REVIEW ARTICLE

Targeting chemokine receptors in allergic disease

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The directed migration of cells in response to chemical cues is known as chemoattraction, and plays a key role in the temporal and spatial positioning of cells in lower- and higher-order life forms. Key molecules in this process are the chemotactic cytokines, or chemokines, which, in humans, constitute a family of approx. 40 molecules. Chemokines exert their effects by binding to specific GPCRs (G-protein-coupled receptors) which are present on a wide variety of mature cells and their progenitors, notably leucocytes. The inappropriate or excessive generation of chemokines is a key component of the inflammatory response observed in several clinically important diseases, notably allergic diseases such as asthma. Consequently, much time and effort has been directed towards understanding which chemokine receptors and ligands are important in the allergic response with a view to therapeutic intervention. Such strategies can take several forms, although, as the superfamily of GPCRs has historically proved amenable to blockade by small molecules, the development of specific antagonists has been has been a major focus of several groups.

In the present review, I detail the roles of chemokines and their receptors in allergic disease and also highlight current progress in the development of relevant chemokine receptor antagonists.

Key words: allergy, asthma, chemokine, G-protein-coupled receptor (GPCR), inflammation, leucocyte.

INTRODUCTION

Allergic diseases such as asthma, allergic rhinitis and atopic dermatitis are typified by an undesirable reaction to antigens (allergens) and are characterized by an influx of eosinophils, lymphocytes, basophils and mast cells to the inflamed tissue. In the last two decades, the incidence of allergic diseases such as asthma has reached epidemic proportions within Western industrialized countries [1]. In the U.K. alone, over 5 million people currently receive treatment for asthma, resulting in increased morbidity and poorer quality of life for the afflicted and a significant financial burden upon healthcare structures [2]. Consequently, much time and effort has been put into dissecting the allergic response in both human and rodent models of disease, with a view to discovering novel therapeutic targets. In the present review, I discuss the roles of chemokines and their receptors in the allergic response and discuss efforts in targeting chemokine receptors with chemokine receptor antagonists.

CHEMOKINES AND THEIR RECEPTORS

Pivotal to leucocyte trafficking within allergic tissues are the chemotactic cytokines, or chemokines, and their receptors which finely tune leucocyte recruitment to both inflammatory sites and secondary lymphoid organs. Chemokines are small proteins, typically of approx. 8–10 kDa, and induce a variety of intracellular signals following binding to their cell-surface receptors. These signals serve to direct cell migration towards the source of chemokine in a process known as chemotaxis [3]. The chemotactic property of chemokines is not specific to immune system cells. For example, in embryogenesis, the temporal and spatial positioning of progenitors is finely tuned by the action of the chemokine stromal-cell-derived factor 1 (CXCL12) and its specific receptor CXCR4 [4]. In addition to cell recruitment, chemokines also play important roles in angiogenesis, where the activation of chemokine receptors on endothelial cells can promote both angiogenic and angiostatic responses [5].

In humans, the chemokine family consists of over 40 members divided into two major and two minor families on the basis of the location of two N-terminal cysteine residues. In the CC family, these cysteine residues are adjacent, whereas, in the CXC family, they are separated by a single amino acid. Two other minor classes, the CX3C and C chemokine families exist which have three members between them. A systemic nomenclature is now in operation in which chemokines are given the prefix CCL (CC ligand), CXCL (CXC ligand), CX3CL (CX3C ligand) and XCL (C ligand), together with an identifying number, updating the anecdotal method of defining a chemokine upon its function [6]. Chemokines exert their biological effects by binding to specific GPCRs (G-protein-coupled receptors), which, in humans, number 19. The prefixes CCR and CXCR are used to define receptors for CC and CXC chemokines respectively (Table 1). Promiscuity is rife among the receptors with each receptor typically having several chemokine ligands, although the ligand repertoire of receptors is class-restricted, i.e. CC chemokines have agonist activity only at CC chemokine receptors and, likewise, CXC chemokines only activate CXC chemokine receptors.

LEUCOCYTE TRAFFICKING DURING THE ALLERGIC RESPONSE

The allergic response can be broadly broken down into two components, the first or early-phase response which peaks at approx.

Abbreviations used: AHR, airways hyperresponsiveness; ASM, airway smooth muscle; BALF, bronchioalveolar lavage fluid; BALT, bronchus-associated lymphoid tissue; CNV, choroidal neovascularization; DC, dendritic cell; FcεRI, high-affinity IgE receptor; HPSC, haemopoietic pluripotent stem cell; IFN-γ, interferon γ; IL, interleukin; PBMC, peripheral blood mononuclear cell; plt, paucity of lymph node T-cells; SCID, severe combined immunodeficiency; Th, T-helper; Treg, regulatory T-cell.

