

Antichemokine immunotherapy for allergic diseases

Andrew D. Luster

Chemokines have emerged as critical regulators of leukocyte function and as such represent attractive new targets for the therapy of allergic diseases. Recent studies have revealed important roles for the chemokine family in both the afferent and efferent limbs of the immune system, orchestrating and integrating innate and acquired immune responses. A subset of chemokines including eotaxin-1 (also called CCL11), eotaxin-2 (CCL24), eotaxin-3 (CCL26), MCP (monocyte chemoattractant protein)-3 (CCL7), MCP (monocyte chemoattractant protein)-4 (CCL13), TARC (thymus- and activation-regulated chemokine) (CCL17), and MDC (macrophage-derived chemokine) (CCL22) are highly expressed in allergic inflammation and are regulated by T helper type 2 cytokines. Receptors for these chemokines, including CCR3 (CC chemokine receptor 3), CCR4 (CC chemokine receptor 4) and CCR8 (CC chemokine receptor 8) are expressed on key leukocytes associated with allergic inflammation, such as T helper type 2 cells, eosinophils, mast cells and basophils, establishing a subset of chemokine/chemokine receptors potentially important in allergic inflammation. Recent data using inhibitory antibodies and chemokine antagonists support the concept that interfering with this subset of chemokines and their receptors represents a new approach to allergy immunotherapy. *Curr Opin Allergy Clin Immunol* 1:561–567. © 2001 Lippincott Williams & Wilkins.

Correspondence to Andrew D. Luster, Center for Immunology and Inflammatory Diseases, Division of Rheumatology, Allergy and Immunology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA
E-mail: luster@helix.mgh.harvard.edu

Current Opinion in Allergy and Clinical Immunology 2001, 1:561–567

Abbreviations

BLC	B lymphocyte chemoattractant
CCR	CC chemokine receptor
CLA	cutaneous lymphocyte antigen
CXCR	CXC chemokine receptor
DC-CK1	dendritic cell-derived CC chemokine 1
ELC	Epstein-Barr virus induced gene ligand 1 chemokine
MCP	monocyte chemoattractant protein
MDC	macrophage-derived chemokine
MIP	macrophage inflammatory protein
RANTES	regulated upon activation in normal T cells expressed and secreted
SLC	secondary lymphoid chemokine
STAT6	signal transducer and activator of transcription factor 6
TARC	thymus- and activation-regulated chemokine
Th2	T helper type 2

