# Lymphocyte trafficking and chemokine receptors during pulmonary disease

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## Introduction

The localization of lymphocytes to tissue during immune/inflammatory responses involves a series of complex mechanisms, including activation of integrin binding, adhesion molecule expression, and tissue-based chemokine production. The regulation of specific molecules and the expression of certain receptors on lymphocytes during the progression of disease determine the type of T lymphocytes, Th1 or Th2, which migrate into the tissue. Although there have been studies that have outlined tissue-specific expression of certain chemotactic molecules, a more logical view may be that the type of immune/inflammatory response induced within the affected tissue would dictate the mediators that are expressed. The trafficking of naive lymphocytes from the blood to lymph nodes is pivotal to the maintenance of effective immune surveillance; however, deciphering the mechanisms involved in lymphocyte recruitment during inflammation may be more pharmaceutically attractive for regulation of chronic debilitating diseases. Functional diversity of T cells has been demonstrated by the observation that naive T lymphocytes are activated and differentiate into Th0 type cells that produce different combinations of cytokines. Subsequently, these cells can further differentiate into either Th1 type cells (IL-2 and IFN) or Th2 type cells (IL-4, IL-5, and IL-13) depending upon the cytokine environment to which the Th0 cells are exposed [1, 2]. Over the years, it has become clear that certain diseases are characterized by the T helper (Th) cytokine phenotype that is produced. In particular, allergy and asthma responses have been identified as a largely Th2-type disease [3, 4]. The following review outlines some of the recent concepts that may dictate how and why certain Th lymphocyte subsets migrate into inflamed tissues and what contributions other disease mediators provide to worsen the disease process.

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## Chemokine and chemokine receptor patterns during Th1- or Th2-type responses

The migration of lymphocytes into tissues appears to be dependent upon the expression of specific chemokines during the progression of the inflammatory disease. Chemokines are a family of small molecular weight cytokines that are important for localization of particular leukocyte populations during immune/inflammatory responses [5–7]. Chemokines are primarily divided into two main groups, CxC and CC, based upon the juxtaposition of the first two-cysteine residues in their sequence (Tab. 1). The responses induced by the chemokines are initiated via specific G protein-coupled receptors on the surface of cells. Although not entirely characterized, it appears that there are no less than six different CxC family receptors and ten different CC family receptors. In the context of allergy, members of the CC subfamily have been implicated as potential mediators of the inflammatory response through their ability to induce migration of eosinophils, T cells and monocytes. In addition to playing a prominent role in the localization of leukocytes to tissue sites, these activating factors are also involved in important biological events, such as eosinophil and mast cell degranulation, differentiation of Th lymphocyte phenotypes, and regulation of antibody isotype switching. Thus, these molecules have important functions in multiple phases of the developing immune response.

Some of the initial studies in chemokine biology outlined the role of early response cytokines, such as TNF and IL-1, for the activation of chemokines [8]. These early studies reported that chemokines could be induced in nearly every cell type. Subsequently, investigators have begun to define the association of certain chemokine profiles with particular types or phases of immune responses. In fact, the preferential expression of certain chemokines during immune responses likely dictates their function [9–11]. For example, the CC chemokine family members, CCL3 and CCL5, are induced by IFN and TNF, but regulated by IL-4, and appear to be closely associated with Th1-type responses. Likewise, the production of CxCR3 ligands, CxCL9, CxCL10, and CxCL11, are specifically activated by IFN and may have critical roles in enhancing Th1-type lymphocyte recruitment and activation. Along with the preferential expression of chemokines during Th1-type responses, there is also the preferential expression of the associated chemokine receptors on Th1-type lymphocytes. A number of studies have shown the preferential expression of CCR1 and CCR5 (which binds CCL3 and CCL5) as well as CxCR3 (which binds CxCL9, CxCL10, and CxCL11) on Th1-type lymphocytes. Thus, the chemokine expression during a Th1-type response correlates directly with the specificity of the chemokine receptors that are expressed on Th1-type lymphocytes.

As there are chemokines associated with Th1-type responses, there also appears to be certain chemokines that are closely associated with Th2-type responses [9, 12, 14]. An extensive amount of work was performed on CC chemokines, and particular members of this family are specifically activated by IL-4 and IL-13 [15–19]. The

*Table 1- Chemokine receptors, their ligands and diseases*

| Chemokine receptor | Ligands | Disease association |
|---|---|---|
| *CC chemokines* | | |
| CCR1 | CCL3, CCL5, CCL6, CCL7, CCL14, CCL15 | viral and fungal disease |
| CCR2 | CCL2, CCL7, CCL12 | asthma, viral, autoimmune |
| CCR3 | CCL5, CCL7, CCL11, CCL24, CCL26 | asthma, parasitic |
| CCR4 | CCL17, CCL22 | asthma, sepsis |
| CCR5 | CCL3, CCL4, CCL5 | HIV, MS |
| CCR6 | CCL20 | asthma, RA |
| CCR7 | CCL19, CCL21 | IPF, neoplasia, IBD |
| CCR8 | CCL1 | asthma, atopy |
| CCR9 | CCL25 | IBD |
| CCR10 | CCL27 | atopic dermatitis |
| *CxC chemokines* | | |
| CxCR1 | CxCL1, CxCL6, CxCL8 | sepsis, pneumonia, RA |
| CxCR2 | CxCL2, CxCL3, CxCL5, CxCL6, CxCL7, CxCL8 | sepsis, pneumonia, COPD |
| CxCR3 | CxCL9, CxCL10, CxCL11 | viral, autoimmune, transplant |
| CxCR4 | CxCL12 | HIV, asthma, metastasis |
| CxCR5 | CxCL13 | lymphoma |
| CxCR6 | CxCL16 | sarcoidosis, RA |

*MS, multiple sclerosis; RA, rheumatoid arthritis; IPF, idiopathic pulmonary fibrosis; IBD, inflammatory bowel disease.*

