Review

Chemokines and their role in airway hyper-reactivity

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Abstract

Airway hyper-reactivity is a characteristic feature of many inflammatory lung diseases and is defined as an exaggerated degree of airway narrowing. Chemokines and their receptors are involved in several pathological processes that are believed to contribute to airway hyper-responsiveness, including recruitment and activation of inflammatory cells, collagen deposition and airway wall remodeling. These proteins are therefore thought to represent important therapeutic targets in the treatment of airway hyper-responsiveness. This review highlights the processes thought to be involved in airway hyper-responsiveness in allergic asthma, and the role of chemokines in these processes. Overall, the application of chemokines to the prevention or treatment of airway hyper-reactivity has tremendous potential.

Keywords: asthma, eosinophils, fibrosis, T cells

Introduction

One of the key features of pulmonary diseases such as allergic asthma, cystic fibrosis and chronic obstructive pulmonary disease is the development of airway hyper-responsiveness (AHR) [1–3]. The factors involved in the development of AHR seem to differ between diseases, so for clarity this review will focus on the development of AHR during allergic asthmatic disease. In the context of asthma, AHR equates to an exaggerated bronchoconstrictor response, not only to allergens to which the subjects are sensitized, but also to a range of non-specific stimuli, including agents as diverse as cold air and methacholine.

Under normal conditions, airway reactivity, the ability to alter the size of the airways reversibly in response to stimuli, is an essential component of homeostasis. For example, when there is a need to move large volumes of air, such as with exercise, bronchial dilation occurs. Conversely, when it is important to limit or decrease the volume of air inspired, such as with exposure to irritating gases, the lung defends itself with coughing and bronchial narrowing. When this response is excessive, it is referred to as airway or bronchial hyper-reactivity or hyper-responsiveness (AHR) and manifests itself as an exaggerated bronchoconstrictor response to various provocative

AHR = airway hyper-responsiveness; BALF = bronchoalveolar lavage fluid; CCR = CC chemokine receptor; CXCR = CXC chemokine receptor; FEV_1 = forced expiratory volume over 1 s; IL = interleukin; LTC_4 = leukotriene C_4; MCP = macrophage chemoattractant protein; MIP = macrophage inflammatory protein; PC_{20} = provocative concentration; PD_{20} = provocative dose; Th cells, T helper cells.

Table 1

Chemokine receptors and their ligands

CXC chemokine receptors	CC chemokine receptors
CXCR1: IL-8, GCP-2	CCR1: MIP-1α, RANTES, MCP-3, MIP-δ
CXCR2: IL-8, GCP-2, GROα,β,γ, ENA-78	CCR2: MCP-1 to MCP-5
CXCR3: IP-10, MIG, ITAC	CCR3: Eotaxin, MCP-3,4, RANTES
CXCR4: SDF-1	CCR4: TARC, MDC
CXCR5: BCA	CCR5: MIP-1α, RANTES, MIP-1β
	CCR6: LARC, MIP-3α
	CCR7: SLC, MIP-3β
	CCR8: I-309, TARC, MIP-1β
	CCR9: MIP-1α,β, MCP-1, MCP-5
	CCR10: SLC, LARC, BLC-1, ESkine
	CCR11: MCP-1 to MCP-5, eotaxin

GCP-2, granulocyte chemotactic protein-2; GRO, growth-related oncogene; IP-10, γ-interferon-inducible protein 10; MIG, monokine-induced by γ-interferon; TARC = T cell and activation-related chemokine; SLC = secondary lymphoid tissue chemokine; SDF-1, stromal cell-derived factor; BCA, B-cell chemoattractant; ENA, epithelial cell-derived neutrophil-activating factor; RANTES, regulated upon activation normal T cell expressed and secreted; ITAC, interferon-inducible T cell α chemoattractant.

agents. Measurements of AHR have traditionally been used to identify individuals who are at risk of developing asthma or related illnesses. The essential feature to these tests is to provide stimuli of varying intensity, such as methacholine, to the airways of the individual and record the decrease in lung function that develops. The resulting stimulus–response curve that develops is then analyzed to determine the quantity of agent required to produce a given degree of obstruction as measured by various spirometric or plethysmographic variables. Such changes are usually expressed as a percentage decrease in forced expiratory volume over 1 s (FEV_1). The three variables that are most often examined in quantifying the magnitude of the response are the concentration of an agonist that induces a fixed decrease in lung function (ie a 20% decrease in FEV_1), the slope of the dose–response curve, and the dose at which a plateau can be produced. Typically, the response is expressed as either a provocative dose (PD_{20}) or a provocative concentration (PC_{20}).

