Promega Notes Magazine **Number 44, Nov. 1993, p.34**

The Chemokine Network

Coordinate Mediators of Early Inflammatory Events

James M. Balwit and Mary Haak-Frendscho
Promega Corporation

A successful host response to infection generally requires the accumulation of immune cells at the site of tissue damage. This cellular accumulation is a critical step in the normal inflammatory process and requires the migration and attachment of specialized inflammatory cells to blood vessels surrounding the site. Here we review a newly recognized superfamily of chemoattractant cytokines, the chemokines, which play an important regulatory role in these early events of inflammation.

Introduction

Inflammation is characterized by the orderly recruitment and deployment of specific subsets of leukocytes to sites of infection or tissue damage. While the tissue surrounding a blood vessel serves as the site for the final events of the inflammatory process, the outcome is largely determined by earlier events that occur within or along the wall of the blood vessel. Initial vascular changes result in higher blood flow, enhanced vascular permeability and selective accumulation of inflammatory cells at post-capillary venules adjacent to the damaged tissue. Accumulation of immune cells, the most prominent feature of inflammation, occurs as leukocytes migrate toward the site of tissue damage and attach to endothelial cells lining the microvessels in these tissues. These crucial, early events are regulated by soluble mediators and adhesion molecules that coordinate the interaction of leukocytes with and prepare them for localized combat with the invading pathogen.

Leukocyte migration

At the onset of inflammation a variety of leukocytes migrate to the post-capillary venules surrounding the damaged tissue. This migration is a *directional*, nonrandom process, as well as a *selective* event. The composition of the leukocyte population and their activities at inflammatory sites are highly regulated, both temporally and in intensity. Among the first immune cells to arrive at the site of tissue damage are neutrophils which initiate a rapid, nonspecific phagocytic response. This initial response is augmented both in magnitude and specificity as monocytes and specific subsets of T and B cells accumulate at the inflammatory site and become activated to produce protective and inflammatory molecules.

Through a process known as chemotaxis, immune cells migrate up concentration gradients of a variety of products present at sites of inflammation. These products, also known as chemoattractants or chemotactic factors, include bacterial products such as formylmethionyl peptides; C5a, a breakdown product of the complement cascade; and phospholipid metabolites secreted by activated macrophages and endothelial cells, including leukotriene B4 and platelet activating factor. More recently, chemoattractant cytokines released by cells at sites of local tissue damage have been recognized as important mediators of inflammation due to their ability to selectively enhance migration of specific

leukocyte subsets. While structurally distinct, these chemotactic factors are related in their ability to have multiple effects on inflammatory tissues; in addition to mediating cell migration, chemoattractants typically activate their target cell and induce leukocyte attachment to endothelial cells.

Leukocyte attachment

The normal flow of blood within vessels rapidly disrupts the fluid-phase concentration gradients of chemoattractants. Moreover, due to the high shear force in blood vessels, cell migration requires that inflammatory cells associate closely with vascular endothelium to avoid being swept past the site of infection or tissue damage. Indeed, attachment of leukocytes to endothelial cells is a requirement for emigration of these cells from blood vessel into the surrounding inflammatory tissues (extravasation). A number of adhesion molecules, including selectins and integrins, mediate the attachment of specific leukocyte subsets to endothelial cells at inflammatory sites. Weak interactions between L selectin, which is constitutively expressed on the surface of leukocytes (e.g., neutrophils), and an endothelial cell glycoprotein, sialyl Lewis^x, allows the leukocytes to roll along the endothelial cells lining the blood vessel. Selectin-mediated rolling decelerates leukocyte flow rate in the vessel, allowing cells to sample the local vascular environment for signaling molecules, even under the conditions of increased blood flow at sites of inflammation. At these sites, activated endothelial cells secrete cytokines and chemotactic factors which rapidly enhance surface expression of beta-2-integrins on leukocytes. These adhesion molecules firmly tether the activated leukocytes to endothelial cells, serving as molecular footholds for migration across the endothelium into the surrounding inflammatory tissues. The fully-activated leukocytes can subsequently enhance the localized inflammatory response by releasing toxic metabolites and protective enzymes, and by secreting a variety of cytokines, including chemokines.