CC chemokines that are preferentially up-regulated by Th2-, but not Th1-, type cytokines include CCL1, CCL2, CCL11, CCL17, and CCL22. Interestingly, these IL-4- and IL-13-induced chemokines bind to a single chemokine receptor (see Tab. 1), which is fairly unusual among chemokine family members that tend to have a promiscuous binding pattern to multiple chemokine receptors. Studies have indicated that CCL2 is involved in allergen-induced T lymphocyte accumulation in the lungs of sensitized mice, whereas CCL11 is most closely associated with eosinophil accumulation during allergic responses. Thus, the Th2 activation pathway, which has been associated with allergen-induced airway hyperreactivity, likely induces preferential chemokine production that is associated with allergic cell recruitment. This area will be of particular interest since studies have previously shown that these Th2-associated chemokines play significant roles in allergen-induced airway inflam-

mation and airway hyperreactivity. Furthermore, analysis of *in vitro*-derived Th2-type cells indicates preferential expression of CCR3 (CCL11), CCR4 (CCL17, CCL22) and CCR8 (CCL1) [12, 20–23]. This receptor expression pattern correlates well with the type of chemokines that are induced by Th2-type responses discussed above. In addition to lymphocyte migration, there is also preferential chemokine receptor expression on effector cells that migrate into the airways and can induce damage, leading to airway hyperreactivity. Overall, the recruitment of multiple cell populations into allergic tissue is mediated by a combination of preferential chemokine production within the inflamed tissue and the receptors expressed on the marginated leukocyte populations.

## Preferential patterns dictate chemokines utilized during asthmatic disease

The above correlations continue as researchers examine chemokines expressed in samples from asthmatic patient populations. Chemokines previously identified in the airways of asthmatics include CCL5/RANTES, CCL11/eotaxin, MIP-1a/CCL3, CCL7/MCP-1, CCL13/MCP-4, CCL24/Eot-2, CCL17/TARC, CCL22/MDC, CCL28 and CxCL10. The importance of individual chemokines and chemokine receptors in allergic airway inflammation has been investigated using knockout mice sensitized and challenged with allergens including ovalbumin (OVA) and cockroach allergen (CRA) or with infectious challenges including *Aspergillus fumigatus* [24–36]. The preferential expression of these chemokines within the airways is believed to regulate recruitment and activation of a range of leukocyte subtypes including eosinophils and Th2 lymphocytes to the lungs.

$CD4^+$ T cells recruited to the lungs following allergen challenge secrete additional Th2 cytokines such as IL-4 and IL-13, and these cytokines are known to modulate chemokine expression in the lungs, resulting in elevated levels of CCL11, CCL13, CCL22, CCL1 and CCL17 via regulation of signal transducers and activators of transcription 6 (STAT6)-mediated transcription pathways [15, 37, 38]. The chemokine receptors expressed on lymphocytes skewed toward a Th2 phenotype correspond to the expression of ligands that have been implicated in the pathogenesis of allergic airway disease. Recent studies have begun to elucidate the role of individual chemokine receptors in directing Th2-type cell trafficking. Lloyd et al. determined that recruitment of these cells in the initial stages of an allergic response is dependent on expression of CCR3 ligands, but that repeated antigen stimulation results in the predominant use of CCR4 pathways possibly due to a progressive increase in recruitment of $CCR4^+$ cells [39]. Studies utilizing neutralizing CCR3 antibody or $CCR3^{-/-}$ mice have demonstrated that there is a significant defect in not only eosinophil accumulation but also the induction of airway hyperreactivity that may be related to T cell accumulation [40, 41]. CCR4 has also been detected on the majority of Th2 cells found in endobronchial biopsies collected from asthmatic

patients after allergen challenge, while <30% of the cells co-expressed CCR8 [12]. CCR3 expression was detected only on eosinophils. In these patients, CCL17 and CCL22 expression was strongly up-regulated in the airway epithelial cells following allergen challenge further indicating a key role for CCR4 in mediating T cell trafficking to the airways. Although the authors were unable to detect elevated levels of the CCR8 ligand CCL1, CCL17 has been shown to induce migration of CCR8 transfectants in addition to CCR4. As indicated above, CCR8 is expressed predominantly on Th2 cells and a recent study in $CCR8^{-/-}$ mice showed that, while the development of a peripheral Th2 response was normal, the response to a localized allergen challenge in the lungs was altered [14]. Allergen-challenged $CCR8^{-/-}$ mice exhibited reduced levels of Th2 cytokines in the lungs possibly due to an inability to recruit Th2-type lymphocytes to the lungs. The resulting alterations in the immune environment also led to attenuation of eosinophil recruitment. Although the cytokines and eosinophil accumulation was altered in allergic airway responses, no alteration in airway physiology was observed. This latter observation has been confirmed in two independent studies in different $CCR8^{-/-}$ mice [42, 43]. Other studies have shown that $CCR8^{+}$ $CD4^{+}$ T cells are directly associated with IL-10 production and appear to be phenotypically similar to T regulatory cells [44, 45]. Thus, these cells may have a significant role in maintaining or skewing the immune response toward a Th2 environment. It is clear that T cells play a critical role in modulating the immune environment within the lungs, and strategies that exploit the role of chemokines in Th2 cell recruitment may prove extremely beneficial in the development of new treatments for asthma.

Other chemokine receptors have also been implicated in mediating Th2 lymphocyte accumulation in the lungs of mice. In addition to their presence on naïve lymphocytes, receptors, such as CxCR4, CxCR5, CCR6 and CCR7, have now been identified on T lymphocytes that are of memory/activated phenotype as well as skewed helper cell populations [46–52]. These receptors may therefore have a role in recruitment of lymphocytes to the airways of asthmatic patients and lead to exacerbation of disease. This notion can already be supported in the existing literature where targeting CxCR4 or CCR6 have had a beneficial effect within models of allergic airway responses. In the case of blocking CxCR4, studies show that there is a beneficial effect regardless of whether the receptor or ligand (CxCL12) is blocked [24, 53]. In contrast to the CxCR4 results, studies with $CCR6^{-/-}$ mice demonstrated an altered migration of $CD4^{+}$ lymphocytes to the lung, suggesting that tissue-specific migration was altered [32, 54]. There may be multiple explanations for results from the CxCR4 and CCR6 studies including interruption of normal trafficking patterns of memory/activated lymphocytes to lymphoid organs or target tissue as well as reduced pulmonary recruitment and activation of eosinophils that express these receptors. Further studies with other homeostatic receptors indicate that CCR7 expression on T cells in both human and mouse studies is also important for development of asthmatic responses [55, 56].

