

Chemokines Active on Eosinophils: Potential Roles in Allergic Inflammation

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Presently, the eosinophil is recognized as a proinflammatory granulocyte implicated in protection against parasitic infection and likely plays a major role in allergic diseases, such as bronchial asthma, allergic rhinitis, and atopic dermatitis (1). The eosinophil is a rich source of cytotoxic proteins, lipid mediators, oxygen metabolites, and cytokines: all with the potential to induce pathophysiology (2). Numerous studies have shown striking eosinophil infiltration into tissues in disease. For example, even in mild asthma (3) eosinophil and lymphocyte infiltration in respiratory epithelium is a consistent finding. Correlations exist between the number of infiltrating eosinophils and disease severity in asthma (3). Pulmonary segmental allergen challenge in sensitive individuals causes eosinophil recruitment into the airways associated with release of biologically active granule proteins and increases in vascular permeability (4, 5). Marked eosinophil infiltration and deposition of granule proteins are found in areas of epithelial desquamation in paranasal sinus tissues in patients with chronic sinusitis (6). Deposition of eosinophil granule proteins is also prominent in pruritic and eczematous lesions of patients with atopic dermatitis (7). In contrast, infiltration of neutrophils is not prominent in chronic allergic inflammation (8, 9). Yet, in spite of numerous studies (10), the mechanisms allowing selective infiltration of eosinophils in allergic diseases have been a mystery for more than two decades (11).

Several mechanisms for selective eosinophil infiltration in disease are known. The migration of leukocytes through the endothelium involves sequential steps in which the cells are initially lightly tethered to the endothelium and roll along its surface. Locally released mediators, some of which may be attached to proteoglycans on the endothelial surface, activate leukocytes leading to increased affinity and/or increased expression of cell surface integrins; this permits a firmer bond between the leukocyte and the endothelial cell and results in successful adhesion and transmigration. These general mechanisms of leukocyte infiltration are applicable to eosinophils and provide opportunities for selective migration. First, eosinophils but not neutrophils express the $\beta 1$ integrin $\alpha_4\beta_1$ [very late antigen (VLA-4)], and the $\beta 7$ integrin, $\alpha_4\beta_7$, and VLA-4 binds to the vascular cell adhesion molecule (VCAM)-1 on endothelial cells. This adhesion pathway may permit selective migration of eosinophils (12). VCAM-1 on endothelial cells is upregulated by IL-4 and IL-13, important cytokines in allergic inflammation,

and increased expression of these cytokines may further enhance eosinophil recruitment (13). This hypothesis is supported by the observation that eosinophil, but not neutrophil, adhesion and transmigration through monolayers of human umbilical vein endothelial cells (HUVEC) is enhanced by IL-4 (14) and by findings that antibodies to VLA-4 block eosinophil infiltration in guinea pigs (15, 16). However, the expression of VCAM-1 in human allergic inflammation is relatively modest compared to the other adhesion molecules, such as selectins and intercellular adhesion molecule (ICAM)-1 (17), raising doubts about the importance of this mechanism for selective eosinophil infiltration in disease. Second, among the eosinophil growth factors, IL-5 possesses chemokinetic and chemotactic activities for eosinophils, but not for other leukocytes (18). Although IL-5 is a relatively weak chemoattractant, it effectively and specifically primes eosinophils for enhanced chemotactic responsiveness to suboptimal concentrations of platelet-activating factor (PAF) and leukotriene B₄ (LTB₄) (19). Thus, a highly effective but nonspecific mediator, such as PAF (20), could combine with a highly selective but weakly chemotactic agent, such as IL-5, to promote the specific eosinophil accumulation. Evidence for the importance of IL-5 in eosinophil-associated inflammation abounds. IL-5 is the predominant eosinophil-active cytokine in the allergen-induced pulmonary late-phase allergic reaction (21). Antibodies against IL-5 prevent both eosinophil migration into the lungs and airway hyperreactivity in allergen-challenged monkeys and guinea pigs (22, 23). Mice rendered IL-5 deficient by homologous gene recombination fail to develop eosinophil infiltration into the lungs, airway hyperresponsiveness, and lung damage in a model of asthma (24). In contrast, mice transgenic for human IL-5 have extremely high numbers of circulating eosinophils yet show no pathology nor organ localization (25), thus pointing to the critical importance of local IL-5 production. Finally, both IL-2 (26) and IL-16 (lymphocyte chemoattractant factor) (27) are exceedingly potent chemoattractants for eosinophils. However, in spite of the potency and specificity of these chemoattractants their roles in the induction of eosinophil tissue infiltration remain obscure.

