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From production to intracellular signalling, the molecules controlling inflammatory cell migration present a significant opportunity for the therapy of common chronic respiratory diseases.

Leukocyte navigation mechanisms as targets in airway diseases

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Respiratory diseases, including asthma and chronic obstructive pulmonary disease, are among the most significant diseases in terms of their disabling effects and healthcare burden. A characteristic feature of almost all respiratory diseases is the accumulation and activation of inflammatory leukocytes in the lung or airway. Recent advances in the understanding of the molecules and intracellular signalling events controlling these processes are now translating to new therapeutic entities. In this article, the process of leukocyte accumulation is summarized, together with the preclinical and clinical evidence supporting the utility of the individual components of this process as targets for disease therapy.

Activation and accumulation of different leukocyte populations are defining features of inflammatory disease. To begin addressing the possibility of targeting leukocyte movement within chronic lung diseases, we will outline the main characteristics of each disease of interest, followed by the differences most common animal models possess in comparison with human pathology. Table 1 summarizes the key features of chronic inflammatory lung diseases.

Heterogeneity of airway disease and animal models

Asthma

Asthma is one of the most common chronic diseases in the world. It is estimated that ~300 million people currently have asthma and that it accounts for ~1 in every 250 deaths worldwide. Furthermore, it is estimated that there could be an additional 100 million people with asthma by 2025 [1]. Bronchial asthma is characterized by airway eosinophilia, goblet cell hyperplasia with mucus hypersecretion and airway hyperresponsiveness (AHR) to inhaled allergens and to non-specific stimuli [2]. In individuals with an allergic predisposition, T cells exposed to allergen on the surface of antigen-presenting cells (mainly dendritic cells) mature preferentially towards the T-helper (Th) 2 subtype. By producing different chemical mediators, these cells influence the activity of mast cells, granulocytes, B cells and local cells (epithelial, fibroblasts and airway smooth muscle cells), causing the pathophysiological characteristics found in asthma [2] (Figure 1).

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TABLE 1

Main characteristics of chronic inflammatory lung diseases

Disease	Major cell types involved	Defining characteristics	Associated risks and causes
Asthma	T cells (Th2>Th1) Eosinophils Mast cells Airway epithelial cells	Airflow limitation is usually reversible Airway hyperresponsiveness Episodes of coughing, wheezing and dyspnea Onset early in life	Allergy, rhinitis or eczema Family history of asthma
Chronic obstructive pulmonary disease	T cells Neutrophils Macrophages Airway epithelial cells	Airflow limitation is progressive and irreversible Atypical responses to noxious particles Main syndromes: chronic bronchitis and emphysema. Onset in midlife	Long smoking history
Idiopathic pulmonary fibrosis	Neutrophils Fibrocytes Fibroblasts	Chronic fibrosing interstitial pneumonia Limited to the lung Dyspnea with worsening of pulmonary function Pulmonary dysfunction is progressive and irreversible Onset late in life	Unknown

The study of respiratory diseases *in vitro* is limited by the fact that probing a certain molecular or cellular response in isolation does not represent well the situation in the lung of the whole animal. By contrast, *in vivo* modelling is able to capture some of the complicated genetic, biochemical and environmental interactions that combine in a disease state. However, no one model portrays all the human characteristics of the disease; each model focuses on certain characteristics more than others [3].

In the majority of current asthma models, mice are intraperitoneally injected with a sensitizing agent [like ovalbumin (OVA), cockroach antigen or *Aspergillus* conidia]. Upon subsequent exposure to the sensitizing agent in an aerosolized form, animals develop AHR and eosinophil recruitment into their airways, which mimics, to a certain extent, human asthma [3]. However, other aspects of these models are very different compared with human disease. For example, in mice, there is an absence of acute bronchoconstriction [3], a dramatic variation of eosinophil influx in different strains of mice [4] and a prominent role for serotonin, which is nonexistent in human asthma [5]. The chronic inflammation of airway walls, and their remodelling processes by fibrosis

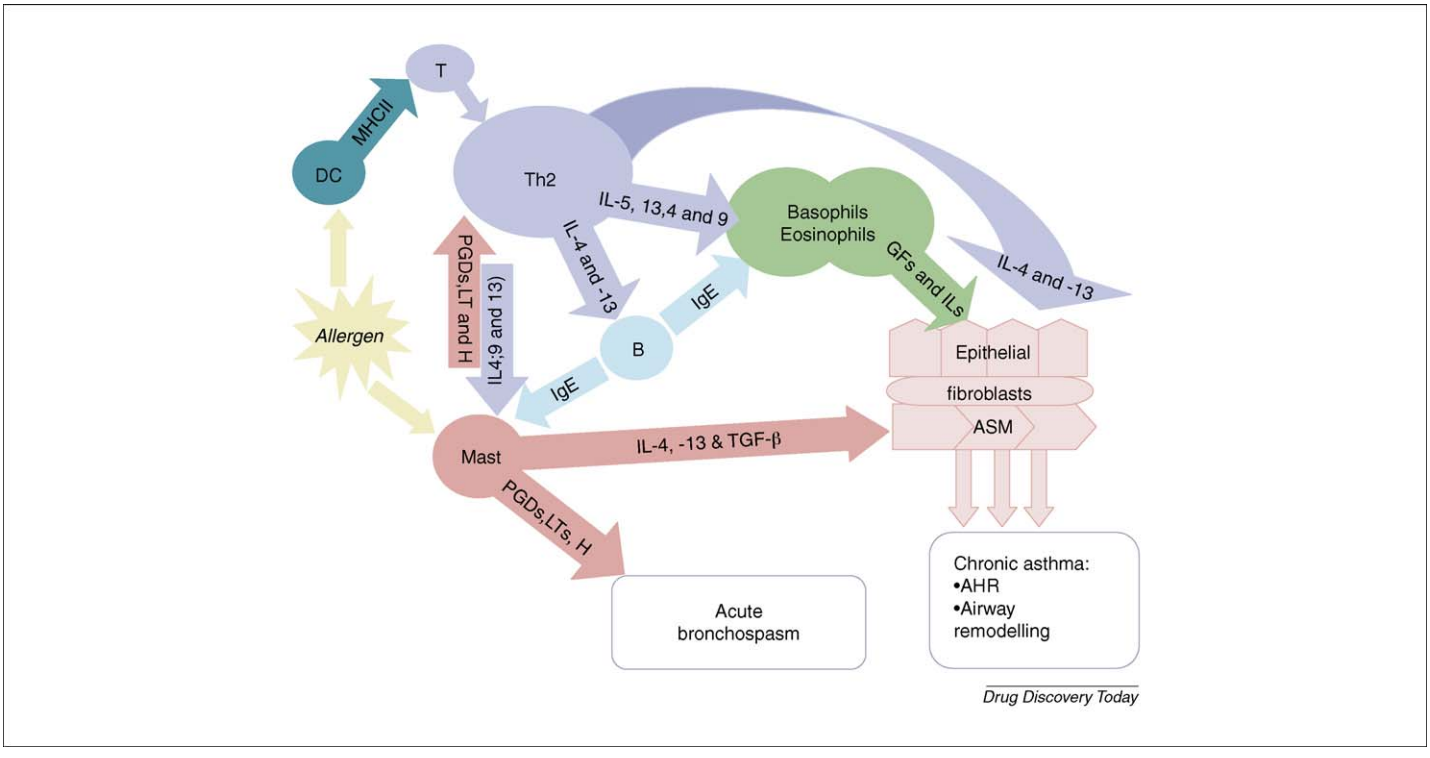

FIGURE 1

The role of T cells in asthma. After allergen exposure, mast cells (Mast) release prostaglandins (PGDs), leukotrienes (LTs) and histamine (H), causing the acute bronchospasm of the airway. For the chronic manifestations, the chemical mediators produced by mast cells in conjunction with T cell interaction with allergen-exposed dendritic cells (DC) preferentially induce T cells into a Th2 subtype. These T cells secrete interleukins (IL-5, IL-4, IL-13 and IL-9), which induce B lymphocyte immunoglobulin (Ig) E synthesis; eosinophil and basophil survival and recruitment; and mast cell maturation and activation. These cells respond by secreting growth factors (GFs) like transforming growth factor-β (TGF-β), and more interleukins. This myriad of immune mediators directly induces lung cells (epithelial cells, fibroblasts and airway smooth muscle (ASM) cells into the asthmatic phenotype, culminating in airway remodelling and hyperresponsiveness (AHR).

and epithelial proliferation, are notably absent [6].The most important disadvantage of the commonly used animal asthma models is that they involve short-term experiments; therefore, they do not portray well most of the lesions that are characteristic of chronic human asthma. However, the recruitment of eosinophils and T cells to the lungs in these models are similar to the human disease [3].

Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is a major cause of chronic morbidity and mortality worldwide. The 2002 World Health Report listed COPD as the fifth leading cause of death in the world, and further increases in its prevalence and mortality are expected in the coming decades [7]. COPD is a disease associated with an abnormal inflammatory response of the lungs to noxious particles or gases, primarily caused by cigarette smoking. It is characterized by progressive airflow limitation that is not fully reversible [7]. This unified definition arises from the fact that the term COPD includes a spectrum of diseases inducing chronic bronchitis and emphysema, and many intermediate states that are difficult to classify. The cell types thought to play the predominant roles in COPD are $CD8^+$ T cells, macrophages and neutrophils, with some of their migration receptors and ligands being reported to be increased in COPD [8,9]. Despite the fact that macrophages accumulate in the alveoli and bronchioli of smokers and COPD patients, and show a positive association between their numbers and the degree of disease, their role is still debated [9]. High levels of neutrophils are also found in the airways of smokers and patients with COPD. In animal models, neutrophil elastase is able to reproduce many of the features of this syndrome [8]. Although neutrophils seem to be important in COPD, studies demonstrating that neutrophil numbers are inversely related to the severity of emphysema indicate that other cell types contribute to the pathology of this disease [9].

Classic models of COPD include elastase instillation to the lungs and cigarette smoke exposure. Modern models of COPD include gene-targeting techniques applied in the context of classic models [10]. Airway wall changes appear to be reversible (once the irritating agent is removed) and much milder in animals than in humans, where the changes are progressive and largely irreversible [11]. Nevertheless, in alveolar spaces, inflammatory cell recruitment and airspace enlargement in response to cigarette smoke are comparable with that observed in humans [10].