## Role of dendritic cells for lymphocyte activation

Chemokines influence the immune response at multiple levels. The presentation of foreign antigen to T cells is the initiating step of the adaptive immune response, and a number of leukocytes are effective antigen-presenting cells (APC). The dendritic cell (DC) is particularly indispensable in this regard, expressing high levels of MHC class II on its surface [57]. Thus, it is not surprising that work has been undertaken to identify chemokines that mediate the trafficking of these highly motile cells from the bone marrow to non-lymphoidal tissues and, following encounter with antigen, to regional lymph nodes. Although differential expression of chemokine receptors during their maturation has not been fully characterized, mature DC express detectable levels of CCR1, CCR2, CCR5 and CCR6, as well as CXCR1, CXCR2 and CXCR4, and the respective ligands for these receptors are effective chemoattractants [6, 58–60]. In particular, immature DC express CCR6, which is down-regulated during the maturation process as the DC migrates to the lymph node to participate in its APC function. Using CCR6-deficient mice, studies have demonstrated defects in DC positioning in the gut mucosa as well as defects in DTH responses centered on T lymphocyte accumulation [61–63]. Thus, CCR6 has the potential to participate in a vast range of immunological responses and, therefore, may not be segregated to only a homeostatic function. Recent data using $CCR6^{-/-}$ mice during allergen-induced airways disease demonstrated a defect in the accumulation of DC subsets in the lungs of challenged animals [54]. These latter data, which suggest that CCR6 is important for DC accumulation in the lungs during allergic responses, have been supported by previous studies. CCR6 is preferentially displayed on myeloid DC populations, and allergen challenge invokes an influx of circulating myeloid DC into the lung [64]. As CCL20 has been shown to be produced by airway epithelial cells [65, 66], CCR6 might be required for localization of DC subsets to the airway, become activated, and acquire antigen for transport back to the draining lymph node. Furthermore, there may be specific defects in defined subsets of DC within the airway and/or decreased accumulation within the draining lymph nodes attributed to the CCR6 deficiency.

A number of chemokines have also been suggested to have a role in determining DC function. DC can produce a number of chemokines that may aid in preferentially recruiting specific T cell subsets to the DC for antigen activation. One of the first chemokines described to be produced, CCL22, which binds to CCR4, has been shown to have a role in allergen-induced airway disease [67–69]. Interestingly, stromal cell-derived factor-1 (SDF-1), which binds CXCR4 and selectively attracts naive T cells, is also an attractant for DC and may be an important mediator to attract these cells for antigen presentation [70–72]. The macrophage is probably the best-studied APC with respect to chemokines. Although they are highly phagocytic, they also express MHC class II and can participate in antigen presentation. A number of the CC chemokines (MCP 1-5, RANTES) were originally described as chemoat-

tractants for monocytes/macrophages, and most CC chemokine receptors (with the notable exception of CCR3) are found on the surface of these cells. Thus, chemokines and their receptors likely play a crucial role in the recruitment and activation of APC, as well as in providing a source of chemokine for recruiting T cells for antigen presentation.

## Viral infection, chemokine production, and exacerbation of the lung disease

The causes of severe asthma exacerbations are poorly understood. While it is clear that environmental levels of allergen can play a role, it is unlikely that this is sole cause of exacerbated asthma. Clinical studies have shown that a decrease in the CD4:CD8 T cell ratio in the bronchoalveolar lavage (BAL) of asthmatic patients is correlated with increased asthma severity. In stable asthmatics the ratio of CD4:CD8 T cells is 3:1; however, this ratio decreases to 1:1 in patients experiencing acute asthma attacks. Additionally, in cases of asthma death the CD4:CD8 T cell ratio is reversed to 1:2–1:6. A possible explanation for the increased recruitment of $CD8^+$ T cells to the BAL of exacerbated patients may be viral infection [73–75]. It has been reported in adults that 80% of asthma exacerbations, as characterized by a decrease in the peak expiratory flow rate, are associated with viral infection. There are a number of respiratory viruses that may play an important role in the exacerbation of the viral responses. The clinical data clearly indicate that one of the common features of most pulmonary viral infections is the early and intense production of chemokines [76–78]. The viruses that have been ascribed to producing chemokines upon infection of target cells include rhinovirus (RV), adenovirus, influenza virus, respiratory syncytial virus (RSV), as well as parainfluenza and SARS. The one characteristic that these viruses have is an often intense inflammatory response that initiates damage in the lungs of susceptible patients. Those most at risk usually have underlying pulmonary diseases, such as asthma, chronic obstructive pulmonary disease (COPD), or are transplant recipients, premature infants, etc., and suffer the most severe disease from the initiation of the anti-viral inflammatory responses [79, 80].

There are a number of chemokines that are initiated by respiratory viruses that appear to be commonly induced, including CCL2, CCL3, CCL5, CxCL8, CxCL9, and CxCL10. Although some of these chemokines are produced during Th2-type allergic responses, many of these have primarily been associated with Th1-type responses, such as those needed for anti-viral responses. A number of studies have identified production of chemokines after viral infection of isolated cell populations via PAMPs, which activate innate molecules such as Toll-like receptors [81–84]. The early and intense up-regulation of these chemokines would normally play an important role in the anti-viral responses. However, during asthmatic responses the overproduction of these chemokines leads to increased leukocyte recruitment and exac-