Chemokines

Chemokines, also known as intercrines, comprise a superfamily of small, secreted proteins that mediate inflammation by inducing chemotaxis and activation of a variety of inflammatory cells. Members of the chemokine superfamily possess a conserved structural motif containing two cysteine pairs. Based on the arrangement of the cysteines within this motif, chemokines are divided into two subfamilies. The first cysteine pair of the C-X-C chemokines (alpha-intercrines) are separated by an intervening amino acid, while the first cysteine residues of the C-C chemokines (beta-intercrines) are adjacent. C-X-C chemokines include IL-8, MGSA, gamma-IP-10, ENA-78, platelet factor 4, platelet basic protein and beta-thromboglobulin. Members of the C-C chemokine subfamily include MIP-1alpha and MIP -1beta, MCAF, MCP-2, MCP-3, RANTES, I-309 and HC14. Chemokines demonstrate 20-45% homology at the amino acid level and are basic heparin-binding proteins.

A number of cells typically present at inflammatory sites secrete chemokines upon induction with inflammatory mediators (Table 1). For example, TNF and IL-1 induce the transcription of IL-8, the prototypical C-X-C chemokine, within one hour of stimulation (1). The 5´ -enhancer-promoter region of the human IL-8 gene contains potential binding sites for a number of known regulatory factors, including NF-kappa-B, AP-3, C/EBP-like factor, octamer binding protein and other nuclear factors (1). Transcriptional control of C-C chemokines has been observed as well, and a number of upstream regulatory sequences have been identified (reviewed in 2 and 3).

Bioactivities of chemokines

Chemokines mediate selective chemotaxis

Chemokines, by definition, demonstrate chemotactic activity and a general pattern of chemokine-mediated chemotaxis has emerged-as a rule, C-X-C chemokines attract neutrophils, but not macrophages, while CC chemokines preferentially induce migration of macrophages. Particular chemokines induce *selective migration of leukocyte subsets* which differ both in phenotypic markers and activation state. This has led to the view that the cellular composition at inflammatory sites depends on the combinatorial effects of multiple chemokines, each with selective chemotactic activities (4). For example, while the C-C chemokines RANTES, MIP-1alpha and MIP-1beta all induce macrophage migration, they have distinct chemoattractant properties for lymphocytes: MIP-1alpha induces the preferential migration of activated CD8+ T cells and B cells, while MIP-1beta selectively induces chemotaxis of activated CD4+ T cells (5). RANTES induces migration of both activated *and* resting T cells, including, perhaps most significantly, resting memory T cells (CD29+ and CD4+/UCHL1+ T cells; 6,7). This pattern of selective migration corresponds to the capacity of these chemokines to enhance the adhesion of specific subsets of activated T cells to IL-1 stimulated endothelial cells: MIP-1alpha and MIP-1beta augment the attachment of activated CD8+ and CD4+ T cells, respectively (6). Moreover, differences in the kinetics of the expression between these chemokines (e.g., RANTES vs. MIP-1alpha and MIP-1beta; 8) may further coordinate the regulation of the migration pattern, and thus the composition of the lymphocyte population at inflammatory sites at any given time.

Models of chemokine-mediated chemotaxis

The classic C-X-C chemokine, IL-8, appears to regulate the migration of neutrophils to inflammatory sites by enhancing the attachment of neutrophils to activated endothelial cells. In this model, neutrophils encounter IL-8 bound to surface glycoproteins of endothelial cells during selectin-mediated rolling (9). Since activated endothelial cells (and other immune cells) at inflammatory sites secrete IL-8, a chemokine concentration gradient forms along the vessel wall, with the IL-8 concentration being highest at the site of tissue damage. As neutrophils sample this solid-phase IL-8 gradient, they become activated and release beta-2-integrins from intracellular storage pools. These adhesion molecules are rapidly expressed and become conformationally activated on the neutrophil surface. The integrins firmly tether neutrophils to activated endothelial cells via intercellular adhesion molecule-1 (ICAM-1), the counter-receptor which is inducibly expressed on the endothelial cell surface. Since surface expression of ICAM-1 on endothelial cells is greatly enhanced by IL-1, TNF-alpha and gamma-IFN, which are secreted at sites of inflammation, neutrophils accumulate at post-capillary venules adjacent to the infected or damaged tissue. In response to the localized, combinatorial effects of chemokines and other inflammatory mediators (4) the gathering neutrophils rapidly undergo shape change, project pseudopods into the intercellular spaces between endothelial cells and migrate across the microvessel wall to enter the infected or damaged tissue.

A similar model of selective chemotaxis has been proposed for the C-C chemokine MIP-1beta and other chemokines containing glycosaminoglycan-binding sites (10). In this model, endothelial cells at inflammatory sites present CD8+ T cells with a gradient of the chemokine immobilized on endothelial surface proteoglycans, such as CD44. The bound chemokine triggers functional activation of the leukocyte integrins, enhancing attachment to the vascular endothelium and migration through the vessel into the surrounding tissue.

