell growth stimulating factor (PBSF).

An important difference between the CXC and the CC chemokines is that the former are mostly active on polymorphonuclear leukocytes (PMNs), whereas the latter chemokines have preferential activity on mononuclear leukocytes, eosinophils, and basophils. Very little is known about the function of C chemokines. Lymphotactin, the first identified C chemokine, induces chemotaxis of T cells.

CXC CHEMOKINES

Chemotactic activity

Most members of the CXC chemokine family have chemotactic activity for neutrophils. IL-8 is considered the most potent chemotactic factor for PMNs. Antibodies against IL-8 and other CXC chemokines block neutrophilic inflammation in various models. Some CXC chemokines are chemotactic for T cells. For example, IL-8 attracts a subpopulation of CD8⁺, CD56⁺ T cells.

Nonchemotactic activity

IL-8 is a potent activator of PMNs. It causes the secretion of granular enzymes (β -glucuronidase, elastase, gelatinase), induces respiratory burst, and stimulates PMNs for phagocytosis. It upregulates the expres-

sion of CR1 and CD11/CD18 on the neutrophil surface. IL-8 has weak histamine-releasing activity for IL-3-primed basophils. However, the preincubation of basophils with IL-8 inhibits histamine release induced with potent activators such as monocyte chemotactic peptide (MCP)-1. IL-8, Gro- α , and platelet factor 4 suppress myeloid colony formation. Stromal cell-derived factor-1 has the opposite effect. Further, it stimulates the growth of pre-B cells. IL-8 and many other CXC chemokines contain an ELR (Glu-Leu-Arg) motif. The ELR chemokines have angiogenic activity. The non-ELR CXC chemokines such as platelet factor 4, inducible protein-10 (IP-10), and monokine induced with γ -interferon (mig) have angiostatic effects. CTAP III has moderate mitogenic activity for fibroblasts and stimulates collagen synthesis. IL-8 and melanocyte growth-stimulating activity (MGSA) possess growth factor-like activity for melanocytes and melanoma cells.

CC CHEMOKINES

Chemotactic activity

Most CC chemokines are chemotactic for monocytes and lymphocytes. The exception is eotaxin, which thus far appears to be specific for eosinophils. A moderate