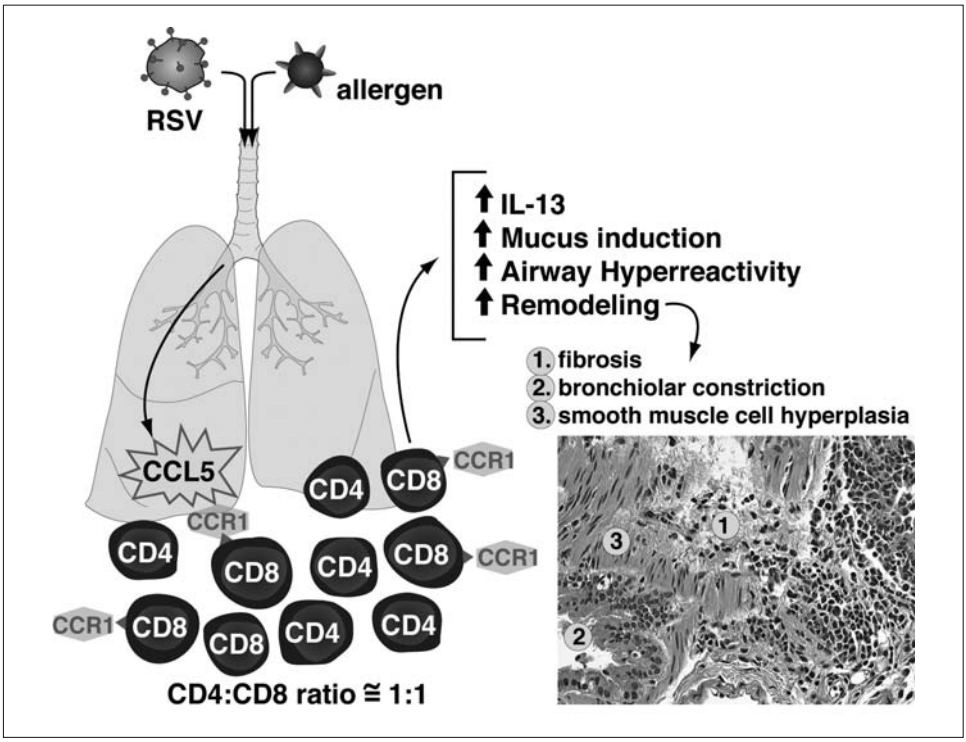


*Figure 1*
*Viral exacerbation of asthma due to Respiratory virus infection.*

erbated disease. As indicated above, the overproduction of these virus-associated chemokines leads to the accumulation of CD8 T cells. The identification of chemokine receptors on CD8 T cells has not been thoroughly investigated. Recent data would indicate that CCR1 appears to be one of the receptors that mediate CD8 T cell recruitment ([85] and unpublished data). Other studies have indicated that CCR2, CCR5, and CxCR3 may individually allow the accumulation of CD8 T cells to a site of viral infection [86–89]. The overall effect of producing specific chemokines that are induced during viral responses may be to elicit certain T cell subsets during disease. Data from our laboratory using RSV infection has shown that CCR1 specifically regulates CD8 T cells that produce Th2-type cytokines, especially IL-13 (unpublished data). This effect would bias the entire pulmonary immune environment toward ineffective clearance of the viral response and lead to enhanced Th2-mediated asthmatic reactions, especially mucus hypersecretion, as illustrated in Figure 1. This mechanism may also operative in other diseases such as COPD, where mucus overproduction is related directly to the intensity of the $CD8^+$

*Table 2- Potential chemokine receptor targets for asthma*

| Receptor targets | Cellular distribution | Aspect of asthmatic response altered | Ligands |
|---|---|---|---|
| CCR1 | Monocytes, T cells eosinophils, neutrophils | Chronic stage remodeling, infectious organism response, mucus hypersecretion, IL-13 production | CCL3, CCL5, CCL6, CCL7, CCL14, CCL15 |
| CCR3 | Eosinophils, basophils, mast cells, Th2 cells | Eosinophil accumulation, basophil recruitment, development of AHR | CCL5, CCL7, CCL8, CCL11, CCL13, CCL24, CCL26 |
| CCR4 | Dendritic cells, Th2 cells | T cell recruitment, airway remodeling, clearance of fungal spores | CCL17, CCL22 |
| CCR6 | Dendritic cells, T cells, B cells, eosinophils | $CD4^+$ T cell recruitment, eosinophil accumulation, development of AHR, IgE | CCL20 |
| CCR7 | T memory Cells, DC, naive T cells | Unknown | CCL19, CCL21 |
| CCR8 | Th2 cells, eosinophils, dendritic cells | Th2 cytokines, eosinophil accumulation | CCL1 |
| CxCR4 | Naïve and Th2 cells, eosinophils, mast cells | Th2 cytokines, eosinophil accumulation, development of AHR, T cell accumulation | CxCL12 |

*AHR, airway hyperresponsiveness*

T cell recruitment [90–92]. In contrast, other recent publications have identified an important role for CxCR3 for the recruitment of cytotoxic $CD8^+$ T cells for clearance of MHV-68 during lung infection [93, 94]. While this subset may be important with clearance of virus, it was also associated with development of chronic cough in non-asthmatic children [95] and may be associate with post-viral syndromes. Thus, there may be a preferential use of chemokine receptors during viral responses that dictate the phenotype of the $CD8^+$ T cell that is recruited to the airways.

## Conclusion

Although a number of chemokine receptors that have been described that mediate the accumulation of T lymphocytes to the lung during disease progression, there

continues to be a paucity of data regarding their specific function during certain pulmonary diseases. Questions persist on whether the differential receptor display on T cell subsets described in *in vitro* experiments, represent those that cause accumulation during disease. The diversity of chemokine production and the promiscuous pattern of chemokine-chemokine receptor interactions have made the identification of individual chemokine or chemokine receptor targets for therapeutic intervention extremely difficult. Interestingly, several of the "lead" candidates for targeting during chronic asthmatic disease are receptors that appear to bind a single or at most two chemokines, including CxCR4, CCR4, CCR6, and CCR8 (Table 2). Choosing the proper targets for specific disease phenotypes will only occur after careful coordinated animal modeling experiments coupled with translational research efforts in human disease.