© 2001 Lippincott Williams & Wilkins
1528-4050

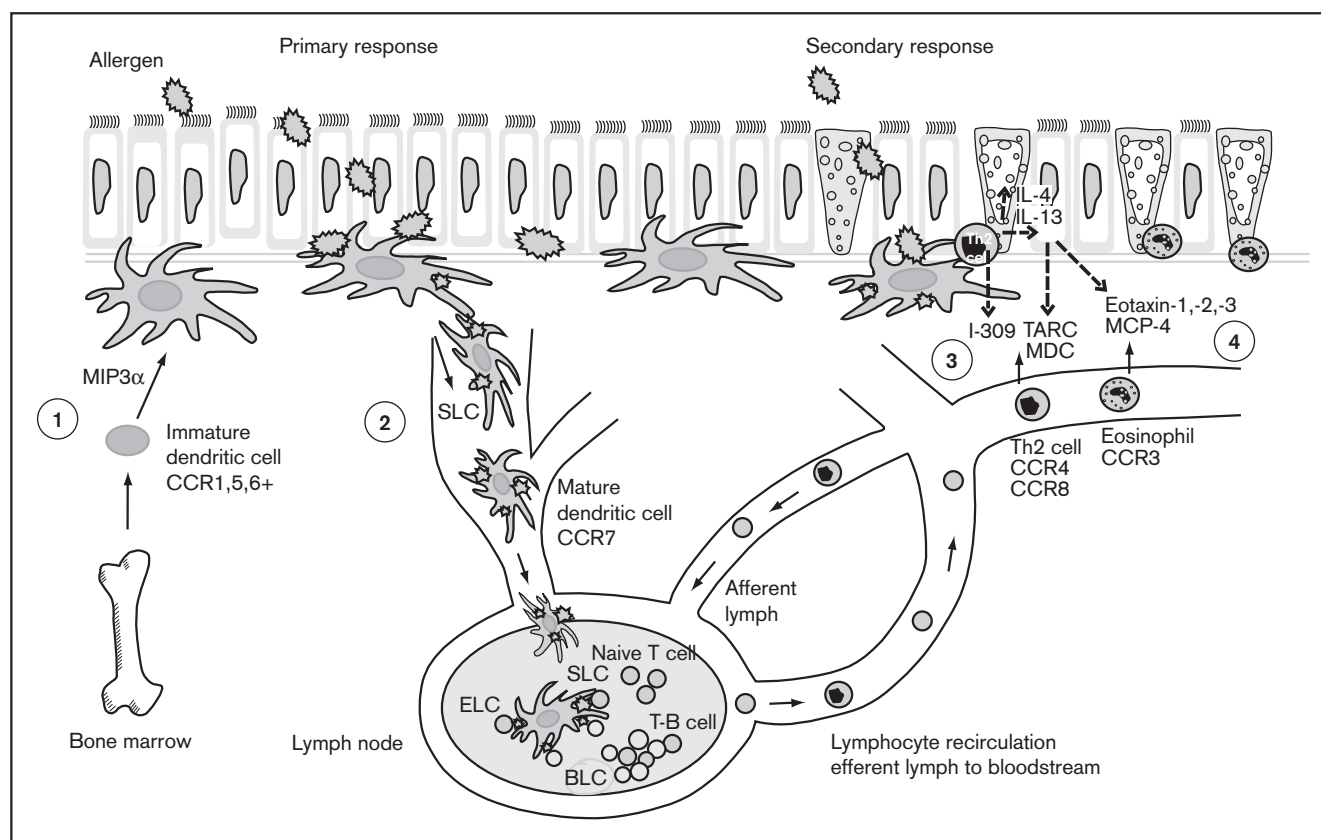
Introduction

Chemokines are secreted basic proteins of 8–10 kDa that represent the largest family of cytokines encoded in the human genome with close to 50 members [1]. They are critically important in regulating leukocyte trafficking *in vivo* and participate in the activation of leukocytes in tissues. Chemokines signal leukocytes through a family of G protein-coupled seven transmembrane receptors consisting of over 20 distinct receptors. The unexpected complexity of the chemokine system appears to allow for the precise control of leukocyte trafficking *in vivo*, which fine-tunes the immune response. The distinct activity of chemokines and the unique pattern of chemokine receptor expression on defined leukocyte subsets have afforded an opportunity to manipulate the immune system in a more defined manner than previously appreciated. This review will present an overview of chemokine function in allergic disease, highlighting potential areas for therapeutic intervention. Particularly attractive chemokine targets that have emerged from human and murine studies will be highlighted.

Chemokine function in allergic inflammation

Chemokines appear to have two main functions in regulating allergic inflammation: leukocyte trafficking and leukocyte activation. Recent murine studies have revealed important roles for the chemokine family in both the afferent and efferent limbs of the immune system, orchestrating both the innate and acquired immune responses (Fig. 1). It has been appreciated for some time now that the primary immune response takes place in the draining lymph nodes and not in the tissue itself. Antigen is picked up by dendritic cells in the tissue and carried into regional lymph nodes, where they activate naïve T and B cells. Activated T and B cells then leave the lymph node and find their way back to the site of inflammation. Once in the tissue, these activated lymphocytes and the cytokines they secrete lead to the accumulation and activation of additional leukocytes. In the case of allergic inflammation, it is the T helper type 2 (Th2) cell, the eosinophil and the mast cell that characterize the tissue inflammatory response.

Whilst the trafficking patterns of dendritic cells have been appreciated for some time now, the molecules that control their movement *in vivo* have only recently been recognized as members of the chemokine family [3]. The chemokine system has emerged as the critical regulator of both dendritic cell and lymphocyte trafficking. It has been hypothesized that macrophage inflam-

Figure 1. The role of chemokines in the generation of allergic pulmonary inflammation

Chemokines function in at least four distinct steps in the development of allergic inflammation. (1) Immature dendritic cells traffic from the bone marrow into the respiratory epithelium. This process is likely under the control of multiple chemokines, including MIP-3 α (CCL20) signaling via CCR6. (2) Once dendritic cells pick up antigen in the tissue they become activated and downregulate their responsiveness to CCR1, CCR5 and CCR6 ligands and become responsive to the CCR7 ligands SLC (CCL21) and ELC (CCL19), which guide the antigen loaded dendritic cell into the lymph node. In the lymph node these dendritic cells activate naive T cells generating T helper type 2 (Th2) cells. (3) Th2 cells leave the lymph node and travel back to the lung where they can be reactivated in the lung by reexposure to inhaled antigen. Activated Th2 cells release the chemokine I-309 as well as Th2 cytokines interleukin (IL)-4 and IL-13, which then induce the release of the eotaxins (CCL11, CCL24, CCL26) and MDC (CCL22) and TARC (CCL17) from respiratory epithelium. (4) These 'Th2-chemokines' then recruit more Th2 cells as well as eosinophils and perhaps basophils and mast cells. BLC (CXCL13), MCP-4 (CCL13). Modified from [2].

matory protein (MIP)-3 α (CCL20) attracts immature dendritic cells into the tissue, a process that is likely amplified by other 'inflammatory' chemokines, such as MIP-1 α (CCL3) [4]. Once in the vicinity of the inflammatory stimulus, immature dendritic cells pick up antigen and then differentiate into cells more capable of activating lymphocytes. During this maturation process, dendritic cells down modulate the expression of CC chemokine receptor 6 (CCR6) and hence responsiveness to MIP-3 α [5]. At the same time, they upregulate their expression of CCR7 allowing them to respond to secondary lymphoid chemokine (SLC) (CCL21) and Epstein-Barr virus induced gene ligand 1 chemokine (ELC) (CCL19). This switch in chemokine receptor expression and chemokine responsiveness results in the dendritic cells leaving the tissue and being drawn into the lymphatics, and ultimately, into the T cell rich regions of lymph nodes.

While the molecular details still remain to be fully elucidated, the expression of chemokines by lymph node stroma and dendritic cells coordinates the juxtaposition of antigen loaded dendritic cells with recirculating T and B cells. Chemokines that may play a role in this process are dendritic cell-derived CC chemokine 1 (DC-CK1) (CCL18), macrophage-derived chemokine (MDC) (CCL22), MIP-3 α , SLC and ELC. ELC and SLC are expressed by T zone lymph node stroma as well as dendritic cells and recruit naive T cells [6,7]. DC-CK1 is expressed by activated mature dendritic cells and also recruits naive T cells [8]. In contrast, MDC is induced in Langerhans cells migrating from contact-sensitized skin during maturation into lymph node dendritic cells, and chemoattracts antigen-specific T cells but not naive T cells [9]. B lymphocyte chemoattractant (BLC) (CXCL13) is expressed from stromal cells in the B cell follicle and

brings together CXCR5-positive T helper cells and B cells [10–13].