Idiopathic pulmonary fibrosis

Idiopathic pulmonary fibrosis (IPF) is characterized by progressive breathing difficulty and worsening of pulmonary function [12]. IPF is a fatal disease, with only 30% of patients surviving 5 years after diagnosis. Until now, there is no treatment that can modify the natural course and usual terminal outcome of this disease. The prevalence of this disease increases with age, with an incidence of 250 cases per 100,000 in the elderly [13]. Pathophysiologically, IPF is caused by dysregulated tissue and vascular remodelling with fibroproliferation and deposition of extracellular matrix. Although the presence of eosinophils, mast cells and fibroblasts, and increased amounts of interleukin (IL)-4 and IL-13 is reported [12], there is no clear understanding of the mechanisms of this disease. Recently, Phillips *et al.* [14] demonstrated that fibrocytes, a circulating population of cells with leukocyte and mesenchymal markers, could have a role in IPF, because they migrate towards fibrotic lungs in an animal model of the disease.

IPF appears to share several clinical and experimental features with pulmonary fibrosis induced by chemical agents. Many animal models exist, including the instillation of fibrogenic agents, bleomycin, inorganic particles fluorescein isothiocyanate (FITC), and exposure to thoracic irradiation [15]. Although most of these agents can replicate the major structural and biochemical abnormalities of the disease, there are serious drawbacks with these models. The insidious progress of human pulmonary fibrosis is almost impossible to reproduce accurately. Also, removal of the inducing agent in some models allows regression of the induced fibrotic changes, which does not occur in human lung fibrosis. However, bleomycin and FITC instillation do show histological characteristics that resemble lesions in human disease [16], indicating that these disease models portray similar pathophysiological features as IPF.

The mechanisms of leukocyte migration as therapeutic targets

In this section, we will introduce the different steps involved in the migration of a leukocyte to a site of inflammation, before reviewing the recent literature on targeting adhesion molecules and chemoattractants in the context of lung disease.

The multistep paradigm of cell migration

The ability of a cell to migrate directionally to chemical cues plays a key role in embryonic development and in many homeostatic processes, such as wound healing and the immune response. Inappropriate cell migration during embryogenesis results in congenital defects, whereas the permanence of chemoattractant signals, and thus the continued migration of inflammatory cells, is a key factor of chronic inflammatory diseases like asthma, COPD and IPF [17].

Leukocyte migration requires interaction with the endothelial lining of blood vessels within inflamed tissue and organs. Selectins are a family of membrane glycoproteins that have a lectin-like domain that binds to proteins containing specific oligosaccharide groups expressed on the endothelium and leukocytes. The selectin family comprises E-selectin (endothelial), P-selectin (platelet and endothelial) and L-selectin (leukocyte). Selectin binding initiates tethering and rolling of the leukocyte on the endothelial surface [18].

When the leukocyte is rolling on the endothelium, its receptors engage with chemoattractants leading to increased affinity of leukocyte integrins. This leads to leukocyte adhesion to the endothelium through the interaction of integrins with their cognate endothelial-cell adhesion molecules [18]. Integrins are heterodimeric proteins, consisting of an α and a β chain. Different α and β subunits can associate, resulting in 24 members of this family, each containing a distinct pattern of subunits, and therefore ligand selectivity [18]. Leukocyte adhesion is followed by a process called transendothelial migration, which encompasses the extravasation of the leukocyte from the blood vessel into the inflamed tissue [17]. Finally, the leukocyte migrates towards the source of the chemoattractant – the site of inflammation. This is called chemotaxis. (Figure 2)

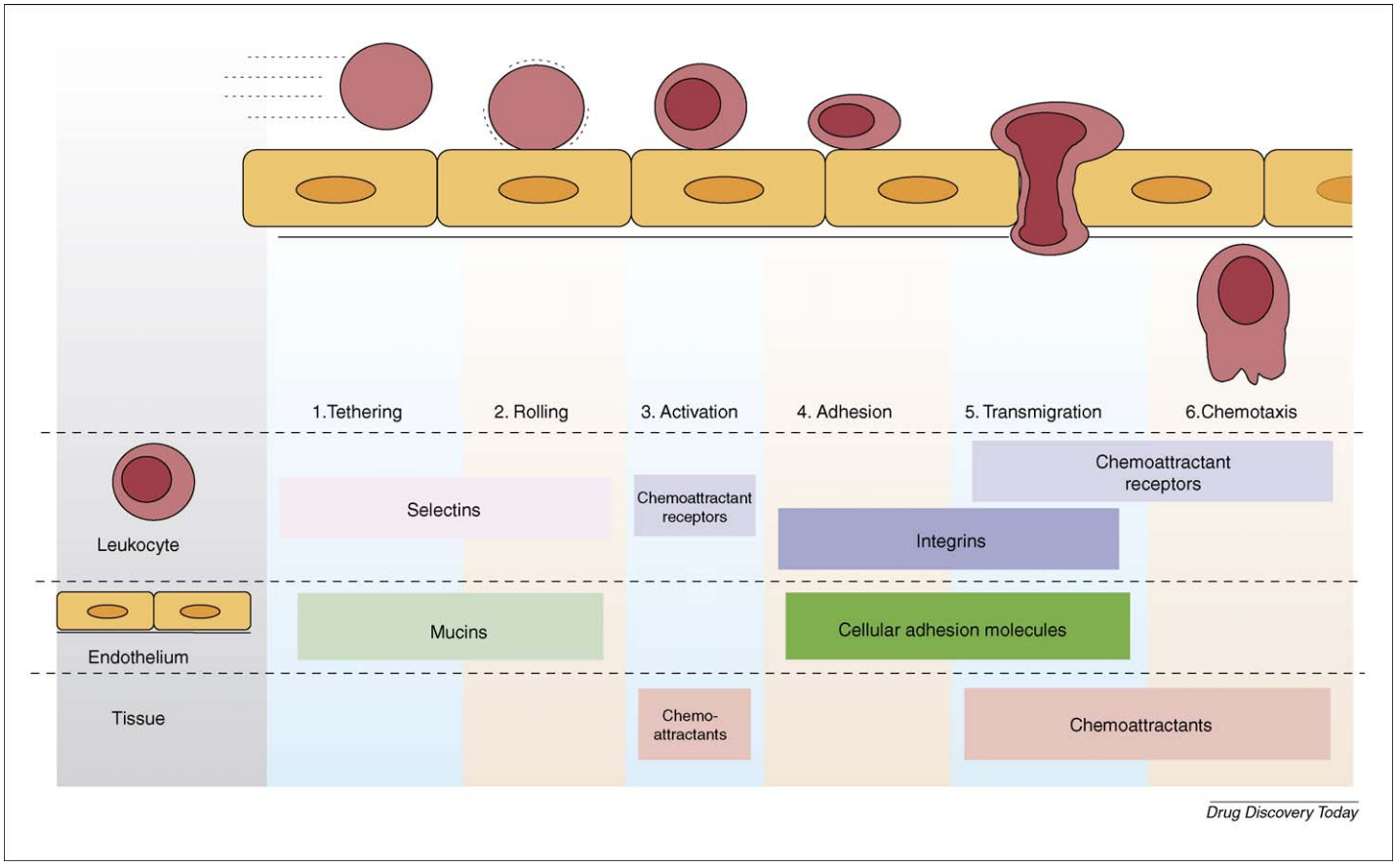

FIGURE 2

Leukocyte chemotaxis. *Tethering and Rolling.* Leukocyte selectins interact with the endothelial cell surface sialylated carbohydrate moieties in mucin-like proteins, making the leukocyte tether and then role along the endothelium. *Activation and Adhesion*. Leukocyte receptors bind chemoattractants secreted locally in an inflamed tissue, activating the leukocyte. This signals a conformational change in the leukocyte integrins, increasing their affinity for the cellular adhesion molecules of the endothelium. *Transmigration*. The leukocyte migrates through the vessel wall into the inflamed tissues. *Chemotaxis*. The leukocyte migrates through a chemoattractant gradient, towards its source.

The different steps involved in leukocyte accumulation in the lung present several possible therapeutic targets. Targeting any of them could potentially diminish recruitment of inflammatory cells to the site of injury and, ultimately, the clinical manifestations associated with these pathologies.

Targeting adhesion molecules

There has been considerable effort to exploit the importance of adhesion in leukocyte migration in the design of new therapeutics for inflammatory diseases. Because cellular adhesion molecule interaction is crucial for exocytosis, cytokine production and respiratory burst, antagonism of these molecules could be therapeutically active for reasons other than inhibition of leukocyte migration [18]. Recent reviews have focused on these molecules within the background of inflammatory disease [19], as well as asthma and COPD specifically [18].

Unfortunately, with a few exceptions, the outcome of clinical trials with adhesion-molecule blockers has been variable and largely disappointing. Since March 2005, the FDA has taken the precautionary measure of placing a clinical hold on investigational new drugs in the α4 integrin antagonist class being tested on human subjects. The reason for the clinical hold had been the uncertainty surrounding the cause of two reports of progressive multifocal leukoencephalopathy in patients taking natalizumab (Tysabri®), a drug targeting multiple sclerosis marketed by Biogen and Elan Pharmaceuticals. Since then, the FDA has lifted the clinical hold, allowing resumption of dosing in open-label extension studies in patients who received the drug in the original trials; however, marketing of the drug for widespread use has been delayed [20]. This has implications not only for the future of this particular drug, but also for adhesion molecule antagonists targeting other inflammatory diseases, such as asthma or COPD. Nevertheless, the concept of pharmacological interference of leukocyte adhesion remains a popular avenue for development of novel immunosuppressants as well as anti-inflammatory drugs.

Targeting chemoattractants and their receptors in airway disease

Chemoattractants vary greatly, from the superfamily of chemokines, which are small, inducible, secreted cytokines, to lipid chemoattractants, such as leukotriene B_4, prostaglandin D_2 and sphingosine-1-phosphate. Although structurally diverse, all these chemoattractants signal through G-protein-coupled receptors (GPCRs) [21]. These receptors are popular drug targets because they are very accessible to small molecule drug design and/or monoclonal antibodies, as outlined in the following sections. The first part of this section will review chemokines and their receptors, with lipid chemoattractants described afterwards.