## References


1 Coffman RL, Mocci S, O'Garra A (1999) The stability and reversibility of Th1 and Th2 populations. *Curr Top Microbiol Immunol* 238: 1–12

2 Powrie F, Mauze S, Coffman RL (1997) $CD4^+$ T-cells in the regulation of inflammatory responses in the intestine. *Res Immunol* 148: 576–581

3 Mazzarella G, Bianco A, Catena E, De Palma R, Abbate GF (2000) Th1/Th2 lymphocyte polarization in asthma. *Allergy 55* (Suppl 61): 6–9

4 Wenzel S (2003) Mechanisms of severe asthma. *Clin Exp Allergy* 33: 1622–1628

5 Sallusto F, Mackay CR, Lanzavecchia A (2000) The role of chemokine receptors in primary, effector, and memory immune responses. *Annu Rev Immunol* 18: 593–620

6 Sallusto F, Lanzavecchia A (2000) Understanding dendritic cell and T-lymphocyte traffic through the analysis of chemokine receptor expression. *Immunol Rev* 177: 134–140

7 Cyster JG (2000) Leukocyte migration: scent of the T zone. *Curr Biol* 10: R30–33

8 Kunkel SL, Lukacs N, Strieter RM (1995) Chemokines and their role in human disease. *Agents Actions* (Suppl 46): 11–22

9 Sallusto F, Kremmer E, Palermo B, Hoy A, Ponath P, Qin S, Forster R, Lipp M, Lanzavecchia A (1999) Switch in chemokine receptor expression upon TCR stimulation reveals novel homing potential for recently activated T cells. *Eur J Immunol* 29: 2037–2045

10 Moser B, Wolf M, Walz A, Loetscher P (2004) Chemokines: multiple levels of leukocyte migration control. *Trends Immunol* 25: 75–84

11 Lukacs NW, Tekkanat KK (2000) Role of chemokines in asthmatic airway inflammation. *Immunol Rev* 177: 21–30

12 Panina-Bordignon P, Papi A, Mariani M, Di Lucia P, Casoni G, Bellettato C, Buonsanti C, Miotto D, Mapp C, Villa A et al (2001) The C-C chemokine receptors CCR4 and CCR8 identify airway T cells of allergen-challenged atopic asthmatics. *J Clin Invest* 107: 1357–1364

13 Campbell JJ, Brightling CE, Symon FA, Qin S, Murphy KE, Hodge M, Andrew DP, Wu L, Butcher EC, Wardlaw AJ (2001) Expression of chemokine receptors by lung T cells from normal and asthmatic subjects. *J Immunol* 166: 2842–2848
14 Chensue SW, Lukacs NW, Yang TY, Shang X, Frait KA, Kunkel SL, Kung T, Wiekowski MT, Hedrick JA, Cook DN et al (2001) Aberrant *in vivo* T helper type 2 cell response and impaired eosinophil recruitment in CC chemokine receptor 8 knockout mice. *J Exp Med* 193: 573–584
15 Goebeler M, Schnarr B, Toksoy A, Kunz M, Brocker EB, Duschl A, Gillitzer R (1997) Interleukin-13 selectively induces monocyte chemoattractant protein-1 synthesis and secretion by human endothelial cells. Involvement of IL- 4R alpha and Stat6 phosphorylation. *Immunology* 91: 450–457
16 Doucet C, Brouty-Boye D, Pottin-Clemenceau C, Canonica GW, Jasmin C, Azzarone B (1998) Interleukin (IL) 4 and IL-13 act on human lung fibroblasts. Implication in asthma. *J Clin Invest* 101: 2129–2139
17 Li L, Xia Y, Nguyen A, Lai YH, Feng L, Mosmann TR, Lo D (1999) Effects of Th2 cytokines on chemokine expression in the lung: IL-13 potently induces eotaxin expression by airway epithelial cells. *J Immunol* 162: 2477–2487
18 Orlofsky A, Wu Y, Prystowsky MB (2000) Divergent regulation of the murine CC chemokine C10 by Th(1) and Th(2) cytokines. *Cytokine* 12: 220–228
19 Zhu Z, Ma B, Zheng T, Homer RJ, Lee CG, Charo IF, Noble P, Elias JA (2002) IL-13-induced chemokine responses in the lung: role of CCR2 in the pathogenesis of IL-13-induced inflammation and remodeling. *J Immunol* 168: 2953–2962
20 D'Ambrosio D, Iellem A, Bonecchi R, Mazzeo D, Sozzani S, Mantovani A, Sinigaglia F (1998) Selective up-regulation of chemokine receptors CCR4 and CCR8 upon activation of polarized human type 2 Th cells. *J Immunol* 161: 5111–5115
21 Zingoni A, Soto H, Hedrick JA, Stoppacciaro A, Storlazzi CT, Sinigaglia F, D'Ambrosio D, O'Garra A, Robinson D, Rocchi M, Santoni A, ZlotnikA, Napolitano M (1998) The chemokine receptor CCR8 is preferentially expressed in Th2 but not Th1 cells. *J Immunol* 161: 547–551
22 Andrew DP, Ruffing N, Kim CH, Miao W, Heath H, Li Y, Murphy K, Campbell JJ, Butcher EC, Wu L (2001) C-C chemokine receptor 4 expression defines a major subset of circulating nonintestinal memory T cells of both Th1 and Th2 potential. *J Immunol* 166: 103–111
23 Sallusto F, Mackay CR, Lanzavecchia A (1997) Selective expression of the eotaxin receptor CCR3 by human T helper 2 cells. *Science* 277: 2005–2007
24 Gonzalo JA, Lloyd CM, Peled A, Delaney T, Coyle AJ, Gutierrez-Ramos JC (2000) Critical involvement of the chemotactic axis CXCR4/stromal cell-derived factor-1 alpha in the inflammatory component of allergic airway disease. *J Immunol* 165: 499–508
25 Gonzalo JA, Lloyd CM, Wen D, Albar JP, Wells TN, Proudfoot A, Martinez AC, Dorf M, Bjerke T, Coyle AJ et al (1998) The coordinated action of CC chemokines in the lung orchestrates allergic inflammation and airway hyperresponsiveness. *J Exp Med* 188: 157–167

