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Chemokine Receptors in Asthma: Searching for the Correct Immune Targets

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The overall incidence, severity, and prevalence of asthma has risen dramatically with a distressing increase in children (1). Asthmatics are presently treated with non-specific inhibitors of the inflammatory responses using inhaled or oral steroids accompanied by bronchodilating reagents but the overall long-term success of these treatments is debated. Although there are several contributing factors that promote severe asthmatic responses, it appears that peribronchial inflammation is a common underlying factor. There is substantial evidence that allergic inflammatory responses are driven by Th2-type cytokines including IL-4, IL-5, and IL-13 (2). The sources for the cytokines include lymphocytes, mast cells, and eosinophils, all of which participate in the allergic asthmatic responses. The mechanism governing the preferential recruitment and activation of these participating cells has been a primary focus directed at discovering therapeutic strategies. A group of cytokines that have prominent effects on leukocyte recruitment and activation are the chemokines. A common conception among researchers to date has been that blocking the correct chemokine or chemokine receptor should significantly attenuate the accumulation and/or activation of leukocyte populations that drive the asthmatic response. Although much of the data have been derived from animal models of asthma, several aspects appear to be promising for attenuating disease.

Chemokines have been traditionally divided into two main groups based upon their sequence homology and the position of the first two cysteine residues, C-X-C (α) and C-C (β). Presently there are 16 CXC ligands (CXCL1–16) and 28 CC ligands (CCL1–28) that have been identified. There are also two minor groups, the CX3C and C, both having only a single identified member. Many of the chemokines originally had multiple names that were assigned to these molecules; however, consensus within the field has standardized their nomenclature (3). These chemokine ligands bind to G protein-coupled 7 transmembrane serpentine receptors, but the function and cellular expression patterns still require classification. There are six known CXC chemokine receptors, 10 known functional CC chemokine receptors, and at least two known receptors that do not signal (DARC and D6) but are able to bind to CC and CXC chemokines possibly acting as decoy receptors. The complexity of understanding chemokine biology comes from the promiscuous binding of a single chemokine to multiple receptors, while individual receptors can bind multiple chemokines. Chemokines have diverse functions during asthmatic responses, which relate to recruitment, cellular activation/degranulation, differentiation, as well as directly altering the immune response (4). The identification of chemokines in the airways of asthmatics after allergen provocation initially suggested that these molecules might have a significant role in the accumulation of leukocytes (5). Furthermore, the expression of distinct chemokine receptors on infiltrating cell populations, especially lymphocytes and eosinophils, provides an attractive opportunity to attenuate the influx of these cell populations.