An exciting development in the area of eosinophil biology has been the identification of chemotactic cytokines termed "chemokines." The chemokines have four conserved cysteine residues that form characteristic disulfide bonds and

are divided into two subfamilies, C-X-C and C-C, by the position of the first two conserved cysteines (28). The C-C subfamily chemokines, typified by regulated upon activation in normal T cells expressed and secreted (RANTES), are potently chemotactic for eosinophils, as well as lymphocytes, but not for neutrophils (29). RANTES and monocyte chemoattractant protein (MCP)-3 are among the most potent chemokines for eosinophil chemotaxis in vitro (29, 30). MCP-2 and macrophage inflammatory protein (MIP)-1 α also induce eosinophil migration (31, 32), but to a much lesser extent than MCP-3 or RANTES. In contrast, MCP-1 and MIP-1 β do not induce eosinophil chemotaxis (33). The bioactivities and/or protein levels of MIP-1 α , RANTES, and MCP-3 were increased in bronchoalveolar lavage (BAL) fluids from patients with asthma (34) consistent with a role for these molecules in disease. In addition, RANTES has been localized in the nasal epithelia of patients with nasal polyps (35), and the expression of MCP-3 mRNA, but not RANTES mRNA, correlated with eosinophil infiltration in allergic skin reactions (36). Intradermal injection of RANTES in dogs caused an eosinophil-rich infiltration within several hours; in contrast, IL-8 injection caused neutrophil infiltration (37). Another C-C subfamily chemokine, eotaxin, was discovered in the guinea pig (38) and is present during allergic airway inflammation (39). Intradermal injection of guinea pig eotaxin or LTB₄ in combination with intravenous injection of IL-5 stimulated a rapid and dramatic increase in the number of eosinophils in the skin (40), whereas intradermal and intravenous injections of IL-5 did not. Murine (41) and human (42) homologues of eotaxin have been recently identified. Eotaxin induces chemotaxis of eosinophils, but not neutrophils, monocytes, or lymphocytes in vitro, indicating a highly specific action of this chemokine. Furthermore, human eotaxin was more effective at inducing eosinophil infiltration than RANTES when injected into the skin of a rhesus monkey (42), and eotaxin was expressed in epithelium and submucosa of human nasal polyp tissues (42) which commonly show striking and selective eosinophil infiltration (43). To add to this increasing list of eosinophil-active chemokines, Uguccioni et al. reported another novel human C-C chemokine, designated MCP-4 in the May issue of the *Journal of Experimental Medicine* (44). MCP-4 shares 60% amino acid sequence identity with MCP-3 and eotaxin and is a potent chemoattractant for eosinophils, lymphocytes, and monocytes (44); with eosinophils, MCP-4 is as potent as eotaxin and likely more potent than MCP-3. Thus, the C-C chemokines, including RANTES, MCP-3, eotaxin and the newly identified MCP-4, are selective and effective eosinophil chemokines in vitro and in vivo.

While identification of C-C chemokines has contributed greatly to our understanding of eosinophil biology, information regarding receptors mediating the functions of these chemokines is relatively sparse. The known C-C chemokine receptors are members of the G protein-coupled receptor superfamily; two of these receptors, CKR-1 (45, 46) and CKR-2 (47), are found on mature and immature myeloid cells, B lymphocytes and monocytic cell lines. CKR-1