26 Stafford S, Li H, Forsythe PA, Ryan M, Bravo R, Alam R (1997) 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* 158: 4953–4960
27 MacLean JA, Ownbey R, Luster AD (1996) T cell-dependent regulation of eotaxin in antigen-induced pulmonary eosinophila. *J Exp Med* 184: 1461–1469
28 Gonzalo JA, Lloyd JM, Kremer L, Finger E, Martinez AC, Siegelman MH, Cybulsky M, Gutierrez-Ramos JC (1996) Eosinophil recruitment to the lung in a murine model of allergic inflammation. The role of T cells, chemokines, and adhesion receptors. *J Clin Invest* 98: 2332–2345
29 Rothenberg ME, Luster AD, Lilly CM, Drazen JM, Leder P (1995) Constitutive and allergen-induced expression of eotaxin mRNA in the guinea pig lung. *J Exp Med* 181: 1211–1216
30 Campbell EM, Charo IF, Kunkel SL, Strieter RM, Boring L, Gosling J, Lukacs NW (1999) Monocyte chemoattractant protein-1 mediates cockroach allergen-induced bronchial hyperreactivity in normal but not CCR2$^{-/-}$ mice: the role of mast cells [In Process Citation]. *J Immunol* 163: 2160–2167
31 Campbell EM, Kunkel SL, Strieter RM, Lukacs NW (1998) Temporal role of chemokines in a murine model of cockroach allergen-induced airway hyperreactivity and eosinophilia. *J Immunol* 161: 7047–7053
32 Lukacs NW, Prosser DM, Wiekowski M, Lira SA, Cook DN (2001) Requirement for the chemokine receptor CCR6 in allergic pulmonary inflammation. *J Exp Med* 194: 551–555
33 Thomas MS, Kunkel SL, Lukacs NW (2002) Differential role of IFN-gamma-inducible protein 10 kDa in a cockroach antigen-induced model of allergic airway hyperreactivity: systemic *versus* local effects. *J Immunol* 169: 7045–7053
34 John AE, Thomas MS, Berlin AA, Lukacs NW (2005) Temporal production of CCL28 corresponds to eosinophil accumulation and airway hyperreactivity in allergic airway inflammation. *Am J Pathol* 166: 345–353
35 Blease K, Mehrad B, Standiford TJ, Lukacs NW, Kunkel SL, Chensue SW, Lu B, Gerard CJ, Hogaboam CM (2000) Airway remodeling is absent in CCR1$^{-/-}$ mice during chronic fungal allergic airway disease. *J Immunol* 165: 1564–1572
36 Schuh JM, Blease K, Hogaboam CM (2002) CXCR2 is necessary for the development and persistence of chronic fungal asthma in mice. *J Immunol* 168: 1447–1456
37 Zhang S, Lukacs NW, Lawless VA, Kunkel SL, Kaplan MH (2000) Cutting edge: differential expression of chemokines in Th1 and Th2 cells is dependent on Stat6 but not Stat4. *J Immunol* 165: 10–14
38 Mathew A, MacLean JA, DeHaan E, Tager AM, Green FH, Luster AD (2001) 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* 193: 1087–1096
39 Lloyd CM, Delaney T, Nguyen T, Tian J, Martinez AC, Coyle AJ, Gutierrez-Ramos JC

(2000) 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* 191: 265–274

40 Humbles AA, Lu B, Friend DS, Okinaga S, Lora J, Al-Garawi A, Martin TR, Gerard NP, Gerard C (2002) The murine CCR3 receptor regulates both the role of eosinophils and mast cells in allergen-induced airway inflammation and hyperresponsiveness. *Proc Natl Acad Sci USA* 99: 1479–1484

41 Senechal S, Fahy O, Gentina T, Vorng H, Capron M, Walls AF, A.R. McEuen, M.G. Buckley, Q. Hamid, B. Wallaert et al (2002) CCR3-blocking antibody inhibits allergen-induced eosinophil recruitment in human skin xenografts from allergic patients. *Lab Invest* 82: 929–939

42 Chung CD, Kuo F, Kumer J, Motani AS, Lawrence CE, Henderson WR Jr, Venkataraman C (2003) CCR8 is not essential for the development of inflammation in a mouse model of allergic airway disease. *J Immunol* 170: 581–587

43 Goya I, Villares R, Zaballos A, Gutierrez J, Kremer L, Gonzalo JA, Varona R, Carramolino L, Serrano A, Pallares P et al (2003) Absence of CCR8 does not impair the response to ovalbumin-induced allergic airway disease. *J Immunol* 170: 2138–2146

44 Freeman CM, Chiu BC, Stolberg VR, Hu J, Zeibecoglou K, Lukacs NW, Lira SA, Kunkel SL, Chensue SW (2005) CCR8 is expressed by antigen-elicited, IL-10-producing $CD4^+CD25^+$ T cells, which regulate Th2-mediated granuloma formation in mice. *J Immunol* 174: 1962–1970

45 Sebastiani S, Allavena P, Albanesi C, Nasorri F, Bianchi G, Traidl C, Sozzani S, Girolomoni G, Cavani A (2001) Chemokine receptor expression and function in $CD4^+$ T lymphocytes with regulatory activity. *J Immunol* 166: 996–1002

46 Debes GF, Hopken UE, Hamann A (2002) *In vivo* differentiated cytokine-producing CD4(+) T cells express functional CCR7. *J Immunol* 168: 5441–5447

47 Campbell JJ, Murphy KE, Kunkel EJ, Brightling CE, Soler D, Shen Z, Boisvert J, Greenberg HB, Vierra MA, Goodman SB et al (2001) CCR7 expression and memory T cell diversity in humans. *J Immunol* 166: 877–884

48 Jourdan P, Vendrell JP, Huguet MF, Segondy M, Bousquet J, Pene J, Yssel H (2000) Cytokines and cell surface molecules independently induce CXCR4 expression on $CD4^+$ $CCR7^+$ human memory T cells. *J Immunol* 165: 716–724

49 Fitzhugh DJ, Naik S, Caughman SW, Hwang ST (2000) Cutting edge: C-C chemokine receptor 6 is essential for arrest of a subset of memory T cells on activated dermal microvascular endothelial cells under physiologic flow conditions *in vitro*. *J Immunol* 165: 6677–6681