Other inflammatory activities of chemokines

In addition to inducing selective chemotaxis, chemokines demonstrate a variety of other bioactivities involved in inflammation. For example, many of the chemokines directly activate granulocytes and/or monocytes, triggering the respiratory burst, degranulation and the release of lysosomal enzymes. Chemokines may also prime immune cells to respond to sub-optimal levels of other inflammatory

mediators (e.g., MCAF pretreatment enhances macrophage tumoricidal activity in response to low concentrations of endotoxin; 11). In addition, several chemokines, including MCAF-1 and RANTES, potent histamine releasing factors for basophils (12).

A number of chemokines appear to play roles in growth inhibition and proliferation of human myeloid progenitor cells (13). While certain chemokines are specific inhibitors of the proliferation of hematopoietic stem cells (14), others stimulate growth of tumor cells (e.g., MGSA; 15). The mitogenic properties of IL-8 have been reported to induce endothelial cell proliferation and angiogenesis (16), which is important both in normal vascular development and wound healing, as well as in inflammatory diseases (e.g., rheumatoid arthritis and atherosclerosis; 16,17) and tumorigenesis.

Chemokine receptors and receptor-mediated events

The specificity of action of C-X-C and C-C chemokines appears to be regulated at the receptor level. Cross-competition for ligand binding sites has not been observed on neutrophils or monocytes of members of the C-X-C and C-C chemokine subfamilies (18). This observation is consistent with the differential chemotactic effects these chemokine subfamilies have on the two cell types (Table 1).

Receptors for C-X-C and C-C chemokines have been cloned from neutrophils and monocytes, respectively. IL-8 binds to two receptors on neutrophils: the high-affinity IL-8 receptor IL8RA (19), and IL8RB, a low-affinity binding site for multiple C-X-C chemokines (20). IL8RA and IL8RB demonstrate 77% homology at the amino acid level (20) and significant identity (29% and 69%, respectively) to the receptors for neutrophil chemoattractants fMet-Leu-Phe and C5a. These receptors are coupled to G proteins and possess seven transmembrane spanning domains. Upon binding their C-X-C chemokine ligands, IL-8 receptors mobilize calcium (19,20) and are rapidly internalized.

A distinct receptor for multiple C-C chemokines has recently been cloned from monocytes (21). This receptor, termed C-C CKR-1, specifically binds the C-C chemokines MIP-1alpha, MIP-1beta, MCAF and RANTES with varying affinities. Binding of C-C chemokines to C-C CKR-1 induces a rapid, transient increase in intracellular calcium, but the binding affinity is not necessarily predictive of signal strength. While MIP-1alpha binds to C-C CKR-1 with the highest affinity and induces the strongest calcium signal, RANTES transmits a more potent signal than MCAF and MIP-1beta, which bind the receptor with higher affinities. C-C CKR-1 demonstrates approximately 32% identity to amino acid sequences of the IL-8 receptors and roughly 23% identity to the receptors for fMet-Leu-Phe and C5a. Interestingly, the predicted extracellular domain of a gene product encoded by the cytomegalovirus (CMV) open reading frame US28 demonstrates nearly 50% amino acid identity to the corresponding region of C-C CKR-1 (21). The CMV product binds C-C chemokines and may confer a selective advantage to the virus in its strategy to evade the antiviral host response.

The IL-8 receptors and the C-C chemokine receptor bind multiple ligands within their corresponding chemokine subfamily. The diverse binding affinities and signaling potentials that each chemokine possesses, as well as the differential expression of the chemokine receptors on target cells, may regulate the combinatorial effect of multiple chemokines on leukocytes at localized sites of inflammation. Another level of regulation in the chemokine network has been proposed based on the observation that erythrocytes express a promiscuous chemokine receptor that binds both C-X-C and C-C chemokines (22,23). By serving as a sink for multiple chemokines in the fluid phase, this promiscuous receptor may play a novel role in the regulation of these inflammatory mediators in circulation. Chemokines bound to the erythrocyte surface via this receptor are inaccessible to their normal target cells (23,24). Thus the erythrocyte chemokine receptor limits the systemic effect of chemokines without disrupting localized pro-inflammatory effects of solid-phase chemokine gradients immobilized on proteoglycans of activated

vascular endothelial cells at inflammatory sites. Interestingly, this erythrocyte chemokine receptor appears to be the Duffy blood group antigen, the receptor required by malarial parasites *Plasmodium vivax and P. knowlesi* for invasion of human erythrocytes, and binding of chemokines to the receptor blocks malarial invasion (25).