How this hyper-responsive state is acquired is poorly understood; however, in general, as the disease process becomes more severe the airways become more responsive. At present it is believed that AHR can result from the coordination of several mechanisms, some or all of which might be operative in individual asthmatics. In asthma a relationship seems to exist between the inflammatory state of the airways and the severity of hyper-responsiveness. In addition, airway remodeling, including smooth muscle hyperplasia/hypertrophy, collagen deposition and subepithelial fibrosis, might contribute to the development of AHR [4–6]. Because recent work in the field of chemokine biology has highlighted a role for these proteins in many of these inflammatory processes, chemokines might be intimately involved in the initiation and maintenance of AHR. In this regard, chemokines could be attractive therapeutic targets for the treatment of pulmonary disease with an AHR component, in particular asthma.

Introduction to chemokines

During the past decade, our understanding of the mechanisms involved in the initiation and maintenance of pulmonary disease has been greatly aided by advances in the field of chemokine biology. Chemokines comprise four supergene families, classified into groups on the basis of the number and arrangement of conserved amino acid sequences at the N terminus. Two of these families (the CC and CXC chemokine groups) contain over 50 identified ligands and at least 14 individual receptors (Table 1). Two additional chemokine families (C and CX_3C chemokines) are small and contain, respectively, lymphotactin and fractalkine as their members. Recent knowledge of this superfamily has grown significantly as a result of the availability of large databases of expressed sequence tags and bioinformatics [7]. Furthermore, characterization of these chemokines *in vivo* has identified multiple roles within inflammation, including the regulation of leukocyte trafficking, the immunomodulation of leukocyte activation, fibrosis, angiogenesis, hematopoiesis and organogenesis [8].

The biological effects of chemokines are mediated by the interaction of these soluble proteins with specific receptors, which belong to the superfamily of seven-transmembrane G-protein-coupled receptors. So far, 11 CC chemokine

receptors, five CXC chemokine receptors, one CX_3C chemokine receptor and one C chemokine receptor have been characterized [7,9]. Chemokine receptors exhibit multiple ligand specificity, although the chemokine–ligand promiscuity does not usually cross the boundaries between CC and CXC, except for the promiscuous duffy antigen receptor complex that is believed to act as a sink for unbound chemokines. Chemokine receptor distribution on leukocytes confers selective chemoattractant activities for leukocyte subsets, making them ideal candidates for a role in leukocyte subset trafficking at sites of inflammation; that is, getting the correct subpopulation of cells to migrate into the tissue. Whereas CXC chemokines such as interleukin-8 (IL-8) activate predominantly neutrophils, CC chemokines such as RANTES and eotaxin target a variety of cell types including macrophages, eosinophils and basophils. However, controversial results have been published regarding this distinct chemokine receptor profile on leukocytes, particularly in allergic diseases. It has been shown recently that, after the appropriate stimuli, the CC chemokine receptors CCR1 and CCR3 can be expressed on neutrophils, indicating a wider role for CC chemokines than mononuclear cell activation and recruitment [10,11]. Furthermore, both the CXC chemokine receptors CXCR1 and CXCR2 have been identified on eosinophils in addition to neutrophils [12]. However, chemokine receptor expression is not limited to inflammatory cells. It is interesting to note that structural cells such as epithelial cells, endothelial cells, smooth muscle cells and fibroblasts also express chemokine receptors and are able to produce chemokines; they are therefore capable of contributing to a wide range of biological functions [13–15].

Once chemokines are released, they can have profound and longlasting biological effects both in the microenvironment of their release and at distant sites. These effects, including leukocyte recruitment and activation, smooth muscle proliferation, regulation of collagen deposition and coordination of fibrosis, might have key individual roles in the establishment and maintenance of AHR [4,5].

Chemokines and leukocyte recruitment in AHR

Studies in both animals and humans have demonstrated a positive correlation between the inflammatory state of the airways and the severity of AHR. However, because the type and cause of this inflammation, as well as the extent and consequences of the inflammatory process, vary between different diseases exhibiting AHR (Table 2), the direct contribution of individual cell types or chemokines to AHR is not yet clearly understood. As discussed above, the distinct pattern of chemokine receptors on leukocytes means that chemokines can exert effects on particular leukocyte subsets. Therefore, the selective recruitment of leukocytes to sites of inflammation in these diseases is strongly influenced by the temporal pattern of chemokine expression.

