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Chemokines and the late-phase reaction

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A. P. Kaplan[1], P. Kuna[2]

[1]Division of Pulmonary and Critical Care Medicine and Allergy and Clinical Immunology, Medical University of South Carolina, Charleston, SC, USA; [2]Division of Allergy and Pulmonology, Medical University of Łodz, Łodz, Poland

Dr Allen P. Kaplan
Department of Medicine
Medical University of
South Carolina
171 Ashley Avenue
Charleston, SC 29425
USA

The recruitment of basophils and eosinophils into tissues in which allergic inflammation occurs is characteristic of late-phase reactions in the nose, bronchial mucosa, or skin. When these cells arrive, usually in conjunction with small numbers of activated T cells and monocytes, they degranulate and thereby perpetuate the inflammatory response. Those cytokine-like molecules which activate basophils are known as "histamine-releasing factors" (HRF) and can be divided into the β chemokines which act via specific chemokine receptors, and the "IgE-dependent" HRF whose receptor is as yet uncharacterized. The activators of eosinophils have no particular designation but are probably a mixture of interleukin (IL-5), and certain β chemokines. As is true of all migrating cells, basophils and eosinophils must be primed by cytokines to heighten their responsiveness, interact with the vascular endothelium, and ultimately squeeze through gap junctions, and migrate into tissues. However, T cells of the Th_2 subclass and monocytes are also critical to the process and are among the many cellular sources of the cytokines and chemokines that characterize allergic inflammation.

Chemokine families

The chemokine group of cytokine-like factors can now be divided into four families, two of which have only a single member. The two major groupings (Table 1) are the α and β chemokines. All chemokines have a molecular weight of approximately 8 kDa, and the α and β subgroups have four cysteine residues located in closely homologous positions within the amino-acid sequence. The α group is also known as the CXC chemokines because the first two cysteines are separated by a single amino acid (designated X), while β chemokines are also known as C-C chemokines because the first two cysteines are adjacent (1). The α and β chemokines are encoded on chromosomes 4 and 17, respectively, and may have evolved from a single generic chemokine sequence which then duplicated, and separated to differing chromosomal locations. Then each of them replicated repeatedly to create a group of homologous proteins on each chromosome. The chemokines have a strong binding affinity to heparin, i.e., negatively charged mucopolysaccharides, and heparin-Sepharose can be used as a purification step in chemokine isolation. Thus, chemokines also bind to heparan sulfate along the surface of cell membranes or within connective tissue, a fact which may be important in their localization at sites of inflammation.

As a group, the α chemokines are chemotactic for neutrophils, and have little or no effect on basophils or eosinophils (2). However, those α chemokines which lack a glu-leu-arg sequence primarily act on activated T cells, but not monocytes. The β chemokines, on the other hand, act on all leukocytes except neutrophils, but each β chemokine has a different profile. The cellular response to chemokines depends on the particular chemokine receptor displayed; thus, the cellular receptor profile dictates the chemokine responsiveness of each cell type. A third chemokine family contains only the C-chemokine lymphotactin. It has only two cysteines and lacks the first and third cysteine

Table 1. Chemokine families

α Chemokines (CXC)	β Chemokines (C-C)	C-chemokine	CX_3C chemokine
Interleukin-8 (NAP-1)	MCP-1–4	Lymphotactin	Fractalkine
NAP-2	RANTES		
GRO α, β, γ	Eotaxins I and II		
β-Thromboglobulin	MIP-1α		
CTAP III	MIP-1β		
Platelet factor 4	I309		
IP10	MDC		
MiG	TARC		
SDF-1	LARC (EXODUS I)		
GCP-2	PARC (EXODUS II)		
I-TAC	TECK		
	MPIF-I		
	MPIF-II		

MCP: monocyte chemoattractant protein; MIP: macrophage inflammatory protein; MDC: macrophage derived chemokine; TECK: thymus expressed chemokine; SDF: stromal cell derived factor; TARC: thymus and activation regulated chemokine; PARC: pulmonary and activation regulated chemokine; LARC: liver and activation regulated chemokine; IP10: interferon-inducible protein 10; MiG: monokine induced by interferon-gamma; I-TAC: interferon inducible T-cell alpha chemoattractant; GRO: growth regulated oncogene; NAP: neutrophil activating peptide; CTAP: connective tissue activating peptide; RANTES: regulated upon activation normal T-cell expressed and secreted; GCP: granulocyte chemotactic peptide.

that are present in the prototypic sequence. It is among the only chemokines which attracts unactivated lymphocytes. The final chemokine family contains fractalkine, a chemokine that has thus far been found only at the cell surface. It has three amino acids separating the first two cysteines (CXXXC) and a lectin domain which attaches it to the cell.