Table 1. Chemokine Bioactivities

C-X-C Chemokines: The Alpha-Intercrine Family
Members of the C-X-C family are encoded on human chromosome 4 (q12-->q21).

Chemokine	Cell Target	Effects
IL-8 (8kDa) (Interleukin-8) **Other names** NAP-1, NAF, LAI, MDNCF, MONAP, LYNAP, 3-10C, 9E3 (chicken), CEF (chicken) **Cell Source** Monocyte/ Macrophages Endothelial cells Fibroblasts Keratinocytes T cells Neutrophils Hepatocytes Chondrocytes	Neutrophils	Chemotaxis; Shape change; (+) respiratory burst; (+) degranulation; (+) lysosomal enzyme release; (+) cytosolic free Ca2+; (+) adherence to endothelial cell monolayers, fibrinogen and subendothelial matrix proteins; (+) binding of complement protein C3bi and LPS; (+) complement receptor type 1; (+) surface expression of beta-2-integrins; (+) phosphorylation of 48kDa cytosolic protein; activation of arachidonate-5-lipoxygenase and release LTB4 and 5-HETE (reviewed in 26,27).
	T cells	Chemotaxis (reviewed in 26).
	Basophils	Chemotaxis; (+)(-) histamine release; (+) leukotriene release (28,29).
	Endothelial cells	(+) proliferation; angiogenesis (16).
	Keratino-cytes	(+) proliferation (reviewed in 27).
	Melanoma cells	(+) adhesiveness and haptotactic response (reviewed in 27).

Chemokine	Cell Target	Effects
MGSA (8.5kDa) (Melanoma Growth Stimulating Activity) **Other names** Gro, KC, MIP-2, CINC	Neutrophils	Chemotaxis; (+) degranulation; (+) lysosomal enzyme release (reviewed in 27).
	Melanoma cells	Autocrine growth factor (15).

(3 isoforms) **Cell Source** Monocyte/Macrophages Endothelial cells Fibroblasts Synovial cells	Fibroblasts	(+) proliferation; co-stimulates myelopoiesis (reviewed in 27).

Chemokine	**Cell Target**	**Effects**
gamma-IP-10 (8.5kDa) (gamma-IFN Inducible Protein-10) **Other names** crg-2, C7 (murine) **Cell Source** Monocyte/Macrophages Endothelial cells Fibroblasts Keratinocytes	Unknown	(+) protein expression in DTH lesions (reviewed in 26).

(+) = increase; (-) = decrease

C-C Chemokines: The Beta-Intercrine Family
Members of the C-C family are encoded on human chromosome 17 (q11-->q21).

Chemokine	**Cell Target**	**Effects**
MIP-1alpha (8kDa) (Macrophage Inflammatory Protein-1alpha) **Other names** pLD 78 (murine), pAT464, SCI, TY5, GOS-J9, LZG2B5 **Cell Source** Monocyte/ Macrophages T cells	Macrophages	Chemotaxis; (+) respiratory burst; (+) degranulation; (+) lysosomal enzyme release; prostaglandin-independent endogenous pyrogen; myelopoeitic enhancing activity (reviewed in 26,27).
	CD8+ T cells	Chemotaxis; (+) adherence to activated endothelial cells (5,6).
	B cells	Chemotaxis(5).
	Stem cells	Growth inhibition (14).
	Basophils	(+) Histamine release (12).

Chemokine	**Cell Target**	**Effects**
MIP-1beta (8kDa) (Macrophage Inflammatory Protein-1beta)	Macrophages	Chemotaxis; (+) respiratory burst; (+) degranulation; (+) lysosomal enzyme release; prostaglandin-independent endogenous pyrogen;

		inhibits MIP-1alpha-mediated chemotaxis (reviewed in 26,27).
Other names Act-2, LH400, HC21, G-26, pAT744, MAD-5, LAG-1, H400	CD4+ T cells	Chemotaxis; (+) adherence to activated endothelial cells (5,6).
Cell Source Monocyte/Macrophages T cells	Basophils	(+) Histamine release (12).

Chemokine	**Cell Target**	**Effects**
MCAF (15kDa) (Macrophage Chemo-tactic and Activating Factor)	Macrophages	Chemotaxis; (+) respiratory burst; (+) N-acetyl-beta-glucuronaminidase release, (+) cytostatic augmenting activity (reviewed in 26,27).
	Basophils	(+) Histamine release (12).
Other names MCP-1, JE, SMC, GDCF, LDCF, HC11, TSG-8		
Cell Source Monocyte/ Macrophages Fibroblasts, Endothelial cells, Keratinocytes, Smooth Muscle, Some tumor cell lines		

Chemokine	**Cell Target**	**Effects**
RANTES (8kDa) (Regulated Upon Activation, Normal T Expressed and presumably Secreted)	Macrophages	Chemotaxis (30).
	Basophils	(+) Histamine release (31).
	Memory T cells	Chemotaxis (6).
	CD4+ T cells	Chemotaxis, (+) adherence to activated endothelial cells(6).
Cell Source T cells Platelets	Eosinophils	Chemotaxis and activation (32).