50 Breitfeld D, Ohl L, Kremmer E, Ellwart J, Sallusto F, Lipp M, Forster R (2000) Follicular B helper T cells express CXC chemokine receptor 5, localize to B cell follicles, and support immunoglobulin production [In Process Citation]. *J Exp Med* 192: 1545–1552

51 Sallusto F, Lenig D, Forster R, Lipp M, Lanzavecchia A (1999) Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature* 401: 708–712

52 Liao F, Rabin RL, Smith CS, Sharma G, Nutman TB, Farber JM (1999) CC-chemokine receptor 6 is expressed on diverse memory subsets of T cells and determines responsiveness to macrophage inflammatory protein 3 alpha. *J Immunol* 162: 186–194
53 Lukacs NW, Berlin A, Schols D, Skerlj RT, Bridger GJ (2002) AMD3100, a CxCR4 antagonist, attenuates allergic lung inflammation and airway hyperreactivity. *Am J Pathol* 160: 1353–1360
54 Lundy SK, Lira SA, Smit JJ, Cook DN, Berlin AA, and Lukacs NW (2005) Attenuation of allergen-induced responses in CCR6$^{-/-}$ mice is dependent upon altered pulmonary T lymphocyte activation. *J Immunol* 174: 2054–2060
55 Bromley SK, Thomas SY, Luster AD (2005) Chemokine receptor CCR7 guides T cell exit from peripheral tissues and entry into afferent lymphatics. *Nat Immunol* 6: 895–901
56 Kallinich T, Schmidt S, Hamelmann E, Fischer A, Qin S, Luttmann W, Virchow JC, Kroczek RA (2005) Chemokine-receptor expression on T cells in lung compartments of challenged asthmatic patients. *Clin Exp Allergy* 35: 26–33
57 Dieu MC, Vanbervliet B, Vicari A, Bridon JM, Oldham E, Ait-Yahia S, Briere F, Zlotnik A, Lebecque S, Caux C (1998) Selective recruitment of immature and mature dendritic cells by distinct chemokines expressed in different anatomic sites. *J Exp Med* 188: 373–386
58 McColl SR (2002) Chemokines and dendritic cells: a crucial alliance. *Immunol Cell Biol* 80: 489–496
59 Sozzani S, Allavena P, Vecchi A, Mantovani A (2000) Chemokines and dendritic cell traffic. *J Clin Immunol* 20: 151–160
60 Sozzani S, Mantovani A, Allavena P (1999) Control of dendritic cell migration by chemokines. *Forum (Genova)* 9: 325–338
61 Iwasaki A, Kelsall BL (2000) Localization of distinct Peyer's patch dendritic cell subsets and their recruitment by chemokines macrophage inflammatory protein (MIP)-3alpha, MIP-3beta, and secondary lymphoid organ chemokine. *J Exp Med* 191: 1381–1394
62 Cook DN, Prosser DM, Forster R, Zhang J, Kuklin NA, Abbondanzo SJ, Niu XD, Chen SC, Manfra DJ, Wiekowski MT et al (2000) CCR6 mediates dendritic cell localization, lymphocyte homeostasis, and immune responses in mucosal tissue. *Immunity* 12: 495–503
63 Varona R, Villares R, Carramolino L, Goya I, Zaballos A, Gutierrez J, Torres M, Martinez AC, Marquez G (2001) CCR6-deficient mice have impaired leukocyte homeostasis and altered contact hypersensitivity and delayed-type hypersensitivity responses. *J Clin Invest* 107: R37–45
64 Kucharzik T, Hudson JT 3rd, Waikel RL, Martin WD, Williams IR (2002) CCR6 expression distinguishes mouse myeloid and lymphoid dendritic cell subsets: demonstration using a CCR6 EGFP knock-in mouse. *Eur J Immunol* 32: 104–112
65 Reibman J, Hsu Y, Chen LC, Bleck B, Gordon T (2003) Airway epithelial cells release MIP-3alpha/CCL20 in response to cytokines and ambient particulate matter. *Am J Respir Cell Mol Biol* 28: 648–654

66 Starner TD, Barker CK, Jia HP, Kang Y, McCray PB (2003) CCL20 is an inducible product of human airway epithelia with innate immune properties. *Am J Respir Cell Mol Biol* 29(5): 627–633

67 Godiska R, Chantry D, Raport CJ, Sozzani S, Allavena P, Leviten D, Mantovani A, Gray PW (1997) Human macrophage-derived chemokine (MDC), a novel chemoattractant for monocytes, monocyte-derived dendritic cells, and natural killer cells. *J Exp Med* 185: 1595–1604

68 Andrew DP, Chang MS, McNinch J, Wathen ST, Rihanek M, Tseng J, Spellberg JP, Elias CG 3rd. (1998) STCP-1 (MDC) CC chemokine acts specifically on chronically activated Th2 lymphocytes and is produced by monocytes on stimulation with Th2 cytokines IL-4 and IL-13. *J Immunol* 161: 5027–5038

69 Gonzalo JA, Pan Y, Lloyd CM, Jia GQ, Yu G, Dussault B, Powers CA, Proudfoot AE, Coyle AJ, Gearing D, Gutierrez-Ramos JC (1999) Mouse monocyte-derived chemokine is involved in airway hyperreactivity and lung inflammation. *J Immunol* 163: 403–411

70 Caux C, Ait-Yahia S, Chemin K, de Bouteiller O, Dieu-Nosjean MC, Homey B, Massacrier C, Vanbervliet B, Zlotnik A, Vicari A (2000) Dendritic cell biology and regulation of dendritic cell trafficking by chemokines. *Springer Semin Immunopathol* 22: 345–369

71 Delgado E, Finkel V, Baggiolini M, Mackay CR, Steinman RM, Granelli-Piperno A (1998) Mature dendritic cells respond to SDF-1, but not to several beta-chemokines. *Immunobiology* 198: 490–500

72 Rubbert A, Combadiere C, Ostrowski M, Arthos J, Dybul M, Machado E, Cohn MA, Hoxie JA, Murphy PM, Fauci AS, Weissman D (1998) Dendritic cells express multiple chemokine receptors used as coreceptors for HIV entry. *J Immunol* 160: 933–3941