Chemokine receptors

There are now known to be four CXC chemokine receptors and eight C-C chemokine receptors (1). A specific receptor for lymphotactin has not been defined, but fractalkine has a receptor designated CX_3CR-1. These receptors are shown in Table 2 and indicate the array of chemokines reactive to each receptor. MCP-1 and MCP-2 act primarily on CCR2, MCP3 acts on CCR1, CCR2, and CCR3, and RANTES acts on CCR1, CCR3, and CCR5. Eosinophils express CCR1 and CCR3, while basophils express CCR2 and CCR3.

All chemokine receptors thus far described have seven transmembrane segments and are linked to G proteins that are required for signal transduction. There is also a chemokine receptor on the red blood cell which is identical to the Duffy antigen. It binds both α and β chemokines and therefore does not discriminate between these two families. The receptor has the typical transmembrane spanning structure but lacks a cytoplasmic domain and is not linked to any G protein. Thus, no signal is transmitted. The red cell may therefore remove freely circulating chemokines to lower their blood concentration, and/or deliver chemokines to more distant sites.

Table 2. Chemokine receptors

CXC chemokine receptors	
CXCR1	IL-8, GCP-2
CXCR2	IL-8, GCP-2, NAP-2, Gro α, β, γ
CXCR3	IP10, MiG
CXCR4	SDF-1
CC chemokine receptors	
CCR1	MCP-3, MIP-1α, RANTES
CCR2	MCP-1, -2, -3, and -4
CCR3	MCP-3, MCP4, eotaxins I and II, RANTES
CCR4	TARC
CCR5	MIP-1α, MIP-1β, RANTES
CCR6	LARC
CCR7	MIP-3β
CCR8	I309
CX_3C chemokine receptor	
CX_3CR1	Fractalkine
C chemokine receptor	Lymphotactin

Chemokines and the recruitment of T cells and monocytes

The cytokines and chemokines that have the potential to recruit and activate T cells and monocytes are numerous, and there are currently no data which would allow one to discern which are the most important. The T-cell infiltrate is skewed toward a Th_2-type response in a milieu that is dominated by IL-4, IL-5, and IL-13 (3, 4). Interferon-gamma (IFN-γ) is absent, but various other cytokines may be present, which, in man, are produced by Th_0, Th_1, or Th_2 cells. These include IL-2, IL-3, and IL-10 as well as various cytokines produced mainly by monocytes (monokines) such as IL-1, tumor necrosis factor-alpha (TNF-α), IL-6, and GM-CSF. The cytokines/chemokines that can recruit activated T cells include:

1) IL-16 (5), which is specifically for CD4+ cells and actually binds to CD4
2) certain α chemokines such as Mig, iTac, and IP-10 (1, 6), and, to a lesser degree, IL-8
3) β chemokines such as MCP-1, -2, -3, and -4; RANTES; MIP-1α; MIP-1β; TARC; and PARC
4) the CX_3C chemokine, fractalkine.

Resting T cells respond to the C chemokine lymphotactin, the β chemokine PARC, and the α chemokine SDF-1 (1). A distinction between CD4 and CD8 cell responsiveness has been noted when MIP-1α and MIP-1β are compared; MIP-1α is preferential for CD8+ cells, while MIP-1β is selective for CD4+ cells (7). The comparative responsiveness of Th_0, Th_1, or Th_2 subsets of lymphocytes has not been reported.

Chemotactic factors for monocytes are virtually exclusively associated with β chemokines, which include MCP-1, -2, -3, and -4; RANTES; MIP-1α; and MIP-1β, as well as I309, MDC, and TECK. Monocytes also may respond to fractalkine and SDF-1.