Table 2

Cellular infiltrate in the airway wall in asthma and chronic obstructive pulmonary disease

Asthma	Chronic obstructive pulmonary disease
T lymphocytes, CD4	T lymphocytes, CD8
CD25	CD25, VLA-1
Eosinophilia	Mild eosinophilia
Activated eosinophils	Non-activated eosinophils
Mast cells	Mast cells
Neutrophils	Neutrophils
	Macrophages

Eosinophil recruitment and AHR

Lung eosinophilia is a fundamental trait of allergic asthma, and infiltration of the airways by eosinophils seems to be central in the pathogenesis of this disease [16–18]. Eosinophils and their products have been identified in sputum, bronchoalveolar lavage fluid (BALF) and biopsy material of the airways of patients with asthma. Furthermore, the number of these cells and the amount of their products correlate with the severity of airway reactivity [16,17,19,20].

Eosinophils contribute to the development of AHR through the activation, degranulation and release of proteins and oxidative products stored in their granules. These proteins include major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN) and eosinophil peroxidase (EPO). In addition, eosinophils generate oxidative products and lipid mediators, including platelet-activating factor and leukotriene C_4 (LTC_4). The generation of these cytotoxic products can cause extensive tissue damage and enhance the accumulation of inflammatory cells. Damage to airway epithelium appears to correlate with airway hyper-reactivity because the loss of epithelium leads to the exposure of 'irritant' receptors of nerves, which might increase the response of the airways to various stimuli.

Several chemokines, including macrophage chemoattractant protein (MCP)-3, macrophage inflammatory protein (MIP)-1α, MCP-4, RANTES and eotaxin, elicit the migration of eosinophils [21–23] and can confer some degree of selectivity on eosinophil recruitment. Specifically, eotaxin, a potent activator of eosinophils and T helper 2 (Th2) lymphocytes, interacts with CCR3 expressed on eosinophils [24–28] to cause both degranulation and chemotaxis of eosinophils [29,30]. Elevated levels of eotaxin detected in the sputum of asthmatics has been shown to be correlated with increased eosinophil numbers and eosinophil cationic protein levels [31]. In several

murine models of asthma, a pronounced lung eosinophilia was associated with an increase in eotaxin expression; a neutralizing antibody against eotaxin significantly inhibited eosinophil infiltration after antigen challenge and decreased AHR in these animals [28]. Contrasting effects on eosinophil recruitment and AHR have been demonstrated in eotaxin gene-deficient mice, possibly owing to the presence of the other, recently identified, CCR3-specific ligands eotaxin-2 and eotaxin-3 [32,33]. In addition to eosinophil chemotaxis and activation, eotaxin, in combination with IL-5, has been shown to mobilize eosinophils from the bone marrow, thereby increasing circulating numbers of eosinophils within the blood [34]. However, eotaxin is not the only chemokine able to modulate eosinophil accumulation within the lung. Murine models of allergic inflammation have shown the movement of eosinophils during the early stages of asthma to be dependent on RANTES and MIP-1α, whereas eotaxin has been shown to be necessary for eosinophil accumulation during chronic stages of the response [35,36]. Therefore, to target chemokines for therapeutic intervention effectively it is essential to understand the temporal pattern of chemokine release.

Chemokine-induced recruitment of Th2 cells and AHR

In addition to eosinophils, T cells constitute a large proportion of the inflammatory cells within the lungs of asthmatics. Indeed, T-cell-mediated immune responses are believed to be important contributors to AHR in asthmatic patients through the release of chemokines and cytokines that enhance lung inflammation, favor the production of IgE, activate eosinophils and mast cells, and directly enhance AHR [37–39]. The observation that T cells have a role in AHR is supported by findings that the transfer of T cells from a hyper-responsive mouse strain into a hyporeactive strain induces non-specific airway reactivity [40]. Furthermore, a characterization of lymphocyte populations in asthmatics and non-asthmatics has demonstrated differences in T cell subtypes in biopsy specimens and BALF from patients with asthma: in asthmatics, significantly higher numbers of Th2-type cells were seen than in control subjects, whereas there was no difference in the number of Th1-type cells [41]. Th2-type cells can be distinguished by the profile of cytokines that they produce, such as IL-4, IL-13 and IL-5, which favor the production of IgE and the growth and activation of eosinophils and mast cells, in addition to enhancing AHR *in vivo* [37–39].