Chemokines and their receptors

Chemokines are a large family of small molecular weight proteins that primarily function as chemoattractants to direct leukocyte cell migration, although they also influence immune and non-immune cell survival and effector responses [22]. Many chemokines have important roles in haematopoiesis, lymphocyte development, and promoting the organization and function of secondary lymphoid organ in mature organisms [23]. Structurally, chemokines are classified into four families based on a conserved cystein motif as follows: C, CC, CXC and CX3C. Chemokines are remarkably diverse, both in terms of individual proteins and their production by different cells and tissues. Chemokine receptors are classified into CR, CCR, CXCR and CX3CR families, determined by the class of their ligand [22].

A summary of the effects of targeting studies on chemoattractant receptors and ligands in respiratory disease models can be seen in Table 2 and Table 3. Table 4 profiles the chemoattractant antagonists currently under development.

CCR2

Exaggerated macrophage migration is a prominent feature in COPD and IPF. The CCR2 ligand CCL2 is implicated as a crucial mediator of macrophage trafficking because $CCR2^{-/-}$ mice have defective macrophage migration [24]. CCL2 and CCR2 mRNA has been found to be increased in patients with COPD [25]. Furthermore, patients with COPD expressed significantly higher levels of CCL2 in induced sputum than control subjects [26].

In IPF models, CCL2 levels in bronchoalveolar (BAL) fluid peak by day 1 and persist until day 7 after FITC administration [27]. Furthermore $CCR2^{-/-}$ mice displayed fewer macrophages in their BAL fluid, and significantly decreased lung remodelling and fibrosis in bleomycin and FITC animal models [27]. The same group showed that fibrocytes are recruited to the alveolar space in response to fibrotic injury in a CCR2-dependent manner [28]. In $CCR2^{-/-}$ mice, lung fibrocytes were diminished after FITC administration, and reconstitution of the $CCR2^{+/+}$ status using $CCR2^{+/+}$ bone marrow cells restored fibrocyte recruitment and fibrotic susceptibility in these animals [29], indicating a predominant role for CCR2 in fibrocyte recruitment in IPF.

CCR3

CCR3 ligands – CCL11, CCL24 and CCL26 – induce migration of human eosinophils. In asthmatic patients, CCL11 is increased in the early phases of allergen-induced eosinophil recruitment, whereas CCL24 and CCL26 are increased in the late phase of bronchial eosinophilia (48h after allergen challenge) [30]. CCL11 is also reported as a potent chemotactic factor for T-helper (Th) 2 cells, inducing adhesion and aggregation of these T lymphocytes through upregulation of adhesion molecule [31]. The targeted disruption of the CCL11 gene in mice led to a delayed infiltration of eosinophils to the airway in an OVA model of asthma [32]. Because the expression of CCR3 has also been found to be elevated in animal models of asthma [33] and in airway smooth muscle cells from asthmatic patients [34], the targeting of

TABLE 2

Chemoattractant receptors as targets in respiratory disease animal models[a]

Target receptor	Disease model	Research tool	Targeting impact	Refs
CCR2	IPF	Gene disruption	Diminished fibrocyte recruitment and fibrotic susceptibility	[27,29]
CCR3	Asthma	Gene disruption	Reduced eosinophil recruitment; increased intraepithelial mast cells. No protection against disease model.	[35]
	IPF	Neutralizing antibodies	Reduced pulmonary fibrosis, decreased eosinophilia and neutrophilia.	[36]
CCR4	Asthma	Neutralizing antibodies	No reduction in leukocyte recruitment. No protection against disease model.	[38]
	Asthma	Gene disruption	No protection against disease model.	[39]
	Asthma	Gene disruption	Lower AHR, inflammation and cytokine levels 30 days post-challenge, no improvement in chronic airway remodelling	[40]
CCR8	Asthma	Gene disruption	Reduced pulmonary eosinophilia and Th2 responses.	[51]
	Asthma	Gene disruption	No protection against disease model.	[52]
CXCR1 and CXCR2	Acute lung injury	Antagonist	Reduction in airway neutrophilia.	[60]
CXCR4	Asthma	Neutralizing antibodies	Reduced lune eosinophilia and AHR.	[70]
	Asthma	Antagonist	Reduced leukocyte recruitment to the lungs and airway eosinophilia. Diminished AHR.	[71]
BLT1	Asthma	Antagonist	Suppression of AHR in a primate model of asthma.	[76]
	Asthma	Gene disruption	Decreased number of T cells in BAL. Effect lost after 3 challenges. No difference in recruitment of leukocytes to the lung parenchyma.	[73]
	Asthma	Gene disruption	Reduced AHR, goblet cell hyperplasia and IL-13 production.	[77–79]
CRTH2	Asthma	Gene disruption	Enhanced eosinophil and macrophage recruitment to the lung. Increased levels of IL-5. No protection against disease model.	[87]
S1PR	Asthma	Inverse agonist	Reduced leukocyte recruitment in airways and airway inflammation. Inhibited AHR and goblet cell metaplasia.	[97]

[a] Abbreviations: AHR, airway hyperresponsiveness; BAL, bronchoalveola; IL, interleukin; IPF, idiopathic pulmonary fibrosis.

TABLE 3

Chemoattractants as targets in the context of respiratory disease animal models[a]

Target Chemoattractant	Disease model	Research tool	Targeting impact	Refs
CCL11	Asthma	Gene disruption	Reduced eosinophil recruitment 18h post-challenge. Similar recruitment at 48h. No protection against disease model.	[32]
	IPF	Gene disruption	Decreased eosinophilia and neutrophilia. Reduced lung fibrosis	[36]
	IPF	Gene overexpression	Increased leukocyte recruitment. Augmentated pulmonary fibrosis	[36]
CCL17	Asthma	Neutralizing antibodies	Reduced eosinophilia, infiltration of $CD4^+$ T cells, Th2 cytokine levels and degree of AHR	[43]
	IPF	Neutralizing antibodies	Decreased leukocyte recruitment. Reduced pulmonary fibrosis.	[46]
CCL22	Asthma	Neutralizing antibodies	Reduction in leukocyte recruitment to the lungs and airway hyper-responsiveness. No effect in leukocyte migration to airway lumen.	[44]
	IPF	Neutralizing antibodies	No protection against disease model.	[46]
TCA-3[b] (CCL1)	Asthma	Neutralizing antibodies	No protection against disease model.	[52]
CXCL9	Asthma	Exogenous chemoattractant	Reduced eosinophil recruitment to the airway. Reduced AHR.	[65,66]
	Asthma	Neutralizing antibodies	Increased eosinophil recruitment to the airway. Increased AHR.	[65,66]
CXCL12	IPF	Neutralizing antibodies	Reduced fibrocyte migration to the lung. Attenuated, but not abrogated, pulmonary fibrosis.	[14]
PGD_2	Asthma	Exogenous chemoattractant	Increased eosinophil recruitment, Th2 cytokine levels and AHR.	[84]

[a] Abbreviations: AHR, airway hyperresponsiveness; IPF, idiopathic pulmonary fibrosis.
[b] Murine ligand.

TABLE 4

Chemoattractant antagonists currently under development

Target	Antagonist - *manufacturer*	Development stage	ClinicalTrials.gov Identifier[a] or Refs
CCL11	CAT-213 – *Cambridge Antibody Technology*	Preclinical	[129]
CXCL8	Monoclonal neutralizing antibody – *Abgenix*	Clinical trial phase II *(COPD)*	[59]
CCR1 and CCR3	UCB35625 – *Banyu Pharm*	Preclinical	[130]
	Met-RANTES - *GSK*	Preclinical	[131]
CCR2	JNJ-27553292 – *Johnson and Johnson*	Preclinical	[132]
CCR3	SB-297006 – *GSK*	Preclinical	[133]
	SB-328437 – *GSK*	Preclinical	[133]
	YM-355179 - *Yamanouchi*	Preclinical	[134]
CCR4	Compound 12 - *Bristol-Myers Squibb*	Preclinical	[135]
CXCR1 and CXCR2	CXCL8(3–74)K11R/G31P – *University of Saskatchewan*	Preclinical	[60]
	Repertaxin - *Dompé*	Clinical trial phase II *(primary graft dysfunction after lung transplantation)*	NCT00224406
	Reparixin - *Dompé*	Clinical trial phase II *(delayed graft function after kidney transplantation)*	NCT0024840
CXCR2	SB-656933 - *GSK*	Preclinical	[136]
CXCR4	AMD070 – *AnorMED*	Clinical trial phase I *(HIV)*	NCT00063804
	SP01A – *Samaritan Pharmaceuticals*	Clinical trial phase II *(HIV)*	NCT00299897
	AMD3100 – *AnorMED*	Clinical trial phase III *(stem cell transplantation in multiple myeloma)*	NCT00248417
BLT1	CP-105696 - *Pfizer*	Clinical trial phase I *(healthy subjects)*	[137]
	LY293111 – *Lilly*	Clinical trial phase I *(cancer – advanced solid tumors)*	NCT00006375
	BIIL-284 – Boehringer Ingelheim	Clinical trial phase II *(cystic fibrosis)*	NCT00060801
CRTH2	Ramatroban (Bay u 3405) - *Bayer*	Clinical trial phase II *(moderate atopic asthma)*	NCT00311051

[a] http://www.clinicaltrials.gov is a website service of the U.S. National Institutes of Health (developed by the National Library of Medicine). The identifier given is a unique code through which clinical trials which are yet to be published can be identified.

the CCR3 receptor appears to be more reasonable than targeting just one of its ligands. In a model of asthma, $CCR3^{-/-}$ mice displayed significantly lower eosinophil recruitment to the lung after allergen challenge, with the majority of the eosinophils being arrested within the blood vessel. Unexpectedly, intraepithelial mast cells were increased in the trachea in these knockout mice. These mice presented no protection against an OVA model of asthma [35], suggesting that CCR3, although central in many ways to inflammation during an allergic response, might not be as suitable a drug target for asthma as predicted.

In the bleomycin model of IPF, pulmonary expression of CCL11 and CCR3 (in eosinophils and neutrophils), was markedly increased [36]. Mice that were either deficient in CCL11 or treated with neutralizing anti-CCR3 antibodies presented reduced pulmonary fibrosis, with decreased eosinophilia and neutrophilia. The same study indicated that mice overexpressing CCL11 presented increased pulmonary pathology [36]. These findings suggest a key role for the CCR3–CCL11 axis within the pathophysiology of pulmonary fibrosis.