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Figure 1  Cellular and molecular events in the allergic immune response

(A) Events of the early phase of the allergic response in which IgE bound by FcεRI on mast cells is cross-linked by allergen (red). The ensuing mast cell activation and degranulation releases a variety of cytokines and mediators with paracrine effects on other cells, such as the stimulation of IgE production by B-cells, the enhanced survival of eosinophils (Eo) and the contraction of smooth muscle. PAF, platelet-activating factor. (B) Events of the late-phase reaction in which antigen uptake by DCs and subsequent presentation to Th2 cells induces the expression of more cytokines and mediators with activities on additional leucocytes [e.g. basophils (Bo) and non-leucocytes (e.g. epithelial cells)]. TGF-β1, transforming growth factor β1.

Table 1  Human chemokine receptors and their expression by leucocytes

<table>
<thead>
<tr>
<th>Chemokine receptor</th>
<th>Chemokine ligands</th>
<th>Leucocyte expression</th>
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<tbody>
<tr>
<td>CCR1</td>
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<td>Mo, DC, Eo, Bo, T, PMN, NK</td>
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<tr>
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<td>CCL2, CCL5, CCL7, CCL13</td>
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<td>CCL17, CCL22</td>
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<td>CCL3, CCL3L1, CCL4, CCL5, CCL11, CCL13</td>
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<td>T, NK, DC, Mo</td>
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15 min after allergen challenge and is initiated by leucocytes known as mast cells. Mast cells are derived from pluripotent stem cells which reside in the bone marrow. They are released into the blood as progenitors which are recruited into tissues, where they mature into long-lived resident mast cells under the influence of specific growth factors and cytokines. Patients with allergic rhinitis can have 50-fold more mast cells in the nasal mucosa in the pollen season when compared with the winter season [7,8], whereas mast cell numbers can be 10-fold higher in the lungs of asthmatic patients when compared with controls [9]. An increase in circulating mast cell progenitors in asthmatic patients has also been reported [10], suggesting the existence of mechanisms responsible for both recruitment and local maturation of mast cell progenitors to maintain basal populations of mast cells in tissues, and also additional mechanisms to increase local mast cell numbers in the allergic setting.

The mast cell is capable of mounting allergen-specific responses conferred by an IgE antibody bound to high-affinity IgE receptors (FcεRI) which is achieved following allergen cross-linking of IgE (Figure 1A). This triggers the degranulation of the mast cells and results in a release of pre-formed mediators, such as histamine, and rapidly synthesized mediators such as LTC4 (leukotriene C4) and PGD2 (prostaglandin D2). These mediators act on structural cells to induce a variety of effects, including smooth muscle contraction, increased microvascular permeability and, in the case of allergic airways, increased mucus production (Figure 1). IL-5 is an important survival factor for eosinophils [11,12] and induces the release of eosinophil progenitors from the bone marrow [13], whereas IL-4 and IL-13 induce naive B-cells to switch to IgE production, driving the allergic response forwards [14,15].

The early phase of the allergic reaction is followed several hours later by a late-phase reaction which is remarkable for an influx of leucocytes to the effected tissue, including T-cells, eosinophils and basophils. Key to the late-phase reaction and subsequent leucocyte recruitment is the interplay between a variety of structural and immune cells, notably that between DCs (dendritic cells) and effector CD4+ Th (T-helper) cells (Figure 1B). DCs act as immune system sentinels with a voracious appetite for antigen. On encountering and taking up antigen, DCs undergo a process of maturation in which they relocate to local
Figure 2  Chemoattractants and their receptors orchestrate leucocyte trafficking in the allergic immune response

Gradients of chemokine are generated in the tissues following exposure to allergen and subsequent interplay between leucocytes and structural cells. Chemokines induce the release of leucocyte progenitors from the bone marrow to the peripheral blood and guide their recruitment along a concentration gradient to the site of allergic inflammation and chemokine generation. Key to this recruitment is the dynamic cell-surface expression of a wide repertoire of chemokine receptors (depicted as serpentine structures) which also mediate DC and T-cell trafficking to secondary lymphoid organs in the adaptive immune response. Some chemokines such as the Th1-associated CXCR3 ligands can inhibit Th2 cell recruitment, thereby fine-tuning the immune response. Eo, eosinophil; MC, mast cell.

lymph nodes via the lymphatic system and present processed antigen to resident T-cells, resulting in T-cell activation and differentiation. In both humans and rodents, distinct subsets of Th cells mediate specific immune responses tailored to the microbe encountered. The presence of specific cytokines dictates which lineage of Th cell will be produced. IFN\(\gamma\) (interferon \(\gamma\)) and IL-12 drive naïve T-cell differentiation towards cells of the Th1 lineage which themselves produce IFN\(\gamma\) and are critical for responses against intracellular pathogens and viruses. IL-4 is a key cytokine for the production of Th2 cells which themselves produce IL-4 and are key for responses to intracellular pathogens such as helminths and the promotion of Ig production by B-cells. Both IFN\(\gamma\) and IL-4 form autocrine positive-feedback loops to amplify Th1 and Th2 differentiation respectively and antagonize each other’s differentiation by a number of mechanisms [16]. A recently discovered Th17 subset of cells (so-called because they produce IL-17) is thought to govern responses to fungi and intracellular bacteria [17]. Th responses are kept in check by the activities of another subset of T-cells known as Tregs (regulatory T-cells) which secrete IL-10 with potent immunosuppressive properties.