binds MIP-1 α , RANTES, and MCP-3, and CKR-2 binds MCP-1 with high affinity and MCP-3 with low affinity. More recently, Power et al. (48) identified a new receptor, called CKR-4, in a human basophil cell line, which reacts with MCP-1, MIP-1 α , and RANTES. In the meantime, by the characteristic pattern of the desensitization of [Ca²⁺]_i signals, Dahinden et al. (30) speculated on the existence of two chemokine receptors on eosinophils: (a) a RANTES receptor that binds RANTES and MCP-3; and (b) a MIP-1 α receptor that binds MIP-1 α , RANTES and, with low affinity, MCP-3. In the May & June issues of the *Journal of Experimental Medicine*, two groups of investigators (49, 50) independently report the cloning and expression of a novel C-C chemokine receptor, designated CKR-3, from peripheral blood eosinophils and from an eosinophil cDNA library. The sequences of CKR-3 identified by these two groups are identical and show 50–60% amino acid identity with CKR-1 and CKR-2B. CKR-3 transfected cells bound eotaxin, MCP-3 and RANTES with high affinity; no binding of MIP-1 α , MIP-1 β , or IL-8 was observed. Eotaxin, RANTES, and to a lesser extent MCP-3 activated CKR-3, as determined by stimulation of an increased [Ca²⁺]_i and by chemotaxis of clones expressing the receptor. The binding affinities of eotaxin, MCP-3, and RANTES for peripheral blood eosinophils (49) and the responses of eosinophils to these three cytokines (50) were similar to the clones expressing CKR-3. Furthermore, on eosinophils CKR-1 is expressed at only 1–5% of the levels of CKR-3 (49). Importantly, CKR-3 was expressed only by eosinophils, and not by neutrophils, monocytes, or lymphocytes, as shown by Western blot analysis (49), flow cytometry, and Northern blot analysis (50).

CKR-3 has features which distinguish it from other C-C chemokine receptors and which suggest a role in the selective eosinophil infiltration into tissues. First, it is expressed at high levels on eosinophils, 40,000 (50) to 400,000 (49) receptors per cell, compared to CKR-1 and CKR-2, which are expressed on monocytes and T cells usually at <3,000 receptors per cell (37, 51). This 10–100-fold excess of CKR-3 over CKR-1 and CKR-2 is consistent with the high potency of CKR-3 ligands as eosinophil chemoattractants. Second, although most chemokine receptors are expressed on a number of leukocyte types, CKR-3 is expressed only on eosinophils. This restricted expression of CKR-3 on eosinophils may determine the highly selective recruitment of eosinophils in allergic inflammation. Third, CKR-3 is the only eotaxin receptor identified to date. This apparent high degree of fidelity contrasts to RANTES, which binds to CKR-1 (45, 46) and CKR-4 (48), and to MCP-3, which binds to CKR-1 (45, 46) and CKR-2 (47). Therefore, an interaction between eotaxin and CKR-3 could lead to selective recruitment of eosinophils, but not of other leukocytes. Finally, CKR-3 is likely largely responsible for mediating the effects of other potent eosinophil chemokines, including RANTES and MCP-3. CKR-3 is expressed at 10–100 times the level of CKR-1 (49), a difference that more than compensates for the fourfold greater affinity of CKR-1 for RANTES and MCP-3 compared to

CKR-3. In addition, MCP-4, the potent C-C chemokine identified by Ugucioni et al. also likely binds to eosinophils via CKR-3, as judged by the results of cross-desensitization of $[Ca^{2+}]_i$ signals (44). Thus, the discovery of CKR-3 provides a potential mechanism for the selective recruitment of eosinophils into tissues during allergic inflammation. It will be important to know whether disrupting this eotaxin-CKR-3 interaction prevents eosinophil specific tissue recruitment.