There are virtually no data that distinguish which of these chemokines contribute to Th_2 lymphocyte recruitment or to monocyte recruitment within late-phase reactions. Most studies have focused on basophils and eosinophils, since the appearance of these cells most distinguishes allergic inflammation from other types of immune reactions, particularly delayed hypersensitivity or inflammation mediated by antibody and/or complement.

Priming of basophils and eosinophils

All migrating cells can be activated by certain inflammatory mediators (often cytokines) without imparting directional migration; i.e., the stimulating substances are not truly chemotactic. Some of these can be considered chemokinetic factors which increase the random movement of cells as a result of cell activation. Cells exposed to such primers typically have evidence of enhanced oxidative metabolism and upregulate the expression of receptors, so that the response to bona fide chemotactic factors and the interaction with adhesion molecules on endothelial cells are enhanced. Three of the cytokines that we associate with allergic responsiveness are primers of both basophils and eosinophils (8, 9); namely, IL-3, IL-5, and GM-CSF. The receptors for these cytokines are also closely related, since they share a common β subunit (8), while three different α subunits interact with the β subunit to confer specifically for each particular cytokine. IL-5 is most closely associated with the Th_2-type response, while IL-3 and GM-CSF can be products of all the T-cell subtypes; i.e., Th_0, Th_1, or Th_2. Table 3 shows one example of basophil priming in which basophils were stimulated with either IL-3 or IL-5, or with buffer, and the secretion of histamine was stimulated by the chemokine MCP-1. A marked augmentation of histamine release was seen when the cells were preincubated with concentrations of IL-3 or IL-5 that do not cause histamine release. IFN-γ, a Th_1 cytokine, has also been reported to prime basophils (but not eosinophils), but it is less likely to have a role in allergic inflammation in which a Th_2 response predominates.

Table 3. Basophil priming

	Buffer	Anti-IgE	MCP-1
Buffer	2.2±0.7	8.8±3.5	16.3±6.8
IL-5	6.2±0.9	30.1±7.4*	35.0±6.0*
IL-3	7.3±1.4	32.2±7.5*	36.5±6.3*

Leukocytes of six donors were preincubated with buffer, IL-5 (100 ng/ml), or IL-3 (5000 U/ml) for 10 min and challenged for 35 min with anti-IgE (0.05 μg/ml) or MCP-1 (20 ng/ml). Mean ± SEM of histamine release is shown. * indicates augmentation ($P \leq 0.05$) as result of preincubation with either IL-5 or IL-3.

Eosinophil viability (antiapoptosis)

When cells migrate into tissue, their contribution to the inflammatory response may depend not only on their activation state, but also on the rate at which they secrete. Basophil degranulation is very rapid; the response is virtually complete within 1 min with some of the β chemokines, and is complete in 3–5 min for IgE-dependent reactions. Eosinophils, on the other hand, secrete slowly; thus, their viability within tissues is important. Here, too, there are major effects of cytokines. It may be no coincidence that the primers IL-3, IL-5, and GM-CSF are the same factors that increase the half-life of eosinophils within tissues (Fig. 1). The mechanism appears to be an active process in which the cascade leading to apoptosis is inhibited (9).

Chemotaxis and activation of basophils

The search for cytokine activators of basophils led to the identification of β chemokines as HRF. The first and most potent of these is MCP-1, which directly stimulates basophils to secrete histamine, as shown in Fig. 2. Subsequently, MCP-3, RANTES, and MIP-1α were shown to function similarly, and their order of potency is MCP-1 ⟩ MCP-3 ⟩ RANTES ⟩ MIP-1α or eotaxins (12). MCP-4 was later shown to be a potent basophil activator, similar to MCP-1 and MCP-3 (13), while MCP-2 is weaker, and more closely resembles RANTES or MIP-1α. Their relative effectiveness may relate to binding affinity as well as to the particular receptors with which each one interacts. Basophils have CCR2 and CCR3 receptors; thus, CCR2 will react with MCP-1, -2, -3, and -4, while CCR3 will react with MCP-3, MCP-4, RANTES, and eotaxins I and II.