Allergic reactions are thought to be an aberrant anti-helminth response, and Th2 cells dominate at the site of allergen sensitization. The subsequent secretion of Th2 cytokines such as IL-4 and IL-13 induces the generation of chemokines by epithelial cells which recruit effector cells such as eosinophils and basophils (Figure 2). Th2-produced IL-5 also promotes eosinophil survival. In the case of an airway from an asthmatic patient, the leukotrienes produced by the incoming eosinophils induce bronchoconstriction and mucus hypersecretion, whereas eosinophil-derived TGF-\(\beta1\) (transforming growth factor \(\beta1\)) drives a pro-fibrotic response. In the case of repeated provocation with allergen, the excessive repair processes result in increased ASM (airway smooth muscle) mass, collagen deposition, angiogenesis and subsequent thickening of the airway wall in a process known as remodelling [18–20].

Although they account for less than 1% of circulating granulocytes in the peripheral blood, basophils are important effector cells in IgE-mediated allergic reactions, producing histamine, cytokines and lipid mediators following FceRI cross-linkage by antigen [21]. They arise from the same progenitor cells as mast cells [22] and are thought to be part of a positive-feedback loop for IgE-mediated immediate-type hypersensitivity reactions [23]. Basophils have been shown to be a predominating antigen-presenting cell in Th2 reactions against both helminths and allergens, presenting peptide in conjunction with MHC class II molecules to naïve CD4\(^+\) T-cells and producing IL-4 in the process [24–26].

**CC CHEMOKINE RECEPTORS IMPLICATED IN THE ALLERGIC RESPONSE**

**CCR1**

As its name suggests, CCR1 was the first of the CC chemokine receptors to be identified and is expressed by mast cells [27], eosinophils [28,29] basophils [30,31] and NKT (natural killer T-) cells [32]. CCR1 binds a variety of chemokine ligands, notably CCL3 and CCL5, both of which are found in the BALF (bronchioalveolar lavage fluid) from allergen-challenged lungs from an asthmatic patient [33,34] and in nasal secretions from allergen-induced late-phase reactions in the skin, CCL3 is produced by influxing neutrophils and basophils [37]. CCR1 and FcεRI are co-localized within the plasma membrane of mast cells [38], and co-stimulation of both receptors results in an enhanced degranulation response and decreased chemotaxis to CCL2. This
is postulated to maintain mast cell numbers at sites of allergic inflammation, thereby focusing the immune response [39]. In a murine model of allergic conjunctivitis, CCR1 deficiency or CCL3 blockade results in a reduction in disease score following co-stimulation of CCR1 and FcεRI, suggesting a potential use for anti-CCR1 therapeutics in allergy [40].

**CCR2**

The chemokine receptor CCR2 binds all four members of the MCP (monocyte chemoattractant protein) family (CCL2, CCL7, CCL8 and CCL13) and is expressed by monocytes [41−45], Th1 cells [46,47], basophils [31] and mast cells [48]. Three of the four CCR2 ligands have been reported to induce basophil degranulation directly, namely CCL2, CCL7 and CCL13 [49−51]. CCR2 appears to be vital for an effective Th1 response, with deletion of CCR2 resulting in impaired responses to intracellular pathogens [52−54], thought to be due to defective trafficking of monocytes with Th1-polarizing potential. As might be anticipated with the reciprocal nature of Th1/Th2 responses, CCR2-deficient mice show enhanced Th2 responses following challenge with ovalbumin [55] and *Aspergillus* [56]. Conversely, deletion of CCL2, the principal CCR2 ligand, results in reduced IL-4 and IL-5 production and an inability to undergo Ig class switching following ovalbumin challenge of the airways [57,58]. This suggests a role for CCR2 in controlling Th2 polarization, supported by the finding that depletion of murine CCL2 reduced AHR (airways hyperresponsiveness) in ovalbumin and cockroach allergen challenge models [59,60]. The precise role for receptor and ligand in inflammation, however, is not straightforward. Similar studies in CCR2-deficient mice have shown enhanced Th2 responses to ovalbumin [55] and *Aspergillus* [56], whereas a direct comparison of CCR2- and CCL2-deficient mice reported intact Th2-mediated responses and lung fibrosis in both animals following challenge with *Aspergillus* [61]. In a non-human primate model of allergic airways disease following challenge with *Ascaris suum* antigen, CCR2 blockade by a specific monoclonal antibody resulted in reduced AHR and macrophage and eosinophil recruitment to the lung, although it is noteworthy than no significant T-cell recruitment was detectable in this model [62].