CCR4

CCR4 and its ligands have perhaps been most widely studied within the context of asthma [33]. In asthmatics, $CCR4^{+}$ T cells are recruited to the lung in response to allergen challenge [37]. Blockade of CCR4 with a specific antibody caused only minor changes in the number of Th2 cells in the BAL fluid of allergen-challenged guinea pigs and failed to inhibit the recruitment of inflammatory leukocytes to the lung [38]. $CCR4^{-/-}$ mice have yielded conflicting evidence. CCR4 deletion has been shown to have no effect on an OVA model of asthma on mice [39]. By contrast, Schuh *et al.* [40] stated that at day 30 after challenge with *Aspergillus* conidia, knockout mice exhibited significantly lower total serum IgE, airway hyper-responsiveness, airway inflammation and Th2 cytokine levels compared with the wild-type controls. Curiously, on days 3 and 7 after challenge, these same knockout mice had significantly higher levels of all of these factors. Accordingly, the chronic airway remodelling seen in this disease model was unchanged in $CCR4^{-/-}$ animals. Despite the conflicting results obtained in knockout animal studies, CCR4 antagonists are now reportedly in clinical trials for asthma [2]. Although antagonizing CCR4 seems to be ineffective in animal models, previous studies have shown that its ligands CCL17 and CCL22 are increased in the airway epithelial cells of asthmatic patients, aiding the recruitment of inflammatory cells to the airway [41,42]. Specific monoclonal antibodies against CCL17 in mice attenuated an OVA model of asthma, diminished the degree of AHR, reduced the infiltration of $CD4^{+}$ cells, decreased Th2 cytokine levels and eosinophil-chemotactic chemokine expression in the lung [43]. Neutralizing antibodies against CCL22 in a rat asthma model did not affect the number of cells recruited to the airway lumen, but did decrease the amount of cells recruited to the lungs and, more importantly, reduced AHR [44].

With regard to COPD, Ritter *et al.* [45] have recently demonstrated that the expression of CCL17 and CCL22 is increased in cigarette-smoke-induced pulmonary inflammation models, although CCR4 expression was unchanged [45]. It is thought that the targets for these chemokines within this context are airway epithelial cells that constitutively express CCR4.

Recent reports have shown an increased expression of CCL22 and CCL17, and of their receptor CCR4, in murine and rat IPF models [46,47]. Additionally, the use of neutralizing antibodies to CCL17, but not CCL22 led to a significant reduction in pulmonary fibrosis [46]. In patients, there was an increased expression of CCR4 on BAL $CD4^{+}$ T cells [48] and an overexpression of CCL22 but not CCL17 [47].

CCR8

The chemokine receptor CCR8 is selectively expressed in Th2 cells under *in vitro* and *in vivo* stimulation conditions [49], and is therefore of crucial importance in asthma. Thymus-derived chemotactic agent 3 (TCA-3), the mouse ligand for CCR8, was found to be elevated in inflamed lung tissues in a mouse model of asthma [50]. Furthermore, $CCR8^{+}$ T cells are increased in human airways upon allergen challenge, but only very low levels of CCL1 (its only known human ligand) were detectable in BAL and in serum of asthma patients [41,42]. A recent study in two different mouse models of asthma showed that CCR8 deficient mice have reduced pulmonary eosinophilia and Th2 responses [51]. By contrast, Chung *et al.* [52] report that in an OVA asthma mouse model, $CCR8^{-/-}$ mice presented no differences compared to wild type controls in the development of pulmonary eosinophilia and Th2 cytokine responses. Moreover, administration of neutralizing anti-TCA-3 antibodies during allergen sensitization and challenge failed to inhibit airway allergic inflammation.

CXCR1 and CXCR2

CXCR1 and CXCR2 are highly homologous but functionally different chemokine receptors; the promiscuous CXCR2 binds six different chemokines and CXCR1 preferentially binds to CXCL6 and CXCL8 with high affinity [53]. Moreover, significant numbers of patients with COPD and children with asthma showed CXCR1 polymorphisms [54]. CXCL8 is an important chemotactic and activating factor for neutrophils, one of the cell types thought to play a predominant role in COPD. The CXCL8-induced effects in neutrophils are predominantly mediated by CXCR1 [55]. Patients with COPD have been shown to express higher levels of CXCL8 than healthy subjects [56]. Furthermore, CXCR1 and CXCR2 mRNA are increased in bronchial biopsy specimens of COPD patients with an exacerbation of the disease [57].

The importance of CXCR2 ligands has recently been highlighted in COPD patients by an increased monocyte chemotaxis to CXCL1 and CXCL7 but not toward CXCL5 or CXCL8 [58]. CXCL1 has been found in higher concentrations in induced sputum but not in BAL fluid of COPD patients when compared with non-smokers and healthy smokers [26]. In 2004, Mahler *et al.* [59] used a monoclonal anti-CXCL8 antibody on 100 patients with stable COPD. Although a larger proportion of patients in the antibody group reported improvements in dyspnea compared with the placebo group, there were no significant differences observed for lung function, health status and adverse events between groups. A promising compound, the CXCR1 and CXCR2 antagonist CXCL8(3–74) K11R/G31P, has shown to abrogate airway neutrophilia in an acute respiratory distress syndrome (ARDS) animal model [60]. Although ARDS is very different from COPD, it shares neutrophilia as one of its key features, suggesting that this antagonist could prove to be useful in the context of COPD.

CXCR3

Although asthma is generally perceived as a Th2 disease, there is mounting evidence for involvement of mediators classically regarded as Th1 mediators. Indeed, polymorphisms of CXCR3, a Th1 mediator, have been associated with the risk of developing asthma [61]. The CXCR3 chemokine receptor expression is highly expressed on activated T cells, where it is known to have a role in intracellular calcium mobilization and chemotaxis [62]. It has recently been shown that CXCR3 was the most abundantly expressed chemokine receptor on human lung mast cells within the airway smooth muscle (ASM) of asthmatic patients. Moreover, mast cell migration was induced by asthmatic ASM cultures predominantly through CXCR3, although blocking this receptor did not completely inhibit chemotaxis [63]. Bronchial biopsies of ASM from asthma patients express increased CXCL10, one of the three known ligands for CXCR3, when compared with samples from healthy controls [63]. This is in agreement with a previous report of increased BAL CXCL10 after segmental allergen challenge in asthmatics [41]. Although the CXCL10–CXCR3 axis has proven to be important for mast cell migration in asthma, its activation does not induce degranulation or cytokine synthesis in these cells [64]. It is interesting to note that treatment of the murine lung with CXCL9, another ligand for CXCR3, induced dose-dependent abrogation of CCL11-, IL-13- and allergen-induced eosinophil recruitment, whereas neutralization with antibodies of CXCL9 increased eosinophil recruitment to the airway [65,66]. Hence, within a single disease state, the same chemokine receptor exerts heterogenous functions and outcomes can differ depending on the ligand.

In COPD, T cells in peripheral airways have increased expression of CXCR3 when compared with non-smoking, but not healthy smoking, controls. One of the ligands for the CXCR3 receptor, CXCL10, is elevated in COPD airways in comparison with smoking and non-smoking controls [67]. In a recent study, ex-smokers with no COPD and no evidence of emphysema were compared with ex-smokers with moderate to severe COPD and evidence of emphysema. CXCR3 and its ligands, CXCL9 and CXCL10, were all found to be upregulated in the COPD group [68].

CXCR4

The CXCL12 receptor, CXCR4, is upregulated in Th2 T lymphocytes [69], suggesting relevance to Th2-mediated diseases like asthma. Neutralizing antibodies to CXCR4 reduced lung eosinophilia and decreased AHR in an OVA model of asthma in mice [70]. More recent studies with AMD3100 (a specific CXCR4 antagonist) examined the role of this receptor in a cockroach allergen-induced mouse model of asthma. When AMD3100 was administered to sensitized mice, Th1-type cytokines were increased and Th2-type cytokines were reduced. Furthermore, this compound significantly reduced airway hyperreactivity, peribronchial and airway eosinophila, and the number of recruited leukocytes to the lung, when compared with controls [71]. The pharmacokinetics and safety of AMD3100 have been assessed in phase I clinical trials, and it was found to be well tolerated [72]. Currently, AMD3100 is in phase III clinical trials for stem cell transplantation for the treatment of multiple myeloma (see Table 4).

In a bleomycin-induced model of IPF, murine fibrocytes trafficked to the lung in a CXCR4-dependent manner. When neutralizing anti-CXCL12 antibodies were administered, fibrocyte migration to the lungs was reduced and pulmonary fibrosis was attenuated, but not abrogated [14]. This result is in contrast to the strong diminution observed in IPF models with CCR2-null mice.

Lipid chemoattractants and their receptors

There is growing appreciation that, during infection and inflammation, lipid chemoattractants, such as sphingosine-1–phosphate and eicosanoids, have a prominent role navigating distinct T-cell subsets at different stages of the immune response to their intended destinations. Well known lipid chemoattractants, like prostaglandins and leukotrienes (formed by the cyclooxygenase and the lipoxygensase pathways respectively), have long been known to induce chemotaxis of leukocytes, but new light has been shed on the importance of these mediators in the initial stages of leukocytes accumulation in allergic pulmonary inflammation [21]. Blocking these mediators would limit the subsequent clinical manifestations associated with leukocyte recruitment.

Leukotriene B4 receptor

Leukotriene (LT) B_4, was originally described as a chemoattractant for myeloid leukocytes. Since then, the LTB_4 receptor, BLT1, has been detected on neutrophils, eosinophils, monocytes and macrophages. High levels of BLT1 were found in murine $CD4^{+73}$ and $CD8^{+74}$ T cells that had been differentiated *in vitro* to effector phenotypes. $CD4^+$ T cells that were activated *in vitro* under non-polarizing (Th0), Th1-polarizing or Th2-polarizing conditions all had increased levels of BLT1 mRNA compared with naïve cells [73]. Marked induction of BLT1 expression has also been observed in $CD8^+$ T cells that are activated *in vitro*. Naïve $CD8^+$ T cells and $CD8^+$ T central memory cells expressed almost no BLT1, whereas BLT1 was upregulated in $CD8^+$ T effector (T_{EFF}) cells [74]. After finding that mouse $CD4^+$ and $CD8^+$ effector T cells express high levels of BLT1, LTB_4 was found be a potent inducer of chemotaxis and adhesion of these cells [73]. Effector T cell chemotactic responses to LTB_4 were comparable with responses to CXCL12, one of the most efficacious T cell chemokines.