Inflammatory or induced chemokine receptors for therapeutic intervention

One of the most time-consuming aspects of chemokine research has been identifying the relevant chemokine or receptor to block during disease progression. Initially, the task of inhibiting peribronchial inflammation seemed straightforward: block the chemokine activity that is produced in excess during the inflammatory response. However, the reality of airway disease progression is that a diverse number of chemokines are induced with many of these chemokines having overlapping/promiscuous receptor binding patterns. Because of this latter issue, the focus of therapeutic intervention has turned attention toward interrupting individual receptor systems that are used by key effector cell populations. One of the first targets, CCR3, is highly expressed on recruited eosinophils (6, 7), the cells that are most characteristic of asthmatic inflammation and most often correlated with the severity of disease (8). Subsequent investigations also found that CCR3 is expressed on mast cells, basophils, and subsets of Th2-type cells, giving more justification for targeting CCR3 for the modulation of the recruitment and/or activation of a number of cell populations involved in pathogenesis of asthma. Although positive data have been generated in allergic animals treated with anti-CC chemokine ligand (CCL)2 11/eotaxin (the selective CCR3 agonist) (9), examination of CCL11−/− mice did not demonstrate a clear role for eotaxin in the pathogenesis of the allergic airways responses.
appears to be centered on an altered innate immune response. The most prominent effect is not on allergic responses per se, but rather a temporally different phase induced by CCR4-mediated T lymphocytes migrated via CCR3-mediated events followed by up-regulated expression (18). Animal models have not always supported a correlation of IL-4-producing lymphocytes and CCR4/CCR8 expression during allergen challenge (16, 17). Although studies using receptors are essential for recruitment of lymphocytes into the airways during allergen challenge (16, 17). Although studies using cells from airways of asthmatic patients have identified a correlation of IL-4-producing lymphocytes and CCR4/CCR8 expression (18), animal models have not always supported a major role for these receptors. Initial studies demonstrated that T lymphocytes migrated via CCR3-mediated events followed by a temporally different phase induced by CCR4-mediated mechanisms (19). However, in CCR4-deficient animals the most prominent effect is not on allergic responses per se, but appears to be centered on an altered innate immune response that has been examined within endotoxin and antifungal responses (20, 21). Likewise, the use of CCR8 \(^{-/-}\) mice has provided disparate results in regards to the overall physiologic outcome, which was not altered upon allergen challenges in CCR8-deficient mice derived from three different laboratories (22–24). However, one of these studies that used three different Th2 cytokine-mediated models demonstrated an alteration in the eosinophil-associated inflammatory response (24). These latter findings are supported by studies demonstrating the expression and function of CCR8 on eosinophils (25, 26) and in studies blocking the CCR8-specific ligand, CCL1 (27). These seemingly conflicting data may be in line with other studies that have demonstrated little correlation of preferential chemokine receptor expression on lymphocytes derived from skewed responses in vivo (28, 29). These differences in experimental data from different models are disturbing but this issue has become a recurrent theme, particularly in regards to data generated with genetically altered animals (e.g., knockouts). Although there have been some successes, targeting specific chemokine receptors induced during the inflammatory response associated with asthma has been inconclusive.

**Homeostatic or constitutive chemokine receptors**

Chemokine receptors have been further divided into different subsets reflecting the role they have in immune and inflammatory responses. Over the past few years, clear delineations have been identified in chemokine receptors that are involved in homeostatic recirculation of lymphocytes and APCs vs those that are involved in migration during immune activation (30, 31). Although, as highlighted above, efforts in asthmatic inflammation have concentrated on targeting chemokine receptors involved in effector/inflammatory cell migration into tissue, an examination of receptors involved in homeostatic migration of leukocytes has been largely ignored during these pulmonary responses. Several chemokine receptors have been classified as having a primarily homeostatic function in the immune response. CCR7/CCL21 interaction was identified as the key molecules for high endothelial venule binding and entrance into the lymph node working in cooperation with L-selectin. CXCR5 has been implicated as a B cell receptor that localizes cells to follicles of secondary lymphoid organs (32). Original observations identified CXCR4 expression on B cells and naive CD4\(^+\) cells and plays a role for entry into the lymph node (33, 34). Both CCR7 and CXCR4 are efficiently down-regulated from the surface of the cells upon Ag activation possibly signaling their ability to emigrate out of the lymph node. Another homeostatic receptor, CCR6, is present on immature dendritic cells and B cells. Once activated, dendritic cells quickly lose CCR6 from the surface and up-regulate CCR7 for directed migration to the lymph node for Ag presentation. Thus, these receptors together appear to be primarily responsible for the directed migration of lymphocytes and APCs into the lymph node for Ag activation and immune surveillance.

In addition to their presence on naive 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 (35–39). Recent investigations have further defined memory cells as having a central memory or effector memory phenotype based upon the up- or down-regulated expression of CCR7, respectively (40), as well as expression on most resting Th1- and Th2-skewed populations (41). Perhaps these receptors that mark memory/activated cells could be the most effective chemokine receptors to be targeted to alter severity of chronic diseases. This notion can already be supported in the existing literature where targeting CXCR4 or CCR6 has had a beneficial effect within models of allergic airway responses (42–44). In the case of blocking CXCR4, studies show that there is a beneficial effect regardless of whether the receptor or ligand (CXC1L12) is blocked (42). An additional effect on skewing the response toward a more desirable Th1 response was also observed using a specific CXCR4 antagonist (43). 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 (44). Because CCR6 is found on multiple cell populations, including B cells, immature dendritic cells, and eosinophils, it may be important to include these interactions in any model of altered inflammation. However, more recent studies have confirmed that altered T cell recruitment into the lung is one of the possible defects in the CCR6 \(^{-/-}\) mice (our unpublished data). 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. Given these results, other homeostatic receptors, especially CXCR5 and CCR7, should also be investigated for their potential role in progression of chronic inflammatory diseases.
Targeting chemokine receptors for attenuating airway remodeling and mucus overproduction