CCL2 has been shown to stimulate degranulation of mast cells in a murine model of allergic conjunctivitis with CCR2 blockade significantly reducing the disease score [63]. A recent report also highlighted a complex role for the CCL2/CCR2 axis in the recruitment of mast cell progenitors to the allergic lung [64]. In this study, sensitization followed by allergen challenge resulted in mast cell progenitor recruitment to the lung which correlated with increased CCL2 levels in the BALF and which was significantly reduced in CCR2- and CCL2-deficient mice. *In vitro* culture of mast cell progenitors revealed that CCR2 was a functional receptor, but became uncoupled from chemotaxis on maturing cells, despite maintaining surface expression levels. Reconstitution of CCL2-deficient mice with wild-type or CCL2-deficient bone marrow suggests that CCL2 produced by both bone marrow-derived and lung stromal cells is required for mast cell progenitor recruitment to the allergic lung. It remains to be seen whether the corresponding chemokine/receptor axes direct mast cell recruitment in humans.

**CCR3**

CCR3 is the principal chemokine receptor expressed by eosinophils [65], and is also expressed by Th2 cells [66], basophils [67] and mast cells [68,69]. CCR3 binds the eotaxin family of chemokines comprising CCL11 [70], CCL24 [71,72] and CCL26 [73,74]. The accumulation of eosinophils within the bronchial wall is a characteristic feature of allergic airways inflammation, although the precise role of the eosinophil in asthmatic disease remains complex and controversial (see below). A critical role for CCR3 in orchestrating eosinophil migration is supported by the use of CCR3-deficient mice which have reduced numbers in the gut and traffic in reduced numbers to the lungs following allergen challenge [75]. Interestingly, in the airways, the tracking defect lies predominantly at the point of migration from the subendothelial space into the lung parenchyma, suggesting that other chemokines may co-operate in general recruitment from the circulation [75].

The route of sensitization is an important consideration in this model as intradermal sensitization favours the development of increased AHR in response to the bronchoconstrictor methacholine which is absent from CCR3-deficient mice [76]. In contrast, in CCR3-deficient mice, intraperitoneal sensitization results in increased numbers of intraepithelial mast cells in the trachea compared with wild-type mice which was accompanied by increased AHR [75]. This suggests that the CCR3/eotaxin axis does not play a major role in mast cell homing to the lung and subsequent differentiation and that, in the absence of CCR3, mast cell progenitors are recruited and retained in the airway epithelium. This is in keeping with a recent report suggesting that immature and mature bone marrow-derived mast cells are unable to migrate *in vitro* to the CCR3 ligands CCL11 and CCL24 [77]. Similarly, skin mast cell numbers were normal in CCR3-deficient mice following epicutaneous ovalbumin sensitization, suggesting that CCR3 does not play a role in mast cell homing to the skin [76]. However, in a murine model of allergic conjunctivitis, antagonism of CCR3 was reported to impair the early-phase reaction thought to be due to the stabilization of mast cells, perhaps suggesting a role for the receptor in mast cell degranulation [78].

In humans, CCL11 plasma levels have been reported to be elevated in acute compared with stable asthmatic patients [79], and increased expression of CCL11 and CCL24 is observed in the allergic lungs of asthmatic patients [80,81] and in their sputum [82,83]. In mice, although functional orthologues of CCL11 and CCL24 are found, CCL26 is apparently a pseudogene [84]. Studies of an ovalbumin-sensitization model of airway disease employing mice either singly or doubly deficient in CCL11 and CCL24 suggest that both ligands need to be depleted for ablation of pulmonary tissue eosinophilia to levels observed in CCR3-deficient mice [85].

**CCR4**

CCR4 specifically binds the chemokines CCL22 and CCL17 [86]. DC maturation is accompanied by the production of CCL17 and CCL22, and elevated levels of both chemokines have been detected in the skin lesions of atopic dermatitis patients [87−89], and in the lungs of both healthy volunteers and asthmatic patients following allergen challenge [90,91]. CCR4 itself is expressed by mast cells [27], monocytes, DCs and natural killer cells [92], and is notably induced on Th2 cells following their polarization *in vitro* [46,47]. Such polarization is also observed *in vivo* with IL-4-producing cells recovered from the BALF of asthmatic and healthy subjects shown to be CCR4+ [93].