It has been described that the number of $BLT1^+$ T cells is moderately increased in the airways of asymptomatic asthmatic patients [75]. In a primate model of asthma, the LTB_4 receptor antagonist CP-105696, suppressed AHR [76]. Subsequently, Tager *et al.* [73] showed that $BLT1^{-/-}$ mice exposed to one or two challenges with OVA had a decreased number of T cells in the BAL fluid compared with wild-type mice, highlighting the importance of this receptor in T cell recruitment to the airway. However, following three challenges with OVA, similar numbers of cells were present in both groups. Curiously, similar number of T cells was present in the lung parenchyma of BLT1-deficient and wild-type mice after one, two or three challenges. In a more recent study by Miyahara *et al.* [77], $CD8^{-/-}$ mice, which have been shown to develop significantly lower AHR, eosinophil inflammation and IL-13 levels in BAL fluid in an asthma model, received either $BLT1^{+/+}$ or $BLT1^{-/-}$ $CD8^+$ T cells. Administration of $BLT1^{+/+}$ $CD8^+$ T cells restored AHR, eosinophilic inflammation, IL-13 levels and T_{EFF} cells in the lung. Mice that received $BLT1^{-/-}$ $CD8^+$ T cells were not reconstituted. It was also shown that $BLT1^{-/-}$ mice developed significantly lower AHR to methacholine, lower goblet cell hyperplasia in the airways, and decreased IL-13 production both *in vivo*

and *in vitro* [78]. As observed in the report by Tager *et al.*, the total number of T cells were the same in the experimental and control groups. However, Miyahara *et al.* found that the numbers of IL-13-producing antigen-specific effector $CD8^+$ or $CD4^+$ T cells that migrated to the lung were significantly lower in the absence of BLT1 expression. Similar results have been obtained in $BLT1^{-/-}$ mice using an OVA induced asthma model [79]. Although these were promising results, it is disappointing to note that that administration of an LTB_4 receptor antagonist, LY293111, to a small group of asthmatic patients did not positively affect their response to allergen challenge [80]. Nevertheless, the targeting of this receptor remains an attractive option to many pharmaceutical companies because compounds targeting the CysLT1 receptor (the receptor for the other members of the leukotriene family, LTC_4, LTD_4 and LTE_4), like montelukast, pranlukast and zafirlukast, have reached the market with sales of more than US$1 billion. The CysLT1 receptor is not believed to be directly involved in chemotaxis of inflammatory cells, but rather mainly to participate in the acute bronchoconstiction induced by different trigger factors in asthma [81].

Chemoattractant receptor-homologous molecule expressed in Th2 cells

Two different receptors have been identified for prostaglandin (PG) D_2 – DP1 and CRTH2 (also called DP2). CRTH2 is its receptor for migration; hence our focus lies with it. However, there is interesting information regarding the DP1 receptor, which is expressed in many cell types and which apparently mediates the upregulation of the CRTH2 receptor in T cells [82]. Mice with a null mutation of the DP1 receptor, showed reduced lung eosinophilia and Th2 cytokine levels [83]. Its possible roles in respiratory diseases are discussed extensively in recent reviews [21,84], but its role within pulmonary pathology remains controversial.

The arachidonic acid metabolite PGD_2 acts as a chemoattractant for many different cell types through the CRTH2 receptor [85]. CRTH2 is expressed in Th2 cells, eosinophils, basophils and parenchymal cells of the digestive system, heart, thymus, spinal cord and brain [21]. In T cells, this receptor is only expressed in Th2 cells, being absent both in Th1 and naïve T cells. In an OVA model of asthma, PGD_2-treatment increased airway eosinophilia, Th2 cytokine production and AHR compared with control animals [84]. Similar results were found in mice overexpressing prostaglandin D synthase [86]. However, it was recently shown, using the same model of asthma, that $CRTH2^{-/-}$ mice had enhanced eosinophil and macrophage recruitment to the lung. The authors also found IL-5 production increased in the knockout animals, which could be an explanation for the effects seen in these mice [87]. In 1996, ramatroban (BAY-u3405), a compound thought to be a specific thromboxane A2 receptor antagonist, attenuated AHR in a small clinical study involving twelve adult asthmatics [88]. Since then, allergic rhinitis and asthma clinical trails have ensued with promising results. Only until recently was the activity of this compound as a CRTH2 receptor antagonist discovered, helping to define the role played by CRTH2 in PGD2-mediated airway inflammation [89]. Ramatroban has opened up a whole realm of possibilities in the development and application of selective, second and third generation CRTH2 antagonists.

Sphingosine 1-phosphate receptors

In 2001, it was reported that sphingosine 1-phosphate (S1P) levels in the airways of asthmatics are higher after segmental antigen challenge. It was also demonstrated that S1P promoted contraction, cell growth and inflammatory cytokine production by human airway smooth muscle cells *in vitro* [90]. S1P is a potent sphingolipid phosphorylated product and the natural ligand for a family of GPCRs, called S1P receptors, of which five receptors are known to date (designated S1P receptor type 1–5 or $S1PR_{1-5}$). The different receptors for S1P, their regulation and their expression profile on different immune cells have been recently reviewed [91]. Interestingly, S1P has been shown to be capable of activating other receptors, in particular CCR3 [92]. Human eosinophils constitutively express $S1PR_1$, and, to a lesser extent, $SIPR_2$ and $SIPR_3$. After stimulation *in vitro* with S1P, the mRNA of all three receptors is upregulated – as are CCR3 and CCL5 mRNA (CCL5 is a known ligand for CCR3). An anti-CCR3 antibody reverses around 50% of S1P-induced eosinophil chemotaxis, implying that S1P signals via CCR3 and S1PRs [92]. Another report has shown that, in mast cells, the cross-linking of the high affinity receptor for IgE activates sphingosine kinase, leading to the generation and secretion of S1P. In turn, S1P activates its receptors on mast cells, to mediate migration towards antigen and degranulation, demonstrating an important role for this chemoattractant within allergic diseases [93]. It was recently demonstrated that S1P inhibited migration induced by platelet-derived growth factor and tumour necrosis factor α induced CCL5 production in cultured human bronchial smooth muscle cells [94]. These results suggest that the functions of S1P in allergic scenarios are complex and heterogenous, showing the capacity to generate migration (e.g. mast cells) as well as to attenuate cell migration (bronchial smooth muscle cells).

FTY720 was synthesized as part of an effort to minimize the toxic properties of a structurally related and highly potent immunosuppressive agent called myriocin [95]. Studies with FTY720 for transplantation and autoimmunity revealed that it causes a rapid loss of lymphocytes from the blood (lymphopenia). Curiously, FTY720 is a substrate for kinases that phosphorylate sphingosine. FTY720-phosphate is structurally similar to S1P and acts as a functional antagonist for the S1PRs [96]. In an OVA model of asthma, FTY270 suppressed the accumulation of inflammatory cells (eosinophils, neutrophils and T cells) in the BAL fluid and in bronchial tissue, blocked AHR to inhaled methacholine, reduced airway inflammation and inhibited goblet-cell hyperplasia [97]. Because of its potent immunosuppressive abilities, it is difficult to assess clearly the mechanisms of action of this compound in animal models of respiratory diseases and, thus, the possible benefit of using such a wide range acting compound for the treatment of diseases like asthma.

Targeting the signalling molecules involved in leukocyte migration

An alternative strategy to targeting chemoattractants and their receptors to disrupt pathogenic cellular recruitment to the airway is to target the biochemical signals that effect cell migration. In the last section, the signalling molecules most commonly associated downstream of the chemoattractant-receptor interaction will be studied, highlighting their role in inflammatory disease.

For a cell to migrate to a chemoattractant source it must be polarized, meaning that the molecular processes at the front (leading edge) differ from those at the back (uropod) [98]. Establishing and maintaining cell polarity in response to extracellular stimuli appears to be mediated by a set of interlinked positive-feedback loops involving phosphatidylinositol 3-kinases (PI3Ks), protein kinase C (PKC), Rho family GTPases, integrins, microtubules and vesicular transport [98] (Figure 3). The relative contribution of the various signals depends on the cell type and the specific stimulus. These intracellular signals result in reorganization of the cytoskeleton and cell adhesion causing the cells to send out pseudopodia and crawl up the chemoattractant gradient. PI3K-dependent signalling events have previously been demonstrated in several cell systems to contribute to several aspects of the migratory machinery including gradient sensing, signal amplification, actin reorganization and hence cell motility [98].

Phosphatidylinositol 3-kinases

A large body of evidence now supports the view that the temporal and spatial regulation of the phosphoinositide lipid products of class 1 phosphatidylinositol 3-kinases (PI3Ks) (Box 1) plays a crucial and early role in both gradient sensing and determining internal polarity in *Dictyostelium*, as well as more complex mammalian cells [99,100]. Chemokines, LTB_4 and PGD_2 are all known to stimulate PI3K activation [101]. Although it is usually assumed that the receptors for these chemoattractants are linked with the p110γ PI3K isoform, several chemokine receptors can also activate other isoforms implicated in cell migration [99,100]. The most conclusive lines of evidence that PI3K contributes to the leukocyte navigation mechanisms are derived from the use of PI3K inhibitors and studies of mice deficient in the PI3K p110γ catalytic isoform (PI3Kγ). For example, use of PI3K$\gamma^{-/-}$ mice has revealed that the directional movement of cells involved in mounting an immune response to a pathogen or foreign body (most notably neutrophils and macrophages) is severely impaired in the absence of PI3Kγ, both *in vitro* and *in vivo* [102]. The movement of neutrophils into sites of inflammation is central to the pathology of several disease conditions including COPD and ARDS. Indeed, a central role for PI3Kγ has been suggested for neutrophil recruitment and activation in a chemokine instillation model of airway inflammation, as well as intraperitoneal LPS model of lung injury in PI3K$\gamma^{-/-}$ mice [103,104]. Recent evidence indicates the effectiveness of PI3Kγ inhibitors in several models of chronic inflammatory diseases, primarily owing to inhibition of neutrophils and to $CD4^+$ T cells migration [102]. These inhibitors also abrogate recruitment of other leukocytes, particularly neutrophils and macrophages, in other models of chronic inflammation [105].