The traditional view of chemokine blockade attenuating only immune cell migration has been expanded to include their effects on structural cell function. Potentially devastating aspects of asthma, as well as other pulmonary diseases, include the induction of airway remodeling (fibrosis) and overproduction of mucus (2). Both of these aspects likely contribute significantly to the pathophysiologic changes in asthma. An increase of interstitial collagen beneath the airway basement membrane and subepithelial fibrosis are present in the airways of allergic asthmatics and correlate to increased responsiveness. Evidence has shown that CCL2 enhances collagen deposition by fibroblasts, increases TGFβ production, and enhances fibroproliferation (45, 46). Thus, increased expression of this chemokine in the lungs of asthmatics might contribute to the airway remodeling that can exacerbate airway hyperresponsiveness. In fact, several studies have now linked CCR2 (CCL2-specific receptor) expression on pulmonary fibroblasts to remodeling diseases of the lung (47–49).

The development of goblet cell metaplasia/hyperplasia and mucus production is one of the hallmarks of chronic asthmatic responses that lead to long-term dysfunction of airway physiology. Using an animal model of chronic Aspergillus fumigatus sensitization and challenge that leads to goblet cell hyperplasia/metaplasia, a significant attenuation of disease was identified using CXCR2−/− mice (50). This mechanism has been supported in respiratory syncytial virus-induced airway hyperactivity and mucus overproduction, where goblet cell metaplasia/hyperplasia, mucus gene, and mucus protein were significantly attenuated when CXCR2 function was blocked in infected mice (51). Interestingly, a strong correlation has been drawn between overexpression of IL-8 (a CXCR2 ligand) and overproduction of mucus (52, 53). Because CXCR2, normally associated with neutrophils, can also be found on activated monocyte/macrophages and eosinophils (25, 54–56), it may have significant relevance in asthma. A second receptor, CCR2, has also been linked with mucus overproduction using a model of transgenic airway overproduction of IL-13 with clear pathologic consequences centered on airway fibrosis and mucus overproduction (48). Although these latter studies did not clearly identify a mechanism, it demonstrated that CCR2, primarily expressed on macrophages, might induce multiple pathologic manifestations within airway disease. Consistent with these latter reports is a recent study demonstrating that instillation of recombinant chemokines KC (a CXCR2 ligand) or monocyte chemoattractant protein-1 (a CCR2 ligand) into the airways of naïve mice induced goblet cell hyperplasia/metaplasia and mucus production accompanied by epidermal growth factor receptor (EGFR) up-regulation within the lungs (57). Thus, the mechanism linking chemokine receptor expression, leukocyte activation, and airway mucus overproduction together may be through an EGFR-mediated event in the epithelium. Perhaps chemokines mediate these airway events via induced release of EGFR ligands, such as heparin binding-EGF, from the infiltrating leukocytes facilitating goblet cell hyperplasia and/or mucus production as previously established (58, 59), an idea supported by recent studies of another 7-transmembrane receptor, platelet activating factor receptor (60). This concept is summarized in Fig. 1 and demonstrates the complexity of a potentially pathologic mechanism stemming from the activation of inflammatory cells via chemokine receptors that subsequently trigger release of epithelial cell-modifying EGFR ligands. Further analyses of these interesting initial observations may make targeting these receptors attractive for attenuating specific aspects of chronic airway disease within particular patient populations with obstructive lung disease.

**FIGURE 1.** Potential activation pathways leading to mucus overproduction within the airways of asthmatics.
Summary

The identification and characterization of various chemokines and their receptors expressed during the progression of asthmatic disease has led to the developing concept that although a number of targets have been suggested, specific delineation of receptors needs to be accomplished. Our developing analyses of the function of chemokine receptors during the progression of asthmatic type responses, especially those involved in the chronic stages, will likely identify a novel function related to unexpected chemokine receptor biology.

References