Several studies support a role for CCR4 and its ligands CCL17 and CCL22 in T-cell trafficking to the allergic murine lung. Antigen-specific Th2 cells from CCR4-deficient mice fail to traffic in significant numbers to the allergic lung of wild-type mice following adoptive transfer [94], whereas antibody neutralization of CCL22 proved effective in the long-term blockade of Th2...
cell recruitment following repeated stimulation with allergen [95]. Similarly, neutralization of CCL17 decreased CD4+ and eosinophil recruitment to the BALF, coupled with reductions in AHR and Th2 cytokine production [96]. In contrast with these studies, CCR4-deficient mice showed no protection against airway inflammation [97]. Likewise, neutralization of CCR4 in guinea pigs with a monoclonal antibody was ineffective in terms of modulation of the allergic response [98]. More recently, CCR4 blockade in a human PBMC (peripheral blood mononuclear cell)-reconstituted SCID (severe combined immunodeficiency) mouse model via a small-molecule antagonist was reported to abolish many of the features of inflammation, including airway eosinophilia, goblet cell hyperplasia, IgE synthesis and bronchial hyperreactivity [99]. The same small-molecule antagonist has also proved effective in the in vitro blockade of responses from CCR4+ T-cells, a population increased in numbers in nasal biopsies of rhinitic patients following allergen challenge, suggesting another potential therapeutic avenue for CCR4 blockade [100].

CCR7

CCR7 binds the chemokines CCL19 and CCL21 [101–103] and plays a key role in the homoeostatic trafficking of B-cells, T-cells, and DCs to secondary lymphoid organs [104,105]. The DDD/1 mouse carries an autosomal recessive mutation known as paucity of lymph node T-cells (plt) [106] in which the gene encoding CCL19 and one of the two genes encoding CCL21 has been deleted, resulting in a lack of CCL19 and CCL21 expression in lymphoid organs, but expression of CCL21 in non-lymphoid organs [107–109]. Together with CCR7-deficient mice, they have proved valuable tools to dissect the role of CCR7 ligands in homoeostasis and inflammation, revealing many roles for ligand and receptor in leucocyte function.

CCR7-deficient mice exhibit a host of defects, including defective migration of positively selected thymocytes from the cortex to the medulla [105], which is essential for the establishment of central tolerance in the thymus [110]. They also have impaired leucocyte migration and poorly formed secondary lymphoid organs [111], although they do develop highly organized BALT (bronchus-associated lymphoid tissue) [112]. Notably, they possess dramatically reduced numbers of Tregs in the lung-draining bronchial lymph nodes, suggesting that Tregs control BALT formation and rely upon CCR7 for homing to the peripheral lymph nodes [112]. CCR7 signalling also appears to have a positive influence on the intranodal motility of T-cells, as CCR7 or plt mice exhibit impaired motility as assessed by intravital two-photon microscopy [113]. Similarly, there is a role for CCR7 in egress from tissues as, although CCR7+ and CCR7− effector T-cells can be recruited to the allergic murine lung, only CCR7+ T-cells are able to exit the lung and enter the afferent lymphatics [114].

DCs play an key role in sensitization to inhaled allergens as their depletion results in a loss of leucocyte recruitment and Th2 cytokine secretion which can be restored by adoptive transfer of DCs [115]. Migration of mature DCs to the draining lymph nodes is heavily dependent upon CCR7 as plt mice and CCR7−/− strains have severely impaired DC trafficking from the skin to the lymph nodes following allergen challenge [107,111,116]. Likewise, in a human PBMC-reconstituted SCID mouse model, neutralization of the CCR7 ligand CCL21 reduced the pulmonary inflammatory response following allergen challenge of mice repopulated with T-cells from allergic donors [117]. DC migration from the lung to the draining bronchial lymph nodes under steady-state conditions is also impaired by CCR7 deletion, explaining the inability of CCR7-deficient mice to induce tolerance to intratracheal instilled ovalbumin [118].

Curiously, in models of airways inflammation in response to inhaled ovalbumin, plt mice exhibit enhanced airway inflammation and AHR compared with controls, despite an initial delayed IgE-specific response [119,120]. This may suggest an additional role for CCR7 in the resolution of inflammation and together with the impaired tolerance and Treg trafficking observed in CCR7-deficient mice might argue against antagonism of CCR7 in allergic disease treatment. ASM has also been reported to express CCR7, and the increased CCL19 found in bronchial biopsies of asthmatic patients may suggest a role for the CCR7−/CCL19 axis in promoting the ASM hyperplasia observed in asthma [121].

CCR8

CCR8 is one of the few monogamous receptors in the chemokine system, with only CCL1 able to induce its activation. It does have additional suitors among the viral chemokines, however, with the HHV-8 (human herpesvirus 8)-encoded chemokine vMIP-I (viral macrophage-inflammatory protein I) acting as a CCR8 agonist [122,123]. Polarization of T-cells in vitro to Th2 subsets results in expression of CCR8 mRNA [46,47]. Conflicting reports exist in the literature as to whether or not increased expression of CCL1 is observed in the lungs of asthmatic patients compared with healthy controls [124–128] and also whether or not increased numbers of CCR8+ T-cells are recruited to the allergic lung. The latter has been problematic due to the lack of a seemingly reliable antibody against human CCR8. For example, in one study examining CCR8 expression on a population of CD4+ CD25+ Tregs, CCR8 surface staining was undetectable using a commercially available antibody, although the cells evidently expressed functional CCR8, as assessed by CCL1-driven actin polymerization [129].