Thus, some activated T cells down-regulate CCR7 and up-regulate CXCR5, they become directed toward the follicle to instruct B cells in class switching and have been called follicular T cells [10,12]. Other T cells down-regulate CCR7 and up-regulate CCR3, CCR4 and CCR8 and are attracted into sites of Th2 inflammation, while other T cells up-regulate CXCR3 and CCR5 and are attracted into sites of Th1 inflammation [14]. T cells, activated in regional lymph nodes following encounter with activated antigen loaded dendritic cells, are recruited to sites of inflammation by sensing chemokine gradients established at these local sites. For Th2 cells, chemokines induced by Th2 cytokines and regulated by the transcription factor STAT6, such as the eotaxins, MDC, TARC, and I-309 (CCL1), appear to control the trafficking of Th2 cells into sites of allergic inflammation [15•]. Once in the tissue, Th2 cells secrete interleukin (IL)-4 and IL-13, and amplify Th2 cell recruitment by inducing the release of STAT6 inducible chemokines, which are active on Th2 cells. Interestingly, many of these same chemokines are also active on eosinophils and mast cells. In this way, Th2 cells control the trafficking of eosinophils, and perhaps mast cells, into sites of allergic inflammation.

The other main function of chemokines relates to their ability to activate leukocytes once in the tissue. Chemokines have been shown to activate leukocytes associated with allergic inflammation, such as eosinophils, basophils and mast cells, by inducing the exocytosis of secondary granules. In fact, chemokines are the major non-IgE histamine releasing factors and may therefore play a very important role in the late phase release of histamine seen in allergic responses. The main eosinophil chemokine receptor is CCR3 [16], and CCR3 ligands, such as eotaxin-1 and -2 and MCP-4, have been shown to activate eosinophil granule release [17] and respiratory burst [18,19]. Lower levels of other receptors such as CCR1, CXCR2, CXCR3 and CXCR4 have been reported on eosinophils, although their significance remains unknown. Basophils express high levels of CCR2 and CCR3, as well as lower levels of CXCR1, CXCR2, CXCR4 and CCR1 [20]. CCR2 agonists, such as MCP-1, MCP-2, MCP-3 and MCP-4, are potent inducers of exocytosis and mediator-release in basophils but weak inducers of a migratory response [21,22]. CCR2 is the only chemokine receptor on basophils capable of inducing strong and consistent mediator release in basophils without the need for priming with other cytokines, such as IL-3. In contrast, CCR3 agonists are potent inducers of basophil chemotaxis but only weak inducers of mediator release, and require preactivation of the basophil with IL-3 or IL-5

[20]. Chemokines can also induce the release of leukotriene (LT)C₄ from primed basophils. Mast cells also express high levels of CCR3. Interestingly, CCR3 is a receptor of importance to eosinophils, basophils and mast cells and therefore represents an attractive therapeutic target.

Chemokines and T helper type 1/T helper type 2 cell balance

Since allergic inflammation is the result of an aberrant response to a foreign antigen, shifting that response to a Th1-type response is one potential therapeutic strategy. However, while chemokines have been implicated in development of Th2 cell responses [23,24], insufficient data exist that they directly control Th2 cell development in a manner that makes this an attractive strategy for therapeutically shifting the Th1/Th2 balance of an immune response.

Chemokine expression in human allergic diseases

A subset of chemokines has been found to be up-regulated in allergic diseases and in animal models of allergic diseases (Table 1). These studies have revealed that chemokines regulated by Th2 cytokines, particularly IL-4 and IL-13, and proinflammatory cytokines, such as tumor necrosis factor and IL-1, are highly expressed in allergic inflammation, such as asthma, atopic dermatitis, and allergic rhinitis.

Asthma

Expression of eotaxin-1, eotaxin-2, MCP-1, MCP-3, MCP-4, TARC, MDC, RANTES (CCL5), and MIP-1 α are increased in the lung and bronchoalveolar lavage (BAL) of asthmatic patients compared to normal controls [25–28]. In addition, levels of eotaxin-1 [29•], eotaxin-3 [30•], RANTES [31], MDC and TARC [32••] are up-regulated in the lung and BAL following aerosol antigen

Table 1. Chemokine expression in human allergic diseases

Disease	Chemokine	Chemokine receptor
Asthma	RANTES, eotaxin-1, eotaxin-2, eotaxin-3, MCP-3, MCP-4	CCR3
	MCP-1	CCR2
	MDC, TARC	CCR4
	I-309	CCR8
Atopic dermatitis	TARC	CCR4
	MIP-3 α	CCR6
	Eotaxin-1, MCP-4	CCR3
Allergic rhinitis	RANTES, eotaxin-1, eotaxin-2, eotaxin-3, MCP-3, MCP-4	CCR3
	MDC, TARC	CCR4

CCR, CC chemokine receptor; I-309 (CCL1); MCP, monocyte chemoattractant protein; MDC, macrophage-derived chemokine; MIP, macrophage inflammatory protein; RANTES, regulated upon activation in normal T cells expressed and secreted; TARC, thymus- and activation-regulated chemokine.

challenge. Levels of eotaxin-1, MCP-4 and RANTES correlated with BAL eosinophilia, suggesting that these chemokines play a pathogenic role in recruiting eosinophils into the lung. T cells recovered from the airway mucosa 24 h after allergen challenge demonstrated that virtually all T cells expressed IL-4 and CCR4 and that CCR8 was seen on 28% of these T cells [32•]. These observations suggest that CCR4 might play an important role in the recruitment or retention of Th2 cells in the allergic lung. However, these observations await validation as another recent study examining chemokine receptor expression on lung T cells isolated from asthmatic patients at baseline (non-antigen challenge) and nonasthmatic study participants found only low levels of CCR4 in both groups [33•].