73 O'Sullivan SM (2005) Asthma death, $CD8^+$ T cells, and viruses. *Proc Am Thorac Soc* 2: 162–165

74 O'Sullivan S, Cormican L, Faul JL, Ichinohe S, Johnston SL, Burke CM, Poulter LW (2001) Activated, cytotoxic CD8(+) T lymphocytes contribute to the pathology of asthma death. *Am J Respir Crit Care Med* 164: 560–564

75 Faul JL, Tormey VJ, Leonard C, Burke CM, Farmer J, Horne SJ, Poulter LW (1997) Lung immunopathology in cases of sudden asthma death. *Eur Respir J* 10: 301–307

76 Lukacs NW, Hogaboam CM, Kunkel SL (2005) Chemokines and their receptors in chronic pulmonary disease. *Curr Drug Targets Inflamm Allergy* 4: 313–317

77 Matsuse H, Behera AK, Kumar M, Rabb H, Lockey RF, Mohapatra SS (2000) Recurrent respiratory syncytial virus infections in allergen-sensitized mice lead to persistent airway inflammation and hyperresponsiveness. *J Immunol* 164: 6583–6592

78 Lau YL, Peiris JS (2005) Pathogenesis of severe acute respiratory syndrome. *Curr Opin Immunol* 17: 404–410

79 Hogg JC (1999) Childhood viral infection and the pathogenesis of asthma and chronic obstructive lung disease. *Am J Respir Crit Care Med* 160: S26–28

80 Peebles RS Jr. (2004) Viral infections, atopy, and asthma: is there a causal relationship? *J Allergy Clin Immunol* 113: S15–18

81 Li J, Ma Z, Tang ZL, Stevens T, Pitt B, Li S (2004) CpG DNA-mediated immune response in pulmonary endothelial cells. *Am J Physiol Lung Cell Mol Physiol* 287: L552–558

82 Koedel U, Rupprecht T, Angele B, Heesemann J, Wagner H, Pfister HW, Kirschning CJ (2004) MyD88 is required for mounting a robust host immune response to *Streptococcus pneumoniae* in the CNS. Brain 127: 1437–1445

83 Monick MM, Yarovinsky TO, Powers LS, Butler NS, Carter AB, Gudmundsson G, Hunninghake GW (2003) Respiratory syncytial virus up-regulates TLR4 and sensitizes airway epithelial cells to endotoxin. *J Biol Chem* 278: 53035–53044

84 Abel B, Thieblemont N, Quesniaux VJ, Brown N, Mpagi J, Miyake K, Bihl F, Ryffel B (2002) Toll-like receptor 4 expression is required to control chronic *Mycobacterium tuberculosis* infection in mice. *J Immunol* 169: 3155–3162

85 John AE, Gerard CJ, Schaller M, Miller AL, Berlin AA, Humbles AA, Lukacs NW (2005) Respiratory syncytial virus-induced exaggeration of allergic airway disease is dependent upon CCR1-associated immune responses. *Eur J Immunol* 35: 108–116

86 Nansen A, Marker O, Bartholdy C, Thomsen AR (2000) $CCR2^+$ and $CCR5^+$ $CD8^+$ T cells increase during viral infection and migrate to sites of infection. *Eur J Immunol* 30: 1797–1806

87 Sorensen TL, Sellebjerg F (2002) Selective suppression of chemokine receptor CXCR3 expression by interferon-beta1a in multiple sclerosis. *Mult Scler* 8: 104–107

88 Amoura Z, Combadiere C, Faure S, Parizot C, Miyara M, Raphael D, Ghillani P, Debre P, Piette JC, Gorochov G (2003) Roles of CCR2 and CXCR3 in the T cell-mediated response occurring during lupus flares. *Arthritis Rheum* 48: 3487–3496

89 Luangsay S, Kasper LH, Rachinel N, Minns LA, Mennechet FJ, Vandewalle A, Buzoni-Gatel D (2003) CCR5 mediates specific migration of *Toxoplasma gondii*-primed CD8 lymphocytes to inflammatory intestinal epithelial cells. *Gastroenterology* 125: 491–500

90 Leckie MJ, Jenkins GR, Khan J, Smith SJ, Walker C, Barnes PJ, Hansel TT (2003) Sputum T lymphocytes in asthma, COPD and healthy subjects have the phenotype of activated intraepithelial T cells ($CD69^+$ $CD103^+$). *Thorax* 58: 23–29

91 Saetta M, Mariani M, Panina-Bordignon P, Turato G, Buonsanti C, Baraldo S, Bellettato CM, Papi A, Corbetta L, Zuin R, Sinigaglia F, Fabbri LM (2002) Increased expression of the chemokine receptor CXCR3 and its ligand CXCL10 in peripheral airways of smokers with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 165: 1404–1409

92 Di Stefano A, Capelli A, Lusuardi M, Caramori G, Balbo P, Ioli F, Sacco S, Gnemmi I, Brun P, Adcocket IM et al (2001) Decreased T lymphocyte infiltration in bronchial biopsies of subjects with severe chronic obstructive pulmonary disease. *Clin Exp Allergy* 31: 893–902

93 Lee BJ, Giannoni F, Lyon A, Yada S, Lu B, Gerard C, Sarawar SR (2005) Role of CXCR3 in the immune response to murine gammaherpesvirus 68. *J Virol* 79: 9351–9355

94 Sarawar SR, Lee BJ, Anderson M, Teng YC, Zuberi R, Von Gesjen S (2002) Chemokine

induction and leukocyte trafficking to the lungs during murine gammaherpesvirus 68 (MHV-68) infection. *Virology* 293: 54–62

95 Hartl D, Griese M, Nicolai T, Zissel G, Prell C, Konstantopoulos N, Gruber R, Reinhardt D, Schendel DJ, Krauss-Etschmann S (2005) Pulmonary chemokines and their receptors differentiate children with asthma and chronic cough. *J Allergy Clin Immunol* 115: 728–736