Studies in mice aimed at validating CCR8 as a target for allergic airways disease suggest that, as is the case with other ligand/receptor axes in the chemokine system, the precise role of CCR8 and CCL1 in disease is complex. Initial studies using knockout mice suggested that the role of the receptor was minimal, as CCR8-deficient mice did not exhibit impaired inflammation of the airways following allergen challenge [130,131]. The finding that CCR8 is also expressed by CD4+ CD25+ Tregs may help to interpret these data, as the inability of Tregs to traffic to sites of inflammation is likely to be disadvantageous [132,133]. In contrast, the CCR8/CCL1 axis appears to be important for the recruitment of eosinophils to the murine lung [134]. Notably, in vitro studies of mast cells report that CCL1 is the predominant chemokine secreted following their activation, and subsequent studies with CCL1 blockade or CCR8-deficient mice have shown a reduction in lung inflammation similar to that detected in mast cell-deficient mice, leading to the hypothesis that that mast cell-derived CCL1 serves to recruit CCR8-expressing CD4+ T-cells to the inflamed lung [127]. CCR8-deficient mice sensitized with Aspergillus fumigatus antigens were shown to clear fungal material more rapidly from the lung than littermates, suggesting that CCR8 may be a useful target in fungal-associated pulmonary diseases [135].

CXCR1 and CXCR2

CXCR1 and CXCR2 were the first chemokine receptors to be identified at the molecular level and bind a number of
CXC chemokines, notably CXCL8 [136,137]. Both receptors are principally expressed by neutrophils and, in patients with severe asthma exacerbations, increased neutrophil recruitment to the bronchial mucosa is accompanied by increased expression of CXCR1 and CXCR2, together with the ligands CXCL5 and CXCL8 [138].

CXCR2 ligands form a subset of CXC chemokines containing an ELR (Glu-Leu-Arg) motif at their N-terminal and have known angiogenic properties. *Ex vivo* culture of human microvascular endothelial cells from healthy lung in a cocktail containing IL-4 and TNFα (tumour necrosis factor α) has been reported to promote tube formation in a CXCR2-dependent manner [139], whereas a recent study reported that the recruitment of bone marrow-derived endothelial progenitor cells to the lungs following allergen challenge could be significantly impaired by CXCR2 blockade [140]. Both studies suggest that CXCR2 may be an attractive target for manipulating the airway remodelling associated with asthma.

Similarly, in a murine model of *A. fumigatus*-induced asthma, CXCR2 deficiency results in significantly reduced production of Th2 cytokines in the allergic lung, together with reduced numbers of eosinophils and T-cells [141]. Notably, the recruitment of neutrophils was unimpaired in these mice and was dependent on the production of two other CXC chemokines, CXCL9 and CXCL10. Neither of these chemokines are known neutrophil attractants, suggesting that they act by inducing the expression of other neutrophil chemoattractants [141]. CXCR2 also appears to play a role in the homing of murine mast cell progenitors to the small intestine, as CXCR2-deficient mice have reduced numbers of intestinal mast cell progenitors [142].

**CXCR3**

CXCR3 is expressed by approx. 40% of freshly isolated T-cells from peripheral blood and is rapidly up-regulated upon polarization to the Th1 phenotype [143,144]. CXCR3 mediates chemotaxis in response to the chemokines CXCL9, CXCL10 and CXCL11 [145,146], and all three chemokines are induced by the archetypal Th1 cytokine IFNγ and their production is typically associated with responses to intracellular pathogens such as viruses. Although atopic dermatitis is thought to be a Th2-type disease, IFNγ is produced in chronic disease and all three CXCR3 ligands have been detected in the lesional skin from atopic dermatitis patients and also skin biopsies exhibiting allergic contact dermatitis reactions [147–149].

CXCR3 ligands are also up-regulated in both human [91] and murine [150] lungs following allergen challenge, but their precise role in disease is unclear. In a study by Thomas et al. [151] of asthmatic patients undergoing segmental allergen challenge, virtually all CD4⁺ cells recovered from the BALF pre-challenge were CXCR3⁺, which was rapidly down-regulated following allergen challenge, suggesting that the receptor underwent endocytosis following engagement with ligand [151]. We and others have shown *in vitro* that CXCR3 ligands are natural antagonists of CCR3-mediated responses such as Th2 cell recruitment [152,153], which, bearing in mind the expression of CCR3 on Th2 cells, may serve to finely tune T-cell polarization and the subsequent immune response *in vivo*.