Allergic rhinitis and sinusitis

A similar set of chemokines is associated with allergic rhinitis and sinusitis. Nasal allergen challenge of allergic rhinitis patients induced the expression of eotaxin-1, RANTES, MCP-3 and MCP-4 from the nasal mucosa and release of these proteins into nasal secretions [34–36]. Similarly, levels of MCP-3 and MCP-4 but not MCP-1 were increased in the ethmoid sinuses of allergic subjects with chronic sinusitis [37].

Atopic dermatitis

In atopic dermatitis lesions, high levels of RANTES, eotaxin, MCP-3, MCP-4, MIP-3 α and TARC are seen in lesional skin [38–43]. Levels of TARC and MIP-3 α are associated with disease activity [42–43] and the proportion of cutaneous lymphocyte antigen (CLA⁺) (skin homing) CCR4⁺ lymphocytes is up-regulated in the blood and lesions of atopic dermatitis patients [44]. Further support for a role of CCR4 in atopic dermatitis comes from a mutant mouse strain (NC/Nga) that spontaneously develops atopic dermatitis-like lesions that contain high levels of TARC and MDC, CCR4 ligands [45]. Taken together, these data suggest that TARC and MDC play important roles in the recruitment of skin homing CLA⁺ CCR4⁺ Th2 cells into atopic dermatitis lesions. Antigen-induced late-phase cutaneous responses in atopic subjects induced the sequential expression of eotaxin-1 associated with early 6-h eosinophils, and of eotaxin-2 and MCP-4 with later 24-h eosinophils [46].

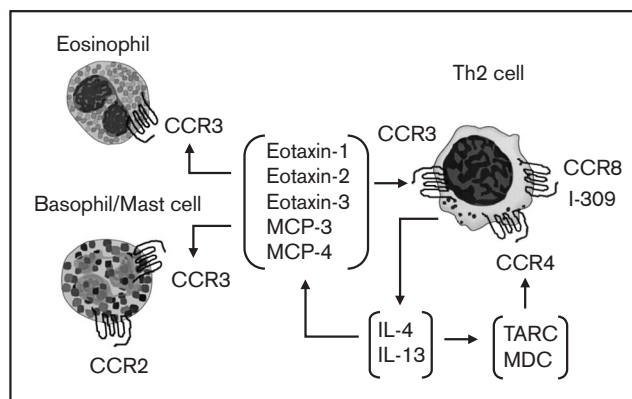
Thus, human studies examining the expression of chemokines in allergic diseases support the emerging concept that CCR3, CCR4, and CCR8 and their chemokine ligands play important roles in the pathogenesis of allergic diseases.

Chemokine targets for allergic inflammation

In thinking about where interruption of the chemokine cascade could effectively inhibit the recruitment and

activation of leukocytes that characterize the allergic response, it is helpful to divide the discussion into the roles of chemokines in different phases of the allergic response (Fig. 1). The first phase relates to the trafficking of dendritic cells or dendritic cell precursors from the bone marrow and blood into the tissue, such as the lung or skin. This process is likely to be under the control of chemokines, but a single chemokine/chemokine receptor pair has not emerged as a dominant player in this process. Immature dendritic cells express several chemokine receptors, including CCR1, CCR2, CCR5 and CCR6 [3]. CCR6 was thought to play an important role in this process but CCR6-deficient mice have been reported to have an impairment in dendritic cell function isolated to the gastrointestinal tract [47,48]. Recently, treatment of rats with the selective CCR1 and CCR5 antagonist Met-RANTES reduced baseline numbers of tracheal intraepithelial dendritic cells by approximately 50% [49]. It is not clear at present if dendritic cells that are recruited into different tissues are responding to the same or different chemokine signals. Thus, currently, selectively interfering with immature dendritic cell trafficking into tissue sites remains an interesting theoretical possibility without a molecular target. The second phase of the response, as outlined in Fig. 1, relates to the trafficking of activated antigen loaded dendritic cells from periphery into draining lymph nodes. Data from CCR7-deficient mice have revealed that this receptor plays an important role in this process [50]. Therefore, inhibiting CCR7 function may be an attractive way of preventing antigens introduced into the lung from being introduced into the immune system and thus preventing a primary immune response. Interruption of CCR7 signaling presents several significant problems, however. The first is that, in most circum-

Figure 2. Key chemokine/chemokine receptor pairs implicated in allergic inflammation



CCR, CC chemokine receptor; I-309 (CCL1); IL, interleukin; MCP, monocyte chemoattractant protein; MDC, macrophage-derived chemokine; TARC, thymus- and activation-regulated chemokine; Th2, T helper type 2.

stances, therapy will need to be aimed at inhibiting the secondary immune response in patients who have demonstrated that they have made a pathologic Th2 response to an antigen. Interruption of CCR7 signaling might prevent the allergic patient from developing an allergic response to a new antigen but probably will be ineffective at preventing the activation of an amnestic response at a body surface. In addition, CCR7 function is important for the recruitment of naïve T cells into lymph nodes and the interruption of CCR7 signaling will likely result in general immunodeficiency.

The third phase relates to the recruitment of Th2 cells into sites of allergic inflammation. Th2 cells express several chemokine receptors, including CCR3, CCR4 and CCR8. Although CCR3 was the first chemokine receptor described to be differentially expressed on polarized Th2 cells [51] most investigators have found little to no CCR3 on T cells found in allergic inflammation [32•,33•]. In addition, mice deficient in CCR3 and CCR4 appear to have normal trafficking of Th2 cells [52]. One study, however, suggested that CCR3 and CCR4 ligands sequentially contribute to the recruitment of Th2 cells in allergic pulmonary inflammation [53]. Mice deficient in CCR8 have an impaired Th2 response, but it remains unclear if this is due to a primary defect in Th2 cell polarization or Th2 cell trafficking [54•]. The author's team has recently demonstrated a dramatic impairment in Th2 cell trafficking in mice deficient in the transcription factor STAT6 using an adoptive transfer protocol [15•]. In this model, chemokines active on Th2 cells and eosinophils, such as the CCR3 ligands eotaxin-1 and eotaxin-2, the CCR4 ligands MDC and TARC and the CCR8 ligand I-309 were not expressed following adoptive transfer of ovalbumin-specific Th2 cells and aerosol ovalbumin challenge. These data suggest that one or more STAT6-inducible chemokines control the trafficking of Th2 cells into the allergic lung. At present, CCR8 may be the best candidate for a therapeutic target aimed at specifically inhibiting Th2 cell trafficking into the lung. CCR4 may play a more prominent role in Th2 cell

trafficking to the skin, although this awaits experimental confirmation.