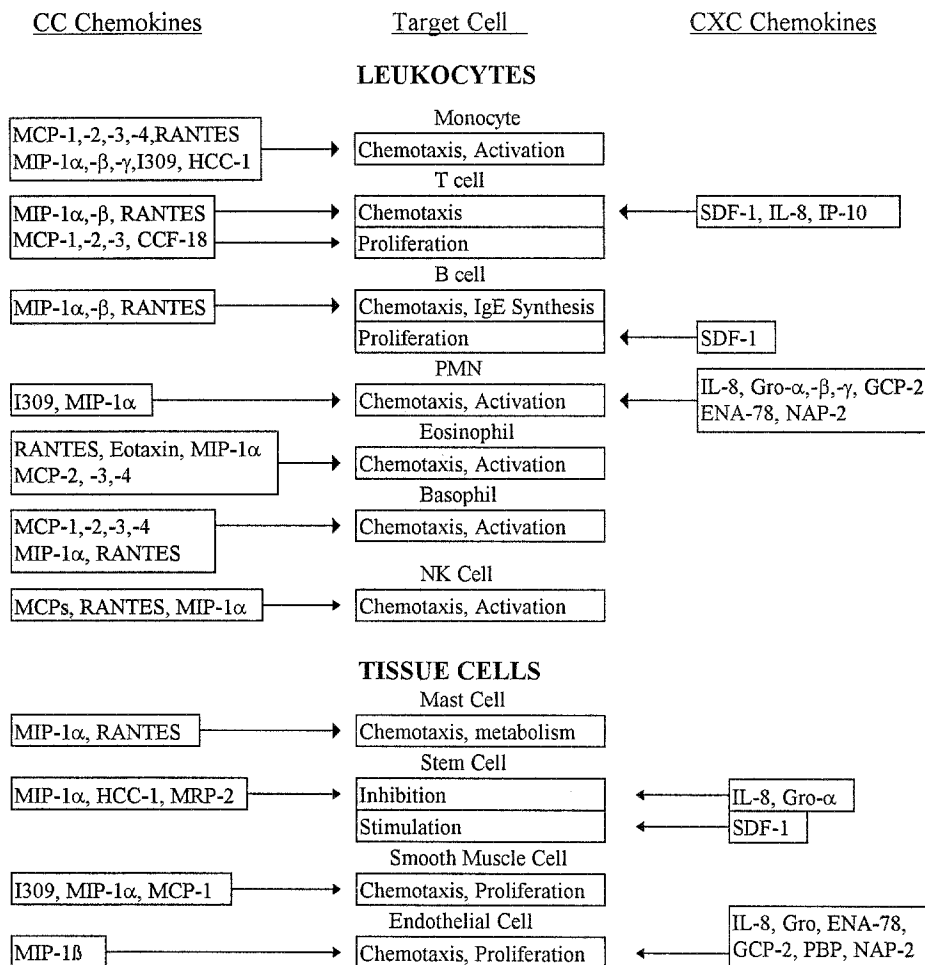


FIG. 2. Chemokines affecting the function of leukocytes and tissue cells. *I309*, TCA-3 (T-cell activation gene-3, mouse homolog); *HCC-1*, hemofiltrate-derived CC chemokine-1; *CCF-18*, CC chemokine F-18; *SDF-1*, stromal cell-derived factor-1; *IP-10*, inducible protein-10; *Gro*, growth factor-related oncogene; *ENA*, epidermal cell-derived neutrophil chemotactic activity; *GCP*, granulocyte chemotactic protein; *PBP*, platelet basic protein; *NAP*, neutrophil-activating peptide.

degree of T-cell selectivity exists for various CC chemokines. Macrophage inflammatory protein (MIP)-1 β is predominantly chemotactic for CD4+ T cells. RANTES selectively attracts memory subtype CD45RO+ CD4+ T cells. RANTES, eotaxin, MCP-3, MCP-4, and to some extent MCP-2 and MIP-1 α , are chemotactic for eosinophils.³

Nonchemotactic activity

Histamine-releasing activity. Many previously described non-IgE-dependent histamine-releasing factors belong to the CC chemokines. MCP-1, MCP-3, MIP-1 α , RANTES, MCP-2 (in order of potency) have histamine-releasing activity.⁴ MCP-1 is one of the most potent secretagogues for basophils.

Activation of eosinophils and other cells. The activation of eosinophils plays a critical role in allergic inflammation. RANTES causes the secretion of eosinophil cationic protein and superoxide from eosinophils. Further,

TABLE I. Functions of chemokines

1. Chemotaxis
2. Immunoregulation
3. Antiviral immunity
4. Regulation of hematopoiesis
5. Regulation of angiogenesis
6. Cell growth and metabolism

it makes eosinophils hypodense. Most CC chemokines, except eotaxin, stimulate monocyte cytotoxic activity and upregulate adhesion molecules. They also augment T-cell proliferation and cytokine production.

Chemokines and viral infection. An emerging and remarkable development in the field of chemokines is their involvement in viral immunity, especially HIV infection. MIP-1 α , MIP-1 β , and RANTES have direct antiviral effects on HIV. They suppress HIV proliferation. Further, three chemokine receptors, CXCR-4 (fusin/