Although lymphocytes have long been known to accumulate at sites of immune and inflammatory reactions, attractants that induce these responses have been identified only recently. RANTES, MIP-1α and MIP-1β were the first chemokines for which lymphocyte-chemotactic activity was reported. The monocyte-chemotactic proteins (MCP-1, MCP-2, MCP-3 and MCP-4) are also potent attractants of T lymphocytes. Gonzalo *et al* [28], using neutralizing antibodies directed against MCP-1 or MCP-5, significantly attenuated the recruitment of both eosinophils and T cells to the lung in a murine model of ovalbumin-induced airway inflammation, and drastically reduced AHR. In contrast, the neutralization of MIP-1α caused only a slight reduction in eosinophilia and AHR, and had no effect on T cell accumulation [28]. In a separate study by Lukacs *et al* [42•], neutralization of MIP-1α or RANTES had no effect on AHR in a murine model of allergy, although eosinophilia was reduced significantly.

The expression of chemokine receptors on lymphocytes and their responsiveness to chemokines vary considerably between subsets. CCR5 is expressed preferentially in Th1 cells, whereas CCR3 and CCR4 seem to be characteristic of Th2 cells [43,44]. It is therefore not suprising that chemokines that preferentially recruit Th2-type cells have recently been identified. A number of chemokines have been shown to have the ability to recruit Th2-type cells preferentially, including monocyte-derived chemokine (MDC) and I-309 [45,46]. T cells recruited to the lung by these chemokines may regulate the persistence and activation of other cells such as eosinophils or mast cells in the airways of patients with asthma via both direct contact and through the release of other inflammatory mediators which contribute to enhanced AHR.

Mast cells and AHR

Mast cells that are located in mucosal and peribronchovascular areas of the lung are known to be important in allergic reactions within the lung. These cells have the capacity to release a variety of mediators that can cause acute bronchospasm, activate and/or attract other inflammatory cells in the lung, and possibly increase AHR [47]. Indeed, there is a strong correlation between amounts of histamine in the airways of allergic asthmatics and sensitivity of the airways to methacholine [48,49].

MCP-1, a CC chemokine that binds CCR2, has been shown to induce AHR by the activation of mast cells in the lung. Activation of mast cells with MCP-1 causes the release of histamine, leukotrienes, platelet-activating factor and various proteases that either directly mediate changes in AHR or further enhance the recruitment of leukocytes to the lungs [36]. Increased levels of MCP-1, in murine models of allergic inflammation, have been shown to activate mast cells directly [36]. In addition, increased levels of MCP-1 have been detected in BALF and bronchial tissue from patients with atopic asthma in comparison with controls [50,51]. With the use of a murine model of cockroach antigen-induced allergic airway inflammation, it has been demonstrated that anti-MCP-1 antibodies inhibit AHR to methacholine and attenuate histamine release into the BALF; furthermore, in normal mice, instillation of MCP-1 induced prolonged airway hyper-reactivity and

histamine release. In addition, MCP-1 directly induced pulmonary mast cell degranulation *in vitro* [36]. In asthmatic patients, histamine and LTC_4 either directly induce AHR or facilitate the recruitment of leukocytes to the lungs to induce AHR indirectly [52,53]. Thus, the induction and evolution of allergic airway inflammation which is dependent on the temporal expression of multiple chemokines and their ligands have been shown to play a key role in the establishment of AHR.

The role of airway remodeling and subepithelial fibrosis in AHR

Although several studies show a direct correlation between AHR and airway inflammation, the causal relationship between leukocyte infiltration and AHR has not been finally settled. There is a discordance in the findings between investigative groups who have studied the relationship between airway inflammation, as assessed by cellular infiltration, and AHR. Some groups have shown a strong relationship between the presence of inflammatory cells and enhanced airway responsiveness [16,17,19,20], whereas other groups have failed to establish such a relationship [18,54–57]. The conflicting evidence might reflect the reality that other factors in addition to, or distinct from, airway inflammation may modulate AHR. Of particular interest is the role that airway remodeling and subepithelial fibrosis play in AHR.