Although C-C chemokines are potent chemotactic factors, not all the known properties of the chemokines involve leukocyte migration. A fascinating spectrum of activities has been attributed to RANTES, including T cell proliferation, IL-2 receptor expression, and IL-2 and IL-5 production, although higher concentrations (1,000 nM) of RANTES were required for these functions than those required for chemotaxis (0.1 nM) (52). RANTES and MIP-1 α at 0.1 nM also stimulated T cells to express matrix metalloproteinases, enzymes required for cells to migrate through the basement membrane barrier (53). Another potentially important function of RANTES and MIP-1 α is reported by Kimata et al. in the May issue of *Journal of Experimental Medicine* (54). They found that surface IgE positive (sIgE⁺) B cells and sIgG⁺ B cells isolated from human tonsil, but not sIgE⁻ B cells or sIgG⁻ B cells, express receptors for RANTES and MIP-1 α . RANTES and MIP-1 α at 100 nM directly stimulated these sIgE⁺ and sIgG⁺ B cells and enhanced IgE and IgG4 production; production of IgG, IgG1, IgG2, IgG3, IgA1 or IgA2 was not affected. A variety of other C-X-C and C-C chemokines tested in their report did not show any effects. These observations suggest that a subpopulation of B cells committed to IgE and IgG4 production specifically express receptors for and respond to RANTES and MIP-1 α . Thus, RANTES and MIP-1 α have the capacity to modulate allergic inflammation by regulating immunoglobulin production. RANTES also induces eosinophil degranulation in vitro (55). Further, it is likely that many of the previously described "histamine-releasing factors" for basophils can be attributed to C-C chemokines, such as MCP-1, MCP-3 and to a lesser extent RANTES (30). Therefore, although the in vivo effects of these chemokines remain to be elucidated, C-C chemokines may modulate allergic inflammation by their non-chemotactic activities, as well as by well-known chemotactic properties.

Thus, considerable evidence indicates important roles for C-C chemokines in allergic inflammation (Fig. 1). Still, questions remain. First, are the involved C-C chemokines specific for allergic inflammation? For example, eotaxin in mice is not restricted to a T_{H2}-type response, and eotaxin is also upregulated by LPS administration, a stimulus favoring neutrophilia rather than eosinophilia (56). Second, are there species specific responses? For example, MIP-1 α is a strong eosinophil chemoattractant in mice; yet, the activity of

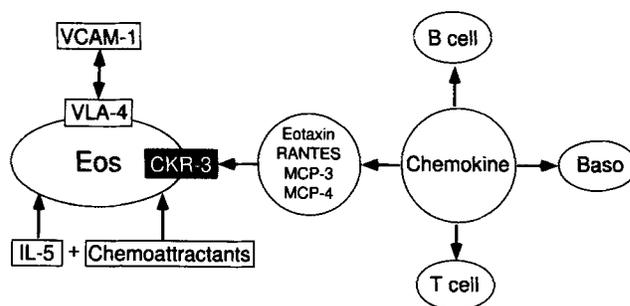


Figure 1. Chemokines and allergic inflammation. Selective tissue infiltration of eosinophils is one of the striking features of allergic inflammation. Chemokines, such as eotaxin, RANTES, MCP-3, and MCP-4, strongly and selectively induce chemotaxis of eosinophils via a unique chemokine receptor, CKR-3. This action of chemokines may be critical for selective eosinophil recruitment. Chemokines also stimulate various effector functions of T cells, B cells, and basophils. See text for details.

MIP-1 α is limited in humans. In mice, the effects of MIP-1 α appear to be mediated through the murine CKR-3 homologue, which also binds and signals with murine eotaxin, rather than through CKR-1 (57). Therefore, information derived from animal experimentation may not be directly applicable to humans. Finally, it is predicted that the total number of chemokines, when finally known, could exceed 100 (58), and the attractive explanation for selective eosinophil tissue infiltration provided by current information may be complicated by new data. For example, deletion of the NH₂-terminal residue of MCP-1, a chemokine not active on eosinophils, converted it to a potent eosinophil chemoattractant (59). It is conceivable that current knowledge of the known chemokines represents only a fraction of their activities. Therefore, a key question remains: will inhibition of a single chemokine or receptor suppress eosinophil-associated inflammation? Currently, eotaxin and CKR-3 show promise as molecules playing pivotal roles in eosinophil infiltration and are exceedingly attractive target(s) for therapeutic intervention in allergic diseases. Inhibition of eosinophil-specific chemokines and cellular infiltration may also provide insight into the pathophysiologic mechanisms of allergic and other disease conditions. There is compelling evidence that links neutrophils and C-X-C chemokines with tissue damage in inflammatory diseases (60), and the disruption of this cascade is beneficial for the host. In allergic diseases, glucocorticoids remain the backbone of therapy; however, the beneficial effects of these agents are counterbalanced by their side effects. The specificity of the newly discovered chemokines and their receptors may permit new therapies for eosinophil-associated disease with glucocorticoid-like actions, but without their serious side effects.

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