PI3K activation seems to be a signalling event shared by most chemokine receptors expressed on T cells, yet paradoxically activation of PI3K by chemokines can be a dispensable signal for directional migration of T cells and eosinophils [99]. However, many reports have used pharmacological tools in *in vitro* assays of migration, which might not accurately reflect physiological settings or events involved. Significant progress has been made in

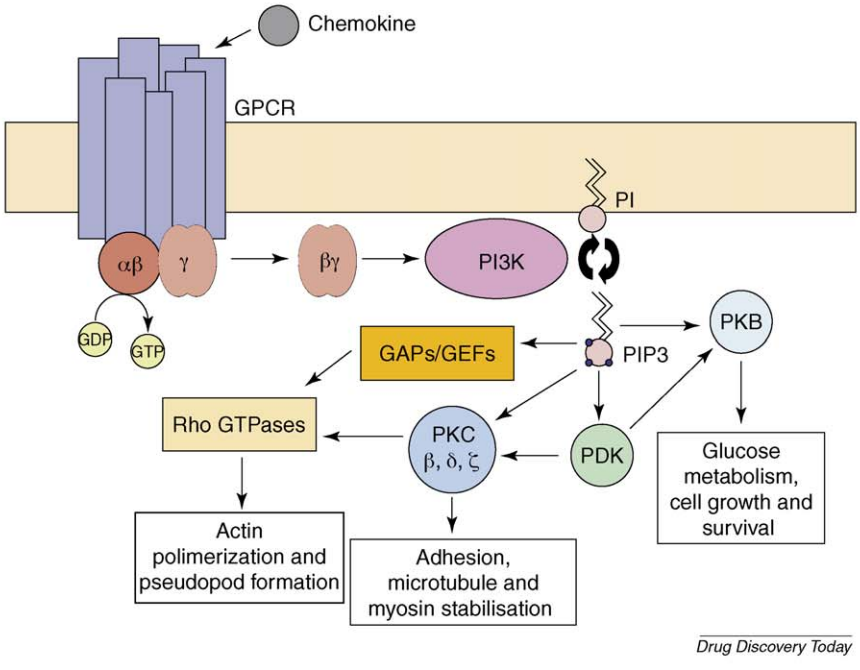

FIGURE 3

Establishing and maintaining cell polarity in response to extracellular stimuli. Chemokine coupling to G-protein-coupled receptor (GPCR) causes the liberation of the βγ subunit from the G protein complex. The βγ subunit then induces the recruitment of PI3K to the cell membrane, where it comes in contact with its membrane bound substrate, phosphatidylinositol (PI). After a series of phosphorylation steps, phosphatidylinositol (3,4,5)-trisphosphate (PIP3) is formed. This second messenger activates a series of downstream proteins, like protein kinase B (PKB), PIP3-dependent protein kinase (PDK), protein kinase C (PKC), guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). The interaction of these molecules, leads to the activation of Rho GTPases, which mediate actin polymerisation and pseudopod formation – the basic mechanisms of chemotaxis.

BOX 1

The class I and II PI3K families

The major products of class I phosphoinositide 3–kinases (PI3Ks) are phosphatidylinositol 3,4,5-trisphosphate [PtdIns(3,4,5)P_3] and its metabolite phosphatidylinositol 3,4-bisphosphate [PtdIns(3,4)P_2]. The levels of PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3 in cells are usually low in resting conditions, but rise sharply after cell stimulation. These lipids then interact with protein effectors via lipid binding domains, such as pleckstrin and phox homology domains. The effects of PtdIns(3,4,5)P_3 are counteracted by the lipid phosphatases phosphatase and tensin homologue deleted on chromosome 10 (PTEN) and src-homology 2-containing inositol 5′phosphatase (SHIP), which convert this lipid to PtdIns(4,5)P_2 and PtdIns(3,4)P_2 respectively. In some leukocytes (e.g. neutrophils), PtdIns(3,4,5)P_3 is located at the leading edge of a migrating cell responding to a chemotactic agent, through the localized action of PI3Ks that reside at the leading edge and the positioning of PTEN at the cell margins and rear [99,106].

Class I PI3Ks consists of two subgroups. The class IA PI3K consists of three catalytic isoforms (p110α, p110β and p110δ), which interact with a regulatory p85 protein that mediates protein–protein interactions. A distinct lipid kinase termed PI3Kγ or (p110γ) is coupled to a p101 regulatory protein and is activated by G protein βγ subunits from G-protein-coupled receptors (GPCRs). Nevertheless, GPCRs, such as receptors for chemokines, are also able to activate the p85–p110 heterodimeric PI3Ks. Expression of p110δ and PI3Kγ is largely (but not exclusively) restricted to leukocytes [99,106].

The class II PI3Ks are structurally distinct and are thought to use only PtdIns and PtdIns(4)*P* as substrates. Mammalian class II PI3Ks predominantly include the ubiquitously expressed PI3KC2α and PI3KC2β. Chemokine receptors and integrins stimulate class II PI3K activity [100].

unravelling the confusion concerning the role of PI3K in leukocyte migration. The *in vitro* migration of PI3Kγ$^{-/-}$ CD4$^+$ and CD8$^+$ T cells to CCL19 and CXCL12 and CCL21 is significantly decreased compared with cells from wild-type mice. By contrast, T cell responses were largely unaffected by PI3K p110δ catalytic isoform deficiency [106]. Hence, in settings where T cell migration required PI3K activation, the p110γ isoform appears to be the predominant isoform required. This correlates with recent observations that p110γ selective inhibitors reduce numbers of CD4$^+$ memory T cells in animal models of systemic lupus [107]. Inhibition of p110δ, using the selective inhibitor IC87114 in a murine model of asthma, resulted in decreased levels of Th2 cytokines, AHR and of migration of leukocytes to the lung [108]. The ability of p110δ to function downstream of chemokines receptors in lymphocyte chemotactic responses is consistent with the finding that a broad spectrum loss-of-function mutant that disrupts all class 1A catalytic isoforms reduced chemotactic responses of leukemic T cells to CXCL12 [99,100]. In short, these studies highlight that individual lymphoid chemokine receptors have differing dependence on PI3K-dependent signals for achieving ordered migration. Hence, a 'one-fits-all' concept of targeting PI3Kγ in airway diseases is probably unwise and the involvement of different chemokines, PI3K signalling pathways and different cell types in distinct types of airway disease must be taken in to consideration.

The class II PI3Ks are structurally distinct members of the PI3K family that have received relatively little attention compared with their famous class I cousins (Box 1). Recent reports have described an important role for class II PI3KC2β and its primary product, phosphatidylinositol 3-phosphate, in regulating cell adhesion, actin reorganization and migration, particularly with regard lysophosphatidic-acid-dependent cell migration and wound healing in nonimmune cell systems [109,110]. This work has important implications for understanding of lymphocyte migration in response to protein and lipid chemoattractants. Indeed, class II PI3K isoforms are activated in leukemic T cell lines by several chemokines as well as in monocytic cell lines by CCL2–CCR2[100] although it is unclear how GPCRs couple to class II PI3Ks.

Protein kinase C

Phospholipase C (PLC) activation, calcium mobilization and activation of PKC isoforms have been proposed as regulators of chemokine-mediated cell adhesion and migration [99,100]. However, studies with mice deficient in PLCβ1 and β3 suggest that the PLC pathway is not required for chemotaxis in neutrophils, although the role of this pathway in T lymphocytes or monocytes was not thoroughly investigated [102]. In this regard, PKCβ1 and PKCδ associate with the microtubules in the uropod (the trailing extension) of migrating T cells [111]. The use of antisense oligonucleotides to specifically reduce PKCβ expression has provided evidence that this isoform is required for monocyte chemotaxis in response to CCL2 [112]. In T-cell models, where CCR4-mediated migration can occur independently of PI3K, pharmacological tools have indicated that PKCδ is required for chemotactic responses to the CCR4 ligands CCL17 and CCL22 [113]. The atypical calcium and DAG-independent ζ PKC isoform has been shown to be essential for neutrophil CXCL8-mediated chemotaxis [100] as well CXCL12-mediated migration and development of human CD34$^+$ progenitor cells [114], where PKCζ appears to be downstream of PI3K. It is not well understood how PKC isoforms regulate cell motility and migration although it is likely that they regulate changes in integrin affinity and lateral mobility [115] and/or exert effects on actin reorganization [116] and phosphorylation of myosin light chain [117].

Several PKC isoforms have been knocked out in mice, including the β, δ and ζ isoforms. No major effects on airway disease have been reported in these mice, possibly because of redundancy in function between individual isoforms [118]. However, in eosinophils of asthmatic patients, there is an increase after allergen exposure in the expression level of PKCζ and of its translocation to the membrane, which relates to the kinase function [119]. Recently it was shown in an OVA model of asthma that mice lacking PKCθ had low levels of Th2 cytokines, reduced eosinophil migration to the airways and lung parenchyma, and lower AHR than control animals [120]. Together, these observations indicate that it might be prudent to examine existing PKC-isoform null mice in closer detail with regard to airway disease and consider gene targeting of multiple PKC isoforms.

Mitogen-activated protein kinases

Other signalling molecules, like the mitogen-activated protein kinase (MAPK) family, have also been implicated in chemotactic signalling mechanisms. Inhibitors of the MAPK (or extracellular signal regulated kinase; ERK)–MAPK kinase (or MAPK/ERK kinase; MEK) pathway were only able to partially attenuate CXCL12–CXCR4- and CCL17–CCR4-mediated lymphocyte chemotaxis

[121]. Furthermore, Ashida *et al.* [122] elucidated that *in vitro*, CCL2–CCR2-mediated chemotaxis was inhibited by using a p38 MAPK inhibitor, but not by a MEK inhibitor. In neutrophils, CXCL1 migratory responses have also demonstrated roles for ERK1/2 [123], while CXCL8 have shown to require p38 and c-Jun amino-terminal kinase (JNK) MAPK signalling but not ERK [124]. Because CXCL1 and CXCL8 are both ligands for CXCR1 and CXCR2, the difference in the signalling could reflect the preference of each chemokine for a particular receptor, and hence a different signalling pathway downstream of each receptor.