Airway wall thickening, airway smooth muscle hypertrophy and subepithelial fibrosis in AHR

Histologic studies have reported a marked increase in the amount of smooth muscle in airways from asthmatic subjects; this abnormality, along with airway inflammation, is thought to contribute to AHR. It is believed that increased smooth muscle mass would allow the development of greater force and enhanced narrowing of the airway lumen to a given contractile stimulus. It has also been shown that smooth muscle cells can display different phenotypes depending on their environment or culture conditions. Smooth muscle cells have been shown to exhibit a classical contractile phenotype and also a proliferative–synthetic phenotype, which are capable of producing proinflammatory cytokines, chemokines and growth factors that further affect the environment within the lung [58]. Airway smooth muscle cells releasing chemokines such as eotaxin, RANTES, MCP-1, MCP-2 and MCP-3 [59–61] augment inflammatory responses within the lung such as leukocyte recruitment and activation, as discussed previously, that further exacerbate AHR. Because the increase in bronchial smooth muscle mass in asthma is due to cell hypertrophy in addition to hyperplasia [62], the potential relevance of phenotype plasticity and its possible relationship to altered function of smooth muscle in disease states has been suggested. Allergic sensitization and exposure to certain cytokines elicit significant functional changes [39,63,64] that can alter both contractile and secretory functions; however, it remains to be seen how chemokines can alter this phenotype.

Table 3

Elevated chemokines in allergic asthmatic lungs shown *in vivo* to participate in AHR

Chemokine elevated in allergic asthmatics	*In vivo* evidence for involvement in AHR so far
Eotaxin	Yes
Eotaxin-2	No
RANTES	Yes
MCP-3	No
MCP-1	Yes
MIP-1α	Yes
CCR3	Yes

Although fibrosis is an essential component of tissue healing and wound repair, clinical studies have demonstrated that the degree of subepithelial fibrosis is correlated with augmented AHR to methacholine [4]. Indeed, a buildup of interstitial collagen beneath the airway basement membrane and subepithelial fibrosis are present in the airways of allergic asthmatics [6]. Infiltrating inflammatory cells such as macrophages, lymphocytes, neutrophils and eosinophils participate in the pathogenesis of lung fibrosis, through the activation of fibroblasts via the release of inflammatory mediators or direct contact [65,66]. Recent evidence has shown that MCP-1 enhances collagen deposition by fibroblasts [67]; therefore, increased expression of this chemokine in the lungs of asthmatics might be responsible for the airway remodelling that can exacerbate AHR.

Chemokine expression in allergic asthma and their therapeutic use

So far, most of the results indicating a role for chemokines in AHR have been obtained through murine models employing chemokine neutralization, transgenic methods or gene knockout methods. The question therefore arises as to why chemokines would be beneficial targets for the therapeutic treatment of AHR in humans. Clinical studies have shown elevated levels of the chemokines and chemokine receptors that have been identified in murine models and in BALF, bronchial biopsies and sputum from allergic asthmatics (Table 3). Eotaxin, CCR3, mRNA and protein have been found to be significantly elevated in bronchial mucosal biopsies from atopic asthmatics in comparison with normal controls [68]. Furthermore, an inverse correlation was made in this study between the expression of eotaxin mRNA and the histamine provoca-

tive concentration causing a 20% decrease in FEV_1 [68]. Significant correlations with clinical parameters of AHR were also found with MCP-1 levels in BALF from patients with allergic asthma [69]. A comprehensive study by Ying *et al* [70••] measured elevated levels of mRNA for eotaxin, eotaxin-2, RANTES, MCP-3, MCP-4 and CCR3 in the bronchial mucosa from allergic asthmatics [67]. In addition, levels of RANTES, MIP-1α and MCP-1 in BALF have been shown to be significantly increased 4 h after challenge with endobronchial allergen in allergic asthmatics compared with levels before the allergen challenge [71]. Therefore the chemokines that have been shown to have a role in AHR in murine models are elevated in human disease and might be potential targets for the development of therapeutic interventions.

Conclusions

Taken together, both experimental evidence from murine models and clinical evidence of elevated chemokine and chemokine receptor levels in the allergic asthmatic lung suggest that chemokines, and their receptors, seem to be effective targets for the development of therapeutic interventions to be used in addition to current therapy for the treatment of AHR. However, it remains to be seen whether the first clinical trials bear out this promise.

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