The rate of basophil activation is much more rapid when compared to an antigen–IgE interaction. With an optimal concentration of MCP-1, for example, 50% of histamine release occurs in 30 s, as shown in Fig. 3. Chemokines with HRF activity are also chemotactic for basophils. Thus, recruitment of basophils into late-phase reactions is likely to depend on these particular chemokines. Basophils are primed by IL-3, IL-5, and/or GM-CSF, and endothelial cells are stimulated by IL-4

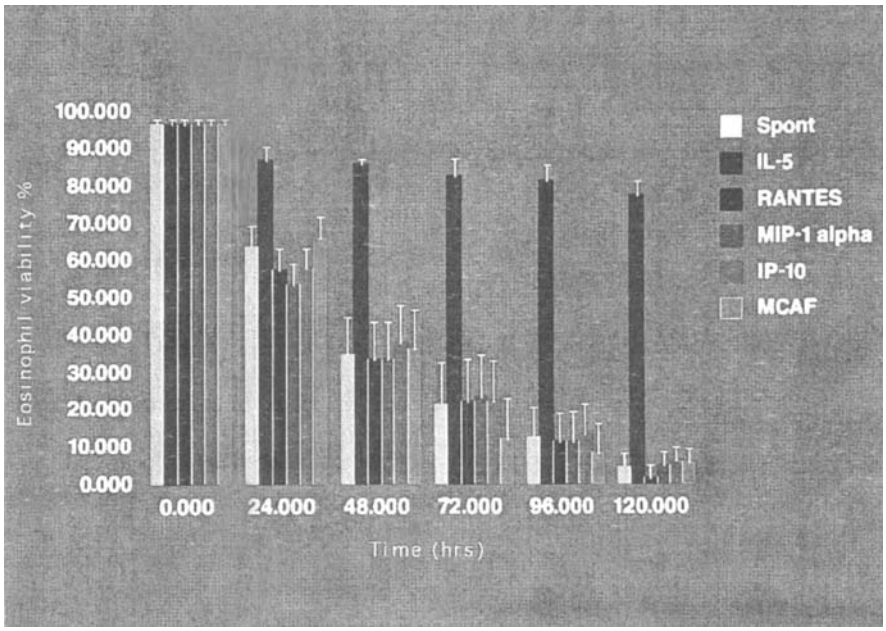

Fig. 1. Effect of chemokines and IL-5 on eosinophil viability. Eosinophils were cultured with buffer (Spont), IL-5 (10 ng/ml), RANTES, MIP-1α, IP-10, and MCAF/MCP-1 (10^{-7} M), and viability was measured as ratio of live to dead cells at start of culture and 24, 48, 72, 96, and 120 h later. Mean value of four experiments in different donors is shown.

and perhaps IL-1 and TNF-α to express VCAM-1 and E selectin and upregulate ICAM-1. Basophils and eosinophils share expression of VLA-4, which is not expressed by neutrophils, and is a major ligand for VCAM-1.

Chemotaxis and activation of eosinophils

The chemokines which are chemotactic for eosinophils include eotaxins I and II, which are selective for eosinophils, as well as RANTES, MCP-3, and MCP-4 (1). These chemokines will also cause eosinophil degranulation and secretion of various granule proteins such as eosinophil major basic protein (MBP) or eosinophil cationic protein (ECP). Eosinophil secretion does not, however, occur by a burst of activity, as does basophil secretion, but consists of a gradual extrusion of proteins that can last for 1 h. IL-5 serves many functions including stimulating eosinophils to secrete, but its actual chemotactic activity when tested in the standard Boyden chamber is weaker