The fourth potential area relates to the trafficking and activation of eosinophils, basophils and mast cells. Once Th2 cells are in the tissue and are activated by antigen, they release cytokines that induce the subsequent release of chemokines leading to the influx of eosinophils. IL-4 and IL-13, via a STAT6-dependent mechanism, induce the release of chemokines active on eosinophils, such as eotaxin-1, eotaxin-2, eotaxin-3, and MCP-4. These chemokines activate eosinophils via CCR3, making CCR3 a good target for interfering with eosinophil recruitment to sites of allergic inflammation [16]. The trafficking of basophils and mast cells in allergic inflammation is less well documented. However, the number of mast cells [55,56] and basophils [57] found in allergic tissue is increased compared to normal tissue and their numbers correlate with disease severity [57], suggesting that these cells are recruited into allergic tissue. As mentioned above, once in the tissue, these cells can be activated by chemokines and, in particular, CCR2 and CCR3 ligands. The contribution that chemokines make to the overall activation of these inflammatory cells in allergic tissue remains to be determined, however.

Chemokine antagonists

Neutralizing antichemokine antibodies and chemokine antagonists have been used in murine models of allergic diseases to establish the principle that antagonizing chemokine action represents a viable therapeutic strategy (Table 2). Antibodies to TARC [58], MDC [59], eotaxin-1 [60], MCP-1 [60,61], MIP-1 α [61], RANTES [61] and MCP-3 [62] have all in their own right inhibited various aspects of allergic pulmonary inflammation and airway hyperreactivity. However, mice with genetic deficiency of these genes or their receptors, such as eotaxin-1 [63,64], CCR2 [65] and CCR4 [52], have not exhibited the phenotypes seen using neutralizing antibodies, raising some concerns that compensatory changes may be seen with long-term depletion of chemokine signaling. Modified chemokines that can

Table 2. Chemokine antagonism in models of allergic pulmonary inflammation

Antagonists	Asthma model	Pulmonary eosinophilia	Th2 cell recruitment	IL-4, IL-5, IL-13	Ig-E	AHR
Met-RANTES	OVA	↓↓↓	↓↓↓	ND	ND	ND
vCC1	OVA	↓↓	No Δ	No Δ	No Δ	↓↓
Anti-TARC Ab	OVA	↓↓↓↓	↓↓	↓↓↓↓	ND	↓↓↓
Anti-MDC Ab	OVA	↓↓	↓	ND	ND	↓↓↓↓
Anti-eotaxin-1 Ab	OVA	↓	↓	No Δ	No Δ	↓↓
Anti-MCP-1 Ab	OVA/SEA	↓↓↓	↓↓↓	↓↓↓	↓↓↓	↓↓↓↓
Anti-MCP-3 Ab	OVA	↓↓↓	ND	ND	ND	ND
Anti-MIP-1 α Ab	SEA	↓↓	No Δ	ND	ND	No Δ
Anti-RANTES Ab	SEA	↓↓	No Δ	ND	ND	No Δ

AHR, airways hyperreactivity; IL, interleukin; MCP, monocyte chemoattractant protein; MDC, macrophage-derived chemokine; MIP, macrophage inflammatory protein; Δ , no change; OVA, ovalbumin; RANTES, regulated upon activation in normal T cells expressed and secreted; SEA, soluble egg antigens from *Schistosoma mansoni*; TARC, thymus- and activation-regulated chemokine; Th2, T helper type 2.

function as antagonists and virally encoded chemokine antagonists have also revealed important roles for chemokines in murine models of allergic pulmonary inflammation. For example, when RANTES is expressed in *Escherichia coli*, the initiating methionine is retained, resulting in Met-RANTES. This protein can block functional responses to RANTES and MIP-1 α . Administration of Met-RANTES during the sensitization and challenge phases in a murine ovalbumin model of allergic pulmonary inhibited T cell and eosinophil recruitment as well as airway hyperreactivity and mucus hypersecretion [60]. Viral-derived CC-chemokine inhibitor is a 35 kDa secreted virulence protein encoded in the pox virus genome [66]. It is a competitive antagonist for at least 15 CC chemokines inhibiting binding to their respective chemokine receptors. Administration of the inhibitor to the respiratory tract of immunized mice prior to antigen challenge reduced pulmonary eosinophilia and airways hyperreactivity [67•]. These studies have established the 'proof of concept' that inhibiting chemokine action in asthma is a viable strategy that should be explored.