**CXCR4**

CXCR4 is another monogamous chemokine receptor, binding the chemokine CXCL12 [154,155] and, unlike most other chemokine receptors, its expression is almost ubiquitous, particularly during embryogenesis where it plays a critical role in haemopoiesis, angiogenesis, neurogenesis and cardiogenesis, as is evident from the lethality of CXCR4 deletion in the mouse [156–158]. Both ligand and receptor are also vital for the homing and retention of HPSCs (haemopoietic pluripotent stem cells) within the bone marrow [159,160]. In the context of allergic disease, allergen challenge of asthmatic patients has been reported to induce a down-regulation in CXCR4 expression on HPSCs and a reduction in levels of bone marrow CXCL12 levels which may promote egress of cells to the periphery perhaps mediated via increased levels of the CCR3 ligand CCL11 present in the circulation [161]. Cell-surface levels of CXCR4 on Th2 cells have been reported to be up-regulated by the Th2 cytokine IL-4 [162], and eosinophils recovered from the BALF of asthmatic patients have increased levels of cell-surface CXCR4 [163], suggesting that the receptor plays a role in leucocyte homing to the inflamed lung. Neutralization of either CXCR4 or CXCL12 by blocking monoclonal antibodies was observed to reduce lung eosinophilia and AHR in murine models of allergic airway inflammation [164].

Since CXCR4 is highly conserved across species, antagonists developed against the human receptor have excellent potency at murine CXCR4, allowing them to be used in disease models. One such compound, named AMD3100, has been reported to show efficacy in a murine model of cockroach allergen-induced inflammation, significantly reducing AHR and associated inflammation [165]. A second-generation derivative, AMD3465, was also reported by the same group to have efficacy in a murine model of Th2-cell-mediated hypersensitivity to *Schistosoma mansoni* egg antigen-coated beads [166]. AMD3100 (also known as Plerixafor or Mozobil®) recently obtained FDA (U.S. Food and Drug Administration) approval for use as an HPSC mobilizer, used in combination with G-CSF (granulocyte colony-stimulating factor) for the treatment of multiple myeloma, and it remains to be seen whether it will have any clinical use in the allergic setting.

**ANTAGONIZING CHEMOKINE RECEPTORS IN ALLERGIC DISEASE**

Chemokine receptors are members of the GPCR superfamily, and have historically proved amenable to antagonism by small molecules, with approx. 50% of currently prescribed drugs acting at GPCRs [167]. This has led to a hive of activity by the pharmaceutical industry to identify potent chemokine receptor antagonists. Chemokine binding to receptors appears to be a complex process involving interactions of the chemokine with a highly negatively charged receptor N-terminus, often rich in acidic residues, sulfated tyrosine resides and O-linked glycosylation [168–170]. This interaction serves to tether the chemokine ligand to the receptor and promotes secondary interactions with other receptor regions, ultimately stabilizing a particular receptor conformation, leading to the activation of G-proteins and intracellular signalling (Figure 3). Studies of several receptors have implicated interactions involving the N-terminus of the chemokine with a hydrophobic binding pocket site composed of the receptor transmembrane helices [171–175]. Such pockets are ‘highly druggable’ by small-molecule antagonists, and several chemokine receptor antagonists have been shown to act as antagonists by binding within these regions [176–180]. More recently, a second class of antagonist-binding site has been described, involving intracellular access of the antagonist to the receptor C-terminus [181]. Although the precise residues making up the binding site remain undetermined, this region contains a highly conserved eight-helix which has been shown to influence...
chemokine binding to the viral chemokine receptor ORF74 [182] and also β-arrestin translocation following activation of the related formyl peptide receptor [183].

One initial hurdle in drug development has been the inability of human antagonists to hit the rodent orthologue with reasonable potency, making target validation in disease models relatively difficult. As a consequence, despite several publications describing in vitro efficacy, descriptions of antagonist efficacy in vivo lag well behind. Table 2 lists small-molecule antagonists which have been reported in the literature to possess efficacy in both in vitro and in vivo models of allergic disease [165,184–196]. Although several compounds have been described with efficacy in vivo, not all have gone on to feature in clinical trials. Reasons for this may include poor pharmacokinetic profiles and safety issues. A notable problem with a variety of prototypic chemokine receptor antagonists has been that they often contain a key quaternary nitrogen residue, postulated to interact with a conserved glutamate residue in the seventh transmembrane helix of the receptor [176,177,197]. Unfortunately, this also often gives them a pharmacophore similar to that observed in blockers of the human ether-a-go-go-related gene, which inhibit a cardiac potassium channel and may induce a fatal sudden cardiac arrhythmia [197,198]. Current antagonist screens utilize a variety of techniques to try to weed out such cross-reactivity at an early stage of drug development [200].