(+) = increase; (-) = decrease

Conclusions

As potent mediators of cell migration and activation, chemokines play a central role in the early events of inflammation. Together with intercellular adhesion molecules, chemokines and their receptors serve

to localize and enhance the inflammatory reaction at the site of tissue damage. A number of these signaling molecules have been implicated in disease processes, ranging from chronic inflammatory diseases, such as psoriasis, rheumatoid arthritis (16) and atherosclerosis (17), to oncogenesis. Continued research on chemokines is required to better understand their regulation and interaction with inflammatory cells and to investigate novel therapies for inflammatory and infectious diseases.

References

1. Mukaida, N. and Matsushima, K. (1992) *Cytokines*, **4**, 41.
2. Widmer, U. *et al*. (1991) *J. Immunol.* **146**, 4031.
3. Nelson, P.J. *et al.* (1993) *J. Immunol.* **151**, 2601.
4. Lasky, L.A. (1993) *Current Biology* **3**, 366.
5. Schall, T.J. *et al.* (1993) *J. Exp. Med.* **177**, 1821.
6. Taub, D.D. *et al.* (1993) *Science* **260**, 355.
7. Schall, T.J. *et al.* (1990) *Nature* **347**, 669.
8. Nelson, P.J. *et al.* (1993) *J. Immunol.* **151**, 2601.
9. Rot, A. (1992) *Immunol. Today* **13**, 291.
10. Tanaka, Y. *et al.* (1993) *Nature* **361**, 79.
11. Singh, R.K. *et al.* (1993) *J. Immunol.* **151**, 2786.
12. Kuna, P. *et al.* (1993) *J. Immunol.* **150**, 1932.
13. Broxmeyer, H.E. *et al.* (1993). *J. Immunol.* **150**, 3448.
14. Graham, G.J. *et al.* (1990) *Nature* **344**, 442.
15. Richmond, A. and Thomas, H.G. (1982) *Cold Spring Harbor Conf. Cell Prolif.* **9**, 885.
16. Koch, A.E. *et al.* (1992) *Science* **258**, 1798.
17. Edgington, S.M. (1993) *Bio/Technology* **11**, 676.
18. Yoshimura, T. and Leonard, E.J. (1990) *J. Immunol.* **145**, 292.
19. Holmes, W.E. *et al.* (1991) *Science* **253**, 1278.
20. Murphy, P.M. and Tiffany, H.L. (1991) *Science* **253**, 1280.
21. Neote, K. *et al.* (1993) *Cell* **72**, 415.
22. Horuk. R. *et al.* (1993) *J. Immunol.* **150** (II), 92A (abstract 511).
23. Neote, K. *et al.* (1993) *J. Biol. Chem.* **268**, 12247.
24. Darbonne, W.C., *et al.* (1990) *J. Clin. Invest.* **88**, 1362.
25. Horuk, R. *et al.* (1993) *Science* **261**, 1182.
26. Miller, M.D. and Krangel, M.S. (1992) *Crit. Rev. Immunol.* **12**, 17.
27. Oppenheim, J.J. *et al.* (1991) *Annu. Rev. Immunol.* **9**, 617.
28. Dahinden, C.A. *et al.* (1989) *J. Exp. Med.* **170**, 1787.
29. Kuna, P. *et al.* (1991) *J. Immunol.* **147**, 1920.
30. Schall, T.J. (1991) *Cytokine* **3**, 165.
31. Kuna, P. *et al.* (1992) *J. Immunol.* **149**, 636.
32. Alam, R. *et al.* (1993) *J. Immunol.* **150**, 3442.

Ordering Information

Product	Size	Cat.#
IL-8, Human, Recombinant (endothelial)	25µg	G5571
IL-8, Human, Recombinant (monocyte)	25µg	G5581
MIP-1alpha, Human, Recombinant	10µg	G5681
MIP-1beta, Human, Recombinant	10µg	G5691
MCAF, Human, Recombinant	2µg	G5351
RANTES, Human, Recombinant	10µg	G5661

anti-MIP-1alpha, IgG (poly)	100µg	G1121