Conclusion

Unraveling the complexity of the chemokine system has provided new insight into the molecular regulation of leukocyte trafficking. The intense interest in the chemokine system has focused attention on the critical importance of leukocyte trafficking in shaping the nature and intensity of an immune response. The specificity of the chemokine system affords a unique opportunity to precisely manipulate leukocyte function in pathologic responses, such as allergic inflammation. In this regard, interfering with CCR3, CCR4 and CCR8 signaling represents attractive new targets for attenuating allergic inflammation associated with allergic diseases.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

- 1 Luster AD. Chemokines: chemotactic cytokines that mediate inflammation. *N Engl J Med* 1998; 388:436–445.
- 2 Lambrecht BN. Allergen uptake and presentation by dendritic cells. *Curr Opin Allergy Clin Immunol* 2001 1:51–59.
- 3 Sozzani S, Allavena P, Vecchi A, Mantovani A. Chemokines and dendritic cell traffic. *J Clin Immunol* 2000; 20:151–160.
- 4 Charbonnier AS, Kohrgruber N, Kriehuber E, et al. Macrophage inflammatory protein 3 α is involved in the constitutive trafficking of epidermal langerhans cells. *J Exp Med* 1999; 190:1755–1768.
- 5 Dieu MC, Vanbervliet B, Vicari A, et al. Selective recruitment of immature and mature dendritic cells by distinct chemokines expressed in different anatomic sites. *J Exp Med* 1998; 188:373–383.
- 6 Ngo VN, Tang L, Cyster J.G. Epstein-Barr virus-induced molecule 1 ligand chemokine is expressed by dendritic cells in lymphoid tissue and strongly attracts naive T cells and activated B cells. *J Exp Med* 1998; 188:181–191.
- 7 Luther SA, Tang HL, Hyman PL, et al. Coexpression of the chemokines ELC and SLC by T zone stromal cells and deletion of the ELC gene in the plt/plt mouse. *Proc Natl Acad Sci U S A* 2000; 97:12694–12699.
- 8 Adema GJ, Hartgers F, Verstraten R, et al. A dendritic-cell-derived C-C chemokine that preferentially attracts naive T cells. *Nature* 1997; 387:713–717.
- 9 Tang H, Cyster J. Chemokine up regulation and activated T cell attraction by maturing dendritic cells. *Science* 1999; 284:819–822.
- 10 Kim CH, Rott LS, Clark-Lewis I, et al. Subspecialization of CXCR5(+) T Cells. B helper activity is focused in a germinal center-localized subset of CXCR5(+) T cells. *J Exp Med* 2001; 193:1373–1382.
- 11 Forster R, Mattis AE, Kremmer E, et al. A putative chemokine receptor, BLR1, directs B cell migration to defined lymphoid organs and specific anatomic compartments of the spleen. *Cell* 1996; 87:1037–1047.
- 12 Schaerli P, Willmann K, Lang AB, et al. CXC chemokine receptor 5 expression defines follicular homing T cells with B cell helper function. *J Exp Med* 2000; 192:1553–1562.
- 13 Ansel KM, Ngo VN, Hyman PL, et al. A chemokine-driven positive feedback loop organizes lymphoid follicles. *Nature* 2000; 406:309–314.
- 14 Sallusto F, Lenig D, Mackay CR, Lanzavecchia A. Flexible programs of chemokine receptor expression on human polarized T helper 1 and T helper 2 lymphocytes. *J Exp Med* 1998; 187:875–883.
- 15 Mathew A, MacLean JA, DeHaan E, et al. Signal transducer and activator of transcription 6 controls chemokine production and T helper cell type 2 cell trafficking in allergic pulmonary inflammation. *J Exp Med* 2001; 193:1087–1096.
- This study was the first to clearly demonstrate that Th2 cell trafficking to the allergic lung is an active process by demonstrating that it is dependent on STAT6 signaling in the lung. In addition, it suggested that chemokines regulated by STAT6 control Th2 cell trafficking.
- 16 Heath H, Qin S, Rao P, et al. Chemokine receptor usage by human eosinophils: The importance of CCR3 demonstrated using an antagonistic monoclonal antibody. *J Clin Invest* 1997; 99:178–184.
- 17 Fujisawa T, Kato Y, Nagase H, et al. Chemokines induce eosinophil degranulation through CCR-3. *J Allergy Clin Immunol* 2000; 106:507–513.
- 18 Elsner J, Hochstetter R, Kimmig D, Kapp A. Human eotaxin represents a potent activator of the respiratory burst of human eosinophils. *Eur J Immunol* 1996; 26:1919–1925.
- 19 Elsner J, Petering H, Kluthe C, et al. Eotaxin-2 activates chemotaxis-related events and release of reactive oxygen species via pertussis toxin-sensitive G proteins in human eosinophils. *Eur J Immunol* 1998; 28:2152–2158.
- 20 Uguccioni M, Mackay CR, Ochensberger B, et al. High expression of the chemokine receptor CCR3 in human blood basophils. Role in activation by eotaxin, MCP-4, and other chemokines. *J Clin Invest* 1997; 100:1137–1143.
- 21 Bischoff SC, Krieger M, Brunner T, Dahinden CA. Monocyte chemotactic protein 1 is a potent activator of human basophils. *J Exp Med* 1992; 175:1271–1275.
- 22 Kuna P, Reddigari SR, Rucinski D, et al. Monocyte chemotactic and activating factor is a potent histamine-releasing factor for human basophils. *J Exp Med* 1992 175:489–493.
- 23 Kennedy KJ, Karpus WJ. Role of chemokines in the regulation of Th1/Th2 and autoimmune encephalomyelitis. *J Clin Immunol* 1999; 19:273–279.
- 24 Gu L, Tseng S, Horner RM, et al. Control of TH2 polarization by the chemokine monocyte chemoattractant protein-1. *Nature* 2000; 404:407–411.
- 25 Lamkhioeu B, Renzi PM, Abi-Younes S, et al. Increased expression of eotaxin in bronchoalveolar lavage and airways of asthmatics contributes to the chemotaxis of eosinophils to the site of inflammation. *J Immunol* 1997; 159:4593–4601.
- 26 Lamkhioeu B, Garcia-Zepeda EA, Abi-Younes S, et al. Monocyte chemoattractant protein (MCP)-4 expression in the airways of patients with asthma. Induction in epithelial cells and mononuclear cells by proinflammatory cytokines. *Am J Respir Crit Care Med* 2000; 162:723–732.
- 27 Alam R, York J, Boyars M, et al. Increased MCP-1, RANTES, and MIP-1 α in bronchoalveolar lavage fluid of allergic asthmatic patients. *Am J Respir Crit Care Med* 1996; 153:1398–1404.
- 28 Ying S, Meng Q, Zeibecoglou K, et al. Eosinophil chemotactic chemokines (eotaxin, eotaxin-2, RANTES, monocyte chemoattractant protein-3 (MCP-3), and MCP-4), and C-C chemokine receptor 3 expression in bronchial biopsies from atopic and nonatopic (intrinsic) asthmatics. *J Immunol* 1999; 163:6321–6329.