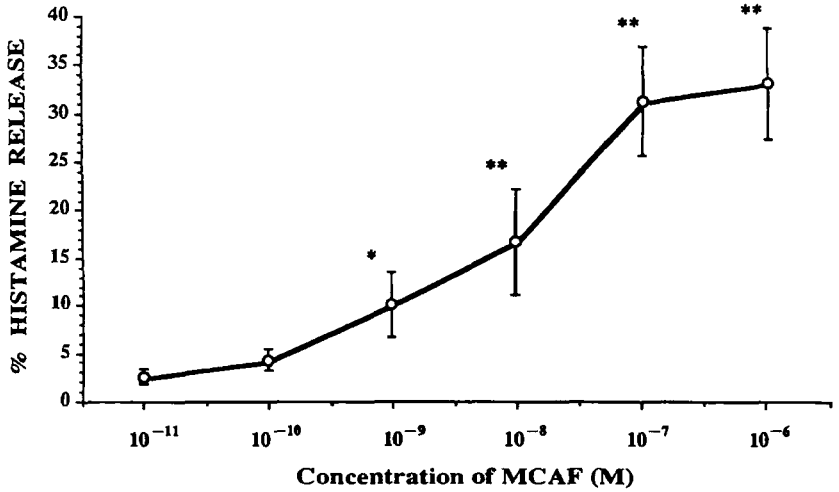

Fig. 2. Dose response of activation of human basophils to secreted histamine by increasing concentrations of MCAF (MCP-1) from 10^{-11} to 10^{-6} M. Results are expressed as mean percent histamine release ± SEM. * $P \leq 0.022$; ** $P \leq 0.004$ *vs* histamine release by 10^{-11} M MCAF (Wilcoxon's rank sum test).

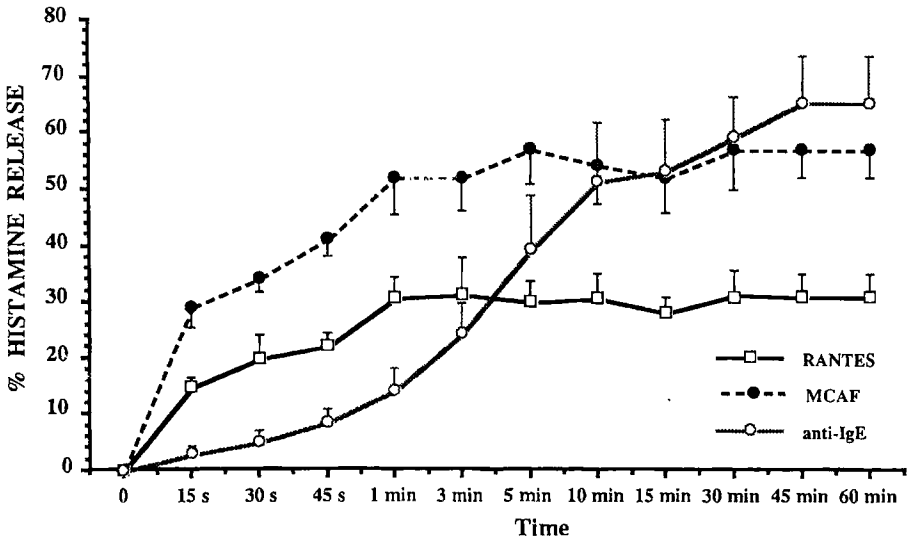

Fig. 3. Time course in which rate of basophil histamine release is compared among anti-IgE (1 μg/ml), RANTES (10^{-7} M), and MCAF (MCP-1) (10^{-7} M). Chemokines MCP-1 or RANTES release 50% of their maximal histamine release within 30 s, while anti-IgE requires 3–5 min. Results are expressed as mean ± SEM.

than that of the β chemokines. However, C5 is critical for recruitment of cells from bone marrow; it is a major eosinophil primer at low concentrations, extends their residence time within tissues, and acts as a direct secretagogue (14).

Relation to allergic diseases

Basophils and eosinophils have been demonstrated within late-phase reactions of the nose, lung, and skin and may perpetuate the inflammatory response by virtue of their local activation and secretion. Although antigen may be present to activate either cell, activation by chemokines can occur in the absence of antigen to perpetuate the process, and IgE antibody is then no longer necessary. Basophils primarily release histamine, leukotriene C_4 (LTC_4), and IL-4, while eosinophils release cationic proteins (MBP, EDN, ECP), oxygen metabolites, LTC_4, and a wide variety of cytokines, chemokines, and growth factors.

One study of allergic rhinitis, in which patients were followed throughout the season, and serial nasal washings were assayed for IL-8 and MCP-1, revealed a marked increase in MCP-1 during the ragweed season, at which time patients were symptomatic, and a small decrease in IL-8 (15). MCP-1 levels decreased markedly during the "off season"; thus, the MCP-1/IL-8 ratio was reversed when "in season" was compared to "off season". The preponderance of β chemokine secretion may be linked to the Th_2 allergic response as the underlying pathogenic mechanism operative in the 20% of the population with allergic diseases.

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