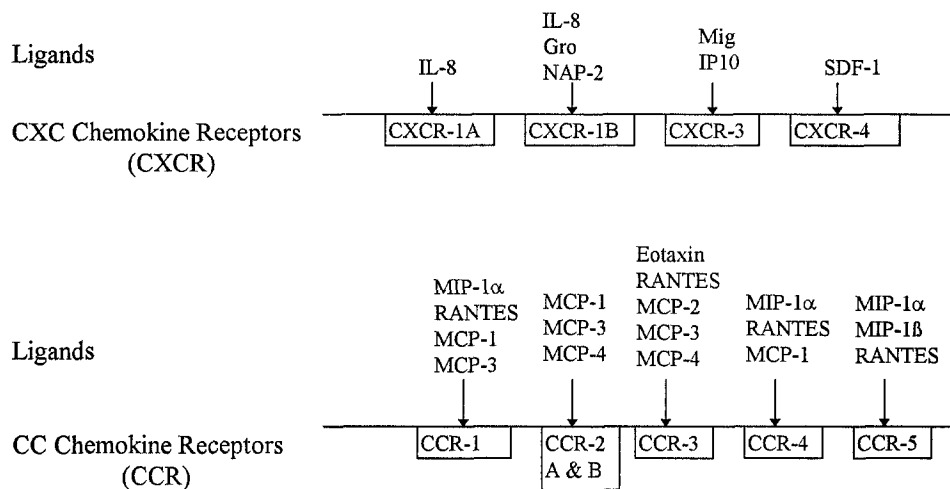


FIG. 3. Chemokine receptors and their ligands. *Gro*, Growth factor-related oncogene; *NAP*, neutrophil-activating peptide; *Mig*, monokine induced with γ -interferon; *IP*, inducible protein; *CXCR-1A*, IL-8R type A; *CXCR-1B*, IL-8R type B; *CXCR-4*, fusin/LESTR; *CCR*, CKR.

LESTR), CCR-5, and to some extent CCR-3 play a critical role in the entry of HIV into T cells and macrophages and subsequent formation of syncytium. Individuals carrying a mutation in CCR-5 are resistant to HIV infections but are otherwise normal. This finding creates an exciting possibility for development and use of CCR-5 antagonists in the treatment of AIDS without interfering with the immune function. Chemokines may be important in immunity against other viruses as well. The knockout mice for MIP-1 α gene have a reduced inflammatory response to influenza virus. Further, they are resistant to coxsackievirus-induced myocarditis.

Chemokine receptors and the signal transduction mechanism

The chemokine receptors belong to the seven-transmembrane-spanning G-protein-coupled receptor superfamily. Ten chemokine receptors have been cloned, and their ligands have been identified (Fig. 3). Many more chemokine receptor-like genes have been cloned, but their ligands are currently unknown. Among the CC chemokine receptors, CCR-3 is expressed in high numbers (40,000 to 60,000 per cell) exclusively on eosinophils; and it binds eotaxin, RANTES, and MCP-3.⁵ It is likely that CCR-3 plays a crucial role in mediating eosinophilic inflammation. The binding of the chemokines to the receptors causes the association of GTP to the α subunit of the heterotrimeric G protein. The GTP-bound α subunit activates phospholipase C and formation of inositol triphosphates (inducible protein 3). Chemokines activate phosphatidyl inositol-3 kinase, which is also linked to phosphatidyl inositol metabolism. The inducible protein 3-induced rise in intracellular Ca^{2+} activates many cellular processes including degranulation of leukocytes.

The chemotaxis of cells involves the formation of uropods and/or lamellipods and coordinated, sequential

attachment and detachment of adhesion molecules. Rho and Rac, belonging to the small family of p21 G proteins, are specifically responsible for the generation of stress fibers and lamellipods, respectively. IL-8 activates Rho in neutrophils. The activated Rho polymerizes actin and leads to the generation of stress fibers. The spatial and temporal coordination of expression of adhesion molecules and the generation of uropods during cell movement is critical. RANTES causes the localization of adhesion molecules such as intercellular adhesion molecule-1, intercellular adhesion molecule-3, CD43, and CD44 in the uropods that are formed de novo in the leading front of a migrating cell. This process promotes the adhesion of the leading edge of the cell and detachment of the rear of the cells.

MCP-1, RANTES, and IL-8 activate tyrosine kinases in monocytes, T cells, and neutrophils, respectively. The tyrosine kinases are linked to the Ras-MAP kinase pathway and are involved in various cellular functions and proliferation. The importance of tyrosine kinases and the Ras-MAP kinase pathway for chemokine functions is currently unknown.

CLINICAL RELEVANCE

CC chemokines attract and activate eosinophils, basophils, and lymphocytes. Because these cells are the hallmark of allergic inflammation, CC chemokines may play an important role in the pathogenesis of allergic diseases.