- 29 Lilly CM, Nakamura H, Belostotsky OI, *et al.* Eotaxin expression after segmental allergen challenge in subjects with atopic asthma. *Am J Respir Crit Care Med* 2001; 163:1669–1675.
- Aerosol antigen challenge induced the expression of eotaxin-1 in the airways of asthmatics, suggesting that this chemokine contributes to the pathogenesis of human asthma.
- 30 Berkman N, Ohnonsa S, Chung FK, Breuer R. Eotaxin-3 but not eotaxin gene expression is upregulated in asthmatics 24 hours after allergen challenge. *Am J Respir Cell Mol Biol* 2001; 24:682–687.
- Aerosol antigen challenge induced the expression of eotaxin-3 in the airways of asthmatics, suggesting that this chemokine contributes to the pathogenesis of human asthma.
- 31 Teran LM, Noso N, Carroll M, *et al.* Eosinophil recruitment following allergen challenge is associated with the release of the chemokine RANTES into asthmatic airways. *J Immunol* 1996; 157:1806–1812.
- 32 Panina-Bordignon P, Papi A, Mariani M, *et al.* The C-C chemokine receptors CCR4 and CCR8 identify airway T cells of allergen-challenged atopic asthmatics. *J Clin Invest* 2001; 107:1357–1364.
- CCR4 and CCR8 but not CCR3 was found on IL-4 secreting T cells in the airways of allergen-challenged atopic asthmatics, suggesting that CCR4 and CCR8 are functionally important for the recruitment of Th2 cells into the asthmatic airway.
- 33 Campbell JJ, Brightling CE, Fiona FA, *et al.* Expression of chemokine receptors by lung T cells from normal and asthmatic subjects. *J Immunol* 2001; 166:2842–2848.
- This study suggested that T cells recruited into the lung express a unique set of adhesion molecules and chemokine receptors, although lung specific or asthma specific adhesion or chemokine receptors were not identified.
- 34 Sim TC, Reece LM, Hilsmeier KA, *et al.* Secretion of chemokines and other cytokines in allergen-induced nasal responses: inhibition by topical steroid treatment. *Am J Respir Crit Care Med* 1995; 152:927–933.
- 35 Christodoulouopoulos P, Wright E, Frenkiel S, *et al.* Monocyte chemotactic proteins in allergen-induced inflammation in the nasal mucosa: effect of topical corticosteroids. *J Allergy Clin Immunol* 1999; 103:1036–1044.
- 36 Rajakulasingam K, Hamid Q, O'Brien F, *et al.* RANTES in human allergen-induced rhinitis: cellular source and relation to tissue eosinophilia. *Am J Respir Crit Care Med* 1997; 155:696–703.
- 37 Wright ED, Frenkiel S, Ghaffar O, *et al.* Monocyte chemotactic protein expression in allergy and non-allergy-associated chronic sinusitis. *J Otolaryngol* 1998; 27:281–287.
- 38 Yawalkar N, Uguccioni M, Scharer J, *et al.* Enhanced expression of eotaxin and CCR3 in atopic dermatitis. *J Invest Dermatol* 1999; 113:43–48.
- 39 Taha RA, Minshall EM, Leung DY, *et al.* Evidence for increased expression of eotaxin and monocyte chemotactic protein-4 in atopic dermatitis. *J Allergy Clin Immunol* 2000; 105:1002–1007.
- 40 Morita E, Kameyoshi Y, Hiragun T, *et al.* The C-C chemokines, RANTES and eotaxin, in atopic dermatitis. *Allergy* 2001; 56:194–195.
- 41 Niwa Y. Elevated RANTES levels in plasma or skin and decreased plasma IL-10 levels in subsets of patients with severe atopic dermatitis. *Arch Dermatol* 2000; 136:125–126.
- 42 Nakayama T, Fujisawa R, Yamada H, *et al.* Inducible expression of a CC chemokine liver- and activation-regulated chemokine (LARC)/macrophage inflammatory protein (MIP)-3 alpha/CCL20 by epidermal keratinocytes and its role in atopic dermatitis. *Int Immunol* 2001; 13:95–103.
- 43 Kakinuma T, Nakamura K, Wakugawa M, *et al.* Thymus and activation-regulated chemokine in atopic dermatitis: Serum thymus and activation-regulated chemokine level is closely related with disease activity. *J Allergy Clin Immunol* 2001; 107:535–541.
- 44 Nakatani T, Kaburagi Y, Shimada Y, *et al.* CCR4 memory CD4+ T lymphocytes are increased in peripheral blood and lesional skin from patients with atopic dermatitis. *J Allergy Clin Immunol* 2001; 107:353–358.
- 45 Vestergaard C, Yoneyama H, Murai M, *et al.* Overproduction of Th2-specific chemokines in NC/Nga mice exhibiting atopic dermatitis-like lesions. *J Clin Invest* 1999; 104:1097–1105.
- 46 Ying S, Robinson DS, Meng Q, *et al.* C-C chemokines in allergen-induced late-phase cutaneous responses in atopic subjects: association of eotaxin with early 6-hour eosinophils, and of eotaxin-2 and monocyte chemoattractant protein-4 with the later 24-hour tissue eosinophilia, and relationship to basophils and other C-C chemokines (monocyte chemoattractant protein-3 and RANTES). *J Immunol* 1999; 163:3976–3984.