When migrating to a site of infection in the presence of multiple chemoattractants, leukocytes must prioritize favouring end-target chemoattractants [formyl-Met-Leu-Phe (fMLP) and C5a] over intermediary tissue derived chemoattractants (CXCL8 and LTB_4). An interesting *in vitro* study highlighted PI3K versus p38 MAPK signalling in migrating neutrophils when comparing chemokine stimulation with fMLP [125]. This study revealed that neutrophils predominantly migrated toward fMLP and C5a via p38 MAPK whereas IL-8 and LTB4-mediated migration was PI3K dependent. When neutrophils were faced with competing gradients of end target and intermediary chemoattractants, PI3K activation was significantly reduced and the cells migrated towards end target chemoattractants.

Within the context of animal models, a p38 inhibitor, SB239063, markedly reduced antigen-induced airway eosinophilia in mice and guinea pigs [126]. Furthermore, inhalation of p38 antisense oligonucleotides in a mouse asthma model caused a significant reduction in lung tissue eosinophilia, as well as decreases in Th2 cytokine levels, AHR and airway mucus hypersecretion [127]. Meanwhile, in allergen-sensitized rats, the JNK inhibitor SP600125 attenuated bronchial infiltration with eosinophils and T lymphocytes, as well as ASM proliferation [128].

Concluding Remarks

Predicting the outcome of targeting specific components in the process of leukocyte navigation presents a significant therapeutic challenge. This area of research and its potential market are of such importance that every leading pharmaceutical company maintains a significant interest in developing agents that regulate leukocyte recruitment as potential anti-inflammatory drugs.

The specificity offered by targeting individual adhesion molecules or chemoattractant receptors is attractive, but their benefits might be limited by the ligand and receptor redundancy seen in most diseases. Nevertheless, the predominately positive results observed in initial trials with the integrin antagonist natalazimab indicate that such therapies can be effective, and such approaches could be applicable in a range of immune-mediated inflammatory disease. Many other compounds are now in clinical trials, including antagonists for lipid mediator and chemokine receptors.

The ubiquity of intracellular signalling molecules suggests that off-target effects are likely to be a significant hurdle for agents inhibiting such pathways. The identification of specific signalling pathways and the role of individual kinase isoforms are necessary to circumvent such limitations and limit potential adverse effects. Compounds targeting these molecules are still in the early stages of development and there are few examples, in other disease areas, of signalling inhibitors that have been developed beyond preclinical studies.

References

1 Masoli, M. *et al.* (2004) The global burden of asthma: executive summary of the GINA Dissemination Committee report. *Allergy* 59, 469–478
2 Heijink, I.H. and Van Oosterhout, A.J. (2005) Targeting T cells for asthma. *Curr. Opin. Pharmacol.* 5, 227–231
3 Szelenyi, I. (2000) Animal models of bronchial asthma. *Inflamm. Res.* 49, 639–654
4 Brewer, J.P. *et al.* (1999) Genetic variability in pulmonary physiological, cellular, and antibody responses to antigen in mice. *Am. J. Respir. Crit. Care Med.* 160, 1150–1156
5 De Bie, J.J. *et al.* (1998) Modulation of airway hyperresponsiveness and eosinophilia by selective histamine and 5-HT receptor antagonists in a mouse model of allergic asthma. *Br. J. Pharmacol.* 124, 857–864
6 Kumar, R.K. and Foster, P.S. (2002) Modeling allergic asthma in mice: pitfalls and opportunities. *Am. J. Respir. Cell Mol. Biol.* 27, 267–272
7 Celli, B.R. and MacNee, W. (2004) Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper. *Eur. Respir. J.* 23, 932–946
8 Stockley, R.A. (2002) Neutrophils and the pathogenesis of COPD. *Chest* 121, 151S–155S
9 Tetley, T.D. (2002) Macrophages and the pathogenesis of COPD. *Chest* 121, 156S–159S
10 Shapiro, S.D. (2000) Animal models for COPD. *Chest* 117, 223S–227S
11 Wright, J.L. and Churg, A. (2002) Animal models of cigarette smoke-induced COPD. *Chest* 122, 301S–306S
12 Gross, T.J. and Hunninghake, G.W. (2001) Idiopathic pulmonary fibrosis. *N. Engl. J. Med.* 345, 517–525
13 Perez, A. *et al.* (2003) The prognosis of idiopathic pulmonary fibrosis. *Am. J. Respir. Cell Mol. Biol.* 29, S19–S26
14 Phillips, R.J. *et al.* (2004) Circulating fibrocytes traffic to the lungs in response to CXCL12 and mediate fibrosis. *J. Clin. Invest.* 114, 438–446
15 Chua, F. *et al.* (2005) Pulmonary fibrosis: searching for model answers. *Am. J. Respir. Cell Mol. Biol.* 33, 9–13
16 Usuki, J. and Fukuda, Y. (1995) Evolution of three patterns of intra-alveolar fibrosis produced by bleomycin in rats. *Pathol. Int.* 45, 552–564
17 Wu, D. (2005) Signaling mechanisms for regulation of chemotaxis. *Cell Res.* 15, 52–56
18 Vanderslice, P. *et al.* (2004) Development of cell adhesion molecule antagonists as therapeutics for asthma and COPD. *Pulm. Pharmacol. Ther.* 17, 1–10
19 Ulbrich, H. *et al.* (2003) Leukocyte and endothelial cell adhesion molecules as targets for therapeutic interventions in inflammatory disease. *Trends Pharmacol. Sci.* 24, 640–647
20 (2006) The trials and tribulations of Tysabri. *Lancet Neurol.* 5, 373
21 Luster, A.D. and Tager, A.M. (2004) T-cell trafficking in asthma: lipid mediators grease the way. *Nat. Rev. Immunol.* 4, 711–724
22 Zlotnik, A. and Yoshie, O. (2000) Chemokines: a new classification system and their role in immunity. *Immunity* 12, 121–127
23 Cyster, J.G. (2005) Chemokines, sphingosine-1-phosphate, and cell migration in secondary lymphoid organs. *Annu. Rev. Immunol.* 23, 127–159
24 Warmington, K.S. *et al.* (1999) Effect of C-C chemokine receptor 2 (CCR2) knockout on type-2 (schistosomal antigen-elicited) pulmonary granuloma formation: analysis of cellular recruitment and cytokine responses. *Am. J. Pathol.* 154, 1407–1416
25 de Boer, W.I. *et al.* (2000) Monocyte chemoattractant protein 1, interleukin 8, and chronic airways inflammation in COPD. *J. Pathol.* 190, 619–626
26 Traves, S.L. *et al.* (2002) Increased levels of the chemokines GROalpha and MCP-1 in sputum samples from patients with COPD. *Thorax* 57, 590–595
27 Moore, B.B. *et al.* (2001) Protection from pulmonary fibrosis in the absence of CCR2 signaling. *J. Immunol.* 167, 4368–4377
28 Moore, B.B. *et al.* (2005) CCR2-mediated recruitment of fibrocytes to the alveolar space after fibrotic injury. *Am. J. Pathol.* 166, 675–684
29 Okuma, T. *et al.* (2004) C-C chemokine receptor 2 (CCR2) deficiency improves bleomycin-induced pulmonary fibrosis by attenuation of both macrophage infiltration and production of macrophage-derived matrix metalloproteinases. *J. Pathol.* 204, 594–604