Allergic rhinitis

The MCP-1 level increases in nasal secretions from patients with allergy in the allergy season. An allergen challenge increases the recovery of MCP-1, MIP-1 α , RANTES, and IL-8 from nasal secretions.⁶ MCP-1 appears late in the late-phase reaction. MIP-1 α shows a biphasic response with an initial peak in the immediate-

phase reaction and the second peak in the late-phase reaction. RANTES peaks at 3 hours after the allergen challenge and precedes the development of the late-phase reaction. The appearance of RANTES before the late-phase reaction may imply a causative effect.

Allergic asthma

Epithelial cells from bronchial biopsy specimens obtained from patients with asthma show increased expression of MCP-1. The concentrations of MCP-1, MIP-1 α , RANTES, and IL-8 in the bronchoalveolar lavage fluid are elevated in patients with mild asthma.⁷ Further, an endobronchial allergen challenge increases the concentration of RANTES in the bronchoalveolar lavage fluid. The eosinophil chemotactic activity of bronchoalveolar lavage fluid from patients with asthma is abolished by antibodies against RANTES and MCP-3. The involvement of CC chemokines has been studied in various animal models. Challenge with the sensitizing allergen induces the production of MCP-1, MCP-3, MIP-1 α , RANTES, and eotaxin in sensitized animals. Pretreatment of animals with antibodies against MIP-1 α , RANTES, and MCP-3 inhibits the influx of eosinophils and other inflammatory cells into the airways.⁸ The latter findings provide a rationale for the development of specific antagonists for CCR-3, which is likely to mediate the action of the CC chemokines on eosinophils.

Atopic dermatitis

Lesional skin scales obtained from patients with atopic dermatitis show high levels of RANTES. The major source of cutaneous RANTES appears to be dermal fibroblasts. The presence of RANTES may explain the dominance of mononuclear cell infiltrates and eosinophils in the lesion. The injection of allergens into the skin of atopic subjects induces cutaneous expression of messenger RNA for MCP-3 and RANTES. The expression of mRNA for MCP-3 peaks at 3 hours, whereas that for RANTES peaks at 24 hours. The expression of mRNA for MCP-3 paralleled the kinetics of eosinophil influx in the late-phase reaction. The latter study further implies the involvement of CC chemokines in the pathogenesis of the late-phase allergic reaction.

Other inflammatory diseases

Chemokines are involved in a variety of infectious and inflammatory diseases. Virtually any inflammatory condition studied has demonstrated increased levels of chemokines. Some of the diseases with increased chemokine production include rheumatoid arthritis, gout, hypersensitivity pneumonitis, pulmonary fibrosis, multiple sclerosis, glomerulonephritis, transplant rejection reactions, and granulomatous diseases such as tubercu-

losis and sarcoidosis. The relative ratio of CXC versus CC chemokines appears to determine the nature of the inflammatory response. For example, IL-8 and Gro are the dominant chemokines in neutrophil-rich inflammatory diseases such as rheumatoid arthritis, bacterial pneumonia, and sepsis. RANTES is also detectable in the synovial fluid from rheumatoid arthritis, but its level is low compared with IL-8. In contrast, RANTES and MCP-1 are abundant in mononuclear cell-rich inflammatory conditions such as granulomatous inflammation, sarcoidosis, and tuberculosis. Interestingly, few eosinophils are present in these conditions. It is likely that the presence of additional cytokines such as IL-5, IL-12, and interferons modulates the response of eosinophils to RANTES. IL-5 promotes RANTES-induced eosinophil chemotaxis. The presence of IL-12, interferon- α , and interferon- γ , which are known to inhibit the synthesis of IL-5, may abrogate the response of eosinophils to RANTES. In the latter environment a mononuclear cell infiltrate without eosinophils dominates the inflammation.

In summary, chemokines have emerged as critical mediators of inflammation. They frequently act as the final common pathway for other cytokines and interleukins and directly activate effector cells of inflammation. Because of their chemotactic activity for eosinophils, T cells, and basophils, CC chemokines such as RANTES, eotaxin, and MCP-3 may play a crucial role in the pathogenesis of allergic inflammation.

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