- 47 Cook DN, Prosser DM, Forster R, *et al.* CCR6 mediates dendritic cell localization, lymphocyte homeostasis, and immune responses in mucosal tissue. *Immunity* 2000; 12:495–503.
- 48 Varona R, Villares R, Carramolino L. CCR6-deficient mice have impaired leukocyte homeostasis and altered contact hypersensitivity and delayed-type hypersensitivity responses. *J Clin Invest* 2001; 107:R37–R45.
- 49 Stumbles PA, Strickland DH, Pimm CL, *et al.* Regulation of dendritic cell recruitment into resting and inflamed airway epithelium: use of alternative chemokine receptors as a function of inducing stimulus. *J Immunol* 2001; 167:228–234.
- 50 Forster R, Schubel A, Breitfeld D, *et al.* CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. *Cell* 1999; 99:23–33.
- 51 Sallusto F, Mackay CR, Lanzavecchia A. Selective expression of the eotaxin receptor CCR3 by human T helper 2 cells. *Science* 1997; 277:2005–2007.
- 52 Chvatchko Y, Hoogewerf AJ, Meyer A, *et al.* A key role for CC chemokine receptor 4 in lipopolysaccharide-induced endotoxic shock. *J Exp Med* 2000; 191:1755–1764.
- 53 Lloyd CM, Delaney T, Nguyen T, *et al.* CC chemokine receptor (CCR)3/eotaxin is followed by CCR4/monocyte-derived chemokine in mediating pulmonary T helper lymphocyte type 2 recruitment after serial antigen challenge in vivo. *J Exp Med* 2000; 191:265–274.
- 54 Chensue SW, Lukacs NW, Yang TY, *et al.* Aberrant in vivo T helper type 2 cell response and impaired eosinophil recruitment in CC chemokine receptor 8 knockout mice. *J Exp Med* 1993:573–584.
- This is the first description of CCR8-deficient mice and the first chemokine receptor knock-out mouse strain to show an impaired allergic pulmonary response characterized by decreased eosinophilic inflammation and decreased levels of the Th2 cell cytokines IL-4, IL-5 and IL-13.
- 55 Gibson PG, Allen CJ, Yang JP, *et al.* Intraepithelial mast cells in allergic and nonallergic asthma. Assessment using bronchial brushings. *Am Rev Respir Dis* 1993; 148:80–86.
- 56 Pesci A, Foresi A, Bertorelli G, *et al.* Histochemical characteristics and degranulation of mast cells in epithelium and lamina propria of bronchial biopsies from asthmatic and normal subjects. *Am Rev Respir Dis* 1993; 147:684–689.
- 57 Maruyama N, Tamura G, Aizawa T, *et al.* Accumulation of basophils and their chemotactic activity in the airways during natural airway narrowing in asthmatic individuals. *Am J Respir Crit Care Med* 1994; 150:1086–1093.
- 58 Kawasaki S, Takizawa H, Yoneyama H, *et al.* Intervention of thymus and activation-regulated chemokine attenuates the development of allergic airway inflammation and hyperresponsiveness in mice. *J Immunol* 2001; 166:2055–2062.
- 59 Gonzalo JA, Pan Y, Lloyd CM, *et al.* Mouse monocyte-derived chemokine is involved in airway hyperreactivity and lung inflammation. *J Immunol* 1999; 163:403–411.
- 60 Gonzalo JA, Lloyd CM, Wen KD, *et al.* The coordinated action of CC chemokines in the lung orchestrates allergic inflammation and airway hyperresponsiveness. *J Exp Med* 1998; 188:157–167.
- 61 Lukacs NW, Strieter RM, Warmington K, *et al.* Differential recruitment of leukocyte populations and alterations of airway hyperreactivity by C-C family chemokines in allergic airway inflammation. *J. Immunol* 1997; 158:4398–4404.
- 62 Stafford S, Li H, Forsythe PA, *et al.* Monocyte chemotactic protein-3 (MCP-3)/fibroblast-induced cytokine (FIC) in eosinophilic inflammation of the airways and the inhibitory effects of an anti-MCP-3/FIC antibody. *J Immunol* 1997; 158:4953–4960.
- 63 Rothenberg ME, MacLean JA, Pearlman E. Targeted disruption of the chemokine eotaxin only partially reduces antigen induced tissue eosinophilia. *J Exp Med* 1997; 185:785–790.
- 64 Yang Y, Loy J, Ryseck RP, *et al.* Antigen-induced eosinophilic lung inflammation develops in mice deficient in chemokine eotaxin. *Blood* 1998; 92:3912–3923.
- 65 MacLean JA, De Sanctis GT, Ackerman KG, *et al.* CC chemokine receptor-2 is not essential for the development of antigen-induced pulmonary eosinophilia and airway hyperresponsiveness. *J Immunol* 2000; 165:6568–6575.
- 66 Smith CA, Smith TD, Smolak PJ, *et al.* Poxvirus genomes encode a secreted, soluble protein that preferentially inhibits beta chemokine activity yet lacks sequence homology to known chemokine receptors. *Virology* 1997; 236:316–327.
- 67 Dabbagh K, Xiao Y, Smith C, *et al.* Local blockade of allergic airway hyperreactivity and inflammation by the poxvirus-derived pan-CC-chemokine inhibitor vCCI. *J Immunol* 2000; 165:3418–3422.
- This study established the principle that a chemokine antagonist delivered to the lung can attenuate allergic pulmonary inflammation.