It can be argued that, in many instances, antagonist discovery and progression to clinical trial has steamrollered ahead in the absence of a detailed understanding of the role of chemokines and their receptors in disease. As our knowledge of the chemokine system has increased, solutions to seemingly intractable paradoxes have been forthcoming. For example, as mentioned above, CCR2-deficient mice show an impaired Th1 response, yet mice deficient in the principal CCR2 ligand CCL2 can mount perfectly robust Th1 responses. Similarly, in plt mice, although migration of DCs to the draining lymph nodes is ablated, Th1 and Th2 responses are apparently enhanced [201]. Both paradoxes have recently been shown to unite around a subset of CD11c⁺CD11b⁺Gr-1⁺ inflammatory DCs which are able to capture large amounts of antigen and whose numbers are substantially increased in plt mice compared with controls. These inflammatory DCs enter the lymph nodes directly from the blood in a CCR2-dependent fashion, therefore bypassing CCR7 homing via the lymphatics [202]. In CFA (complete Freund’s adjuvant)-immunized ccr2⁻/⁻ mice, CD11c⁺CD11b⁺Gr-1⁺ DCs are able to migrate to the draining popliteal lymph nodes (where CCL8 is highly expressed), but are unable to traffic to inflamed footpads (where CCL2 is the most abundant chemokine). Likewise, since we know now that a substantial number of Tregs express CCR4 and CCR8, targeting either receptor may have unwanted side effects [133,203,204]. A greater understanding of such intricacies in the chemokine system might present clues as to why, to date, blockade of seemingly logical targets has been unsuccessful in the clinical treatment of inflammatory disease [205,206].

To date, the only small-molecule chemokine receptor antagonist reported to have featured in any clinical trial of allergic disease is the CCR3 antagonist GSK766994 from GlaxoSmithKline. Despite being orally active in a brown rat model of asthma and possessing a reasonable half-life, this compound sadly did not show efficacy in a Phase III clinical trial for the treatment of allergic rhinitis [192]. One reason put forward for the failure of this and other chemokine antagonists in the clinic is the apparent redundancy in the chemokine system, meaning that blocking a single chemokine receptor in heterogeneous diseases such as asthma may prove unfruitful. This is particularly true in humans where immune responses are likely to be much more heterogeneous that in those observed using inbred rodent strains. In such a scenario, the use of a broad-spectrum antagonist hitting several receptors may be appealing. For example, the molecule UCB35625 antagonizes both CCR1 and CCR3 [207], with both receptors implicated in mast cell and eosinophil activation [31,41,42,78]. The activity of broad-specificity antagonists need not be limited to chemokine receptors. For example, a dual antagonist of CCR3 and the H₁ histamine receptor has been described which may impede both leucocyte recruitment and the vasodilatory and bronchoconstrictory properties of histamine [208]. Such compounds may provide the basis of novel therapeutic opportunities for the treatment of allergy, allowing the sparing of corticosteroids with obvious benefits for patients.
In asthma, efforts have centred upon CCR3 in the belief that targeting eosinophil recruitment is likely to be beneficial in disease treatment. However, the precise role of the eosinophil in asthmatic disease remains complex and controversial. Although deletion or neutralization of the eosinophil survival factor IL-5 suggested a link between eosinophil activation and AHR in murine studies [165], targeting CCR3 may provide ‘added value’. In asthma, efforts have centred upon CCR3 in the belief that targeting eosinophil recruitment is likely to be beneficial in disease treatment. However, the precise role of the eosinophil in asthmatic disease remains complex and controversial. Although deletion or neutralization of the eosinophil survival factor IL-5 suggested a link between eosinophil activation and AHR in murine studies [165], targeting CCR3 may provide ‘added value’. In asthma, efforts have centred upon CCR3 in the belief that targeting eosinophil recruitment is likely to be beneficial in disease treatment. However, the precise role of the eosinophil in asthmatic disease remains complex and controversial. Although deletion or neutralization of the eosinophil survival factor IL-5 suggested a link between eosinophil activation and AHR in murine studies [165], targeting CCR3 may provide ‘added value’. In asthma, efforts have centred upon CCR3 in the belief that targeting eosinophil recruitment is likely to be beneficial in disease treatment. However, the precise role of the eosinophil in asthmatic disease remains complex and controversial. Although deletion or neutralization of the eosinophil survival factor IL-5 suggested a link between eosinophil activation and AHR in murine studies [165], targeting CCR3 may provide ‘added value'.
Of note is the fact that CCR3 activation has been recently implicated in AMD (age-related macular degeneration), with a recent high-profile study suggesting that CCR3 signaling in retinal endothelial cells drives CNV (choroidal neovascularization) in both humans and mice in the distinct absence of inflammation and leucocyte recruitment [218]. Use of a prototypic CCR3 antagonist in a murine model of CNV was a notably more effective inhibitor of CNV than the current clinically approved anti-VEGF (vascular endothelial growth factor)-A treatment, highlighting the point that antagonist development in one field of research may have promising applications in another [218]. The fact that two chemokine receptor antagonists have to date received regulatory approval (Miraviraco has been licensed for the treatment of HIV-1 infection [219] and Plerixafor/Mozobil® has been approved for haemopoietic stem cells mobilization [220]) has no doubt given ‘a shot in the arm’ to the field and revived optimism that antagonists showing efficacy in the inflammatory setting will soon be forthcoming.

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