30 Ravensberg, A.J. *et al.* (2005) Eotaxin-2 and eotaxin-3 expression is associated with persistent eosinophilic bronchial inflammation in patients with asthma after allergen challenge. *J. Allergy Clin. Immunol.* 115, 779–785
31 Jinquan, T. *et al.* (1999) Eotaxin activates T cells to chemotaxis and adhesion only if induced to express CCR3 by IL-2 together with IL-4. *J. Immunol.* 162, 4285–4292
32 Rothenberg, M.E. *et al.* (1997) Targeted disruption of the chemokine eotaxin partially reduces antigen-induced tissue eosinophilia. *J. Exp. Med.* 185, 785–790
33 Lukacs, N.W. (2001) Role of chemokines in the pathogenesis of asthma. *Nat. Rev. Immunol.* 1, 108–116
34 Joubert, P. *et al.* (2005) CCR3 expression and function in asthmatic airway smooth muscle cells. *J. Immunol.* 175, 2702–2708
35 Humbles, A.A. *et al.* (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. U. S. A.* 99, 1479–1484
36 Huaux, F. *et al.* (2005) Role of Eotaxin-1 (CCL11) and CC Chemokine Receptor 3 (CCR3) in Bleomycin-Induced Lung Injury and Fibrosis. *Am. J. Pathol.* 167, 1485–1496
37 Kallinich, T. *et al.* (2005) Chemokine-receptor expression on T cells in lung compartments of challenged asthmatic patients. *Clin. Exp. Allergy* 35, 26–33
38 Conroy, D.M. *et al.* (2003) CCR4 blockade does not inhibit allergic airways inflammation. *J. Leukoc. Biol.* 74, 558–563
39 Chvatchko, Y. *et al.* (2000) A key role for CC chemokine receptor 4 in lipopolysaccharide-induced endotoxic shock. *J. Exp. Med.* 191, 1755–1764
40 Schuh, J.M. *et al.* (2002) Airway hyperresponsiveness, but not airway remodeling, is attenuated during chronic pulmonary allergic responses to Aspergillus in $CCR4^{-/-}$ mice. *FASEB J.* 16, 1313–1315
41 Bochner, B.S. *et al.* (2003) Release of both CCR4-active and CXCR3-active chemokines during human allergic pulmonary late-phase reactions. *J. Allergy Clin. Immunol.* 112, 930–934
42 Panina-Bordignon, P. *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
43 Kawasaki, S. *et al.* (2001) Intervention of thymus and activation-regulated chemokine attenuates the development of allergic airway inflammation and hyperresponsiveness in mice. *J. Immunol.* 166, 2055–2062
44 Gonzalo, J.A. *et al.* (1999) Mouse monocyte-derived chemokine is involved in airway hyperreactivity and lung inflammation. *J. Immunol.* 163, 403–411
45 Ritter, M. *et al.* (2005) Elevated expression of TARC (CCL17) and MDC (CCL22) in models of cigarette smoke-induced pulmonary inflammation. *Biochem. Biophys. Res. Commun.* 334, 254–262
46 Belperio, J.A. *et al.* (2004) The role of the Th2 CC chemokine ligand CCL17 in pulmonary fibrosis. *J. Immunol.* 173, 4692–4698
47 Inoue, T. *et al.* (2004) CCL22 and CCL17 in rat radiation pneumonitis and in human idiopathic pulmonary fibrosis. *Eur. Respir. J.* 24, 49–56
48 Pignatti, P. *et al.* (2005) Role of the Chemokine Receptors CXCR3 and CCR4 in Human Pulmonary Fibrosis. *Am. J. Respir. Crit. Care Med.* 173, 310–317
49 Zingoni, A. *et al.* (1998) The chemokine receptor CCR8 is preferentially expressed in Th2 but not Th1 cells. *J. Immunol.* 161, 547–551
50 Mathew, A. *et al.* (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
51 Chensue, S.W. *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
52 Chung, C.D. *et al.* (2003) CCR8 is not essential for the development of inflammation in a mouse model of allergic airway disease. *J. Immunol.* 170, 581–587
53 Holmes, W.E. *et al.* (1991) Structure and functional expression of a human interleukin-8 receptor. *Science* 253, 1278–1280
54 Stemmler, S. *et al.* (2005) Association of interleukin-8 receptor alpha polymorphisms with chronic obstructive pulmonary disease and asthma. *Genes Immun.* 6, 225–230
55 Green, S.P. *et al.* (1996) Interleukin-8 (IL-8), melanoma growth-stimulatory activity, and neutrophil-activating peptide selectively mediate priming of the neutrophil NADPH oxidase through the type A or type B IL-8 receptor. *J. Biol. Chem.* 271, 25400–25405
56 Pignatti, P. *et al.* (2005) Downmodulation of CXCL8/IL-8 receptors on neutrophils after recruitment in the airways. *J. Allergy Clin. Immunol.* 115, 88–94
57 Qiu, Y. *et al.* (2003) Biopsy neutrophilia, neutrophil chemokine and receptor gene expression in severe exacerbations of chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* 168, 968–975
58 Traves, S.L. *et al.* (2004) Specific CXC but not CC chemokines cause elevated monocyte migration in COPD: a role for CXCR2. *J. Leukoc. Biol.* 76, 441–450
59 Mahler, D.A. *et al.* (2004) Efficacy and safety of a monoclonal antibody recognizing interleukin-8 in COPD: a pilot study. *Chest* 126, 926–934
60 Gordon, J.R. *et al.* (2005) The combined CXCR1/CXCR2 antagonist CXCL8(3-74)K11R/G31P blocks neutrophil infiltration, pyrexia, and pulmonary vascular pathology in endotoxemic animals. *J. Leukoc. Biol.* 78, 1265–1272
61 Cheong, H.S. *et al.* (2005) CXCR3 polymorphisms associated with risk of asthma. *Biochem. Biophys. Res. Commun.* 334, 1219–1225
62 Loetscher, M. *et al.* (1996) Chemokine receptor specific for IP10 and Mig: structure, function, and expression in activated T-lymphocytes. *J. Exp. Med.* 184, 963–969
63 Brightling, C.E. *et al.* (2005) The CXCL10/CXCR3 axis mediates human lung mast cell migration to asthmatic airway smooth muscle. *Am. J. Respir. Crit. Care Med.* 171, 1103–1108
64 Brightling, C.E. *et al.* (2005) Differential expression of CCR3 and CXCR3 by human lung and bone marrow-derived mast cells: implications for tissue mast cell migration. *J. Leukoc. Biol.* 77, 759–766
65 Fulkerson, P.C. *et al.* (2004) Negative regulation of eosinophil recruitment to the lung by the chemokine monokine induced by IFN-gamma (Mig, CXCL9). *Proc. Natl. Acad. Sci. U. S. A.* 101, 1987–1992
66 Thomas, M.S. *et al.* (2004) Regulation of cockroach antigen-induced allergic airway hyperreactivity by the CXCR3 ligand CXCL9. *J. Immunol.* 173, 615–623
67 Saetta, M. *et al.* (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
68 Grumelli, S. *et al.* (2004) An immune basis for lung parenchymal destruction in chronic obstructive pulmonary disease and emphysema. *Plos. Med.* 1, e8
69 Jagodzinski, P.P. and Trzeciak, W.H. (2000) Differential expression of chemokine receptor CXCR4 in Th1 and Th2 subtypes of $CD4^+$ lymphocytes. *Folia Histochem. Cytobiol.* 38, 21–23
70 Gonzalo, J.A. *et al.* (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
71 Lukacs, N.W. *et al.* (2002) AMD3100, a CXCR4 antagonist, attenuates allergic lung inflammation and airway hyperreactivity. *Am. J. Pathol.* 160, 1353–1360
72 Hendrix, C.W. *et al.* (2004) Safety, pharmacokinetics, and antiviral activity of AMD3100, a selective CXCR4 receptor inhibitor, in HIV-1 infection. *J. Acquir. Immune Defic. Syndr.* 37, 1253–1262
73 Tager, A.M. *et al.* (2003) Leukotriene B4 receptor BLT1 mediates early effector T cell recruitment. *Nat. Immunol.* 4, 982–990
74 Goodarzi, K. *et al.* (2003) Leukotriene B4 and BLT1 control cytotoxic effector T cell recruitment to inflamed tissues. *Nat. Immunol.* 4, 965–973
75 Islam, S.A. *et al.* (2005) The leukotriene B4 lipid chemoattractant receptor BLT1 defines antigen-primed T-cells in humans. *Blood* 107, 444–453
76 Turner, C.R. *et al.* (1996) *In vitro* and *in vivo* effects of leukotriene B4 antagonism in a primate model of asthma. *J. Clin. Invest.* 97, 381–387
77 Miyahara, N. *et al.* (2005) Leukotriene B4 receptor-1 is essential for allergen-mediated recruitment of $CD8^+$ T cells and airway hyperresponsiveness. *J. Immunol.* 174, 4979–4984
78 Miyahara, N. *et al.* (2005) Requirement for the leukotriene B4 receptor-1 in allergen-induced airway hyperresponsiveness. *Am. J. Respir. Crit. Care Med.* 172, 161–167
79 Terawaki, K. *et al.* (2005) Absence of leukotriene B4 receptor 1 confers resistance to airway hyperresponsiveness and Th2-type immune responses. *J. Immunol.* 175, 4217–4225
80 Evans, D.J. *et al.* (1996) Effect of a leukotriene B4 receptor antagonist, LY293111, on allergen induced responses in asthma. *Thorax* 51, 1178–1184
81 Dahlen, S.E. (2006) Treatment of asthma with antileukotrienes: first line or last resort therapy? *Eur. J. Pharmacol.* 533, 40–56
82 Tanaka, K. *et al.* (2004) Effects of prostaglandin D2 on helper T cell functions. *Biochem. Biophys. Res. Commun.* 316, 1009–1014
83 Matsuoka, T. *et al.* (2000) Prostaglandin D2 as a mediator of allergic asthma. *Science* 287, 2013–2017
84 Spik, I. *et al.* (2005) Activation of the prostaglandin D2 receptor DP2/CRTH2 increases allergic inflammation in mouse. *J. Immunol.* 174, 3703–3708
85 Hirai, H. *et al.* (2001) Prostaglandin D2 selectively induces chemotaxis in T helper type 2 cells, eosinophils, and basophils via seven-transmembrane receptor CRTH2. *J. Exp. Med.* 193, 255–261
86 Fujitani, Y. *et al.* (2002) Pronounced eosinophilic lung inflammation and Th2 cytokine release in human lipocalin-type prostaglandin D synthase transgenic mice. *J. Immunol.* 168, 443–449
87 Chevalier, E. *et al.* (2005) Cutting edge: chemoattractant receptor-homologous molecule expressed on TH2 cells plays a restricting role on IL-5 production and eosinophil recruitment. *J. Immunol.* 175, 2056–2060

88 Aizawa, H. *et al.* (1996) BAY u3405, a thromboxane A2 antagonist, reduces bronchial hyperresponsiveness in asthmatics. *Chest* 109, 338–342
89 Shiraishi, Y. *et al.* (2005) Prostaglandin D2-induced eosinophilic airway inflammation is mediated by CRTH2 receptor. *J. Pharmacol. Exp. Ther.* 312, 954–960
90 Ammit, A.J. *et al.* (2001) Sphingosine 1-phosphate modulates human airway smooth muscle cell functions that promote inflammation and airway remodeling in asthma. *FASEB J.* 15, 1212–1214
91 Rosen, H. and Goetzl, E.J. (2005) Sphingosine 1-phosphate and its receptors: an autocrine and paracrine network. *Nat. Rev. Immunol.* 5, 560–570
92 Roviezzo, F. *et al.* (2004) Human eosinophil chemotaxis and selective *in vivo* recruitment by sphingosine 1-phosphate. *Proc. Natl. Acad. Sci. U. S. A.* 101, 11170–11175
93 Jolly, P.S. *et al.* (2004) Transactivation of sphingosine-1-phosphate receptors by FcepsilonRI triggering is required for normal mast cell degranulation and chemotaxis. *J. Exp. Med.* 199, 959–970
94 Kawata, T. *et al.* (2005) Sphingosine 1-phosphate inhibits migration and RANTES production in human bronchial smooth muscle cells. *Biochem. Biophys. Res. Commun.* 331, 640–647
95 Napoli, K.L. (2000) The FTY720 story. *Ther. Drug Monit.* 22, 47–51
96 LaMontagne, K. *et al.* (2006) Antagonism of sphingosine-1-phosphate receptors by FTY720 inhibits angiogenesis and tumor vascularization. *Cancer Res.* 66, 221–231
97 Sawicka, E. *et al.* (2003) Inhibition of Th1- and Th2-mediated airway inflammation by the sphingosine 1-phosphate receptor agonist FTY720. *J. Immunol.* 171, 6206–6214
98 Ridley, A.J. *et al.* (2003) Cell migration: integrating signals from front to back. *Science* 302, 1704–1709
99 Ward, S.G. (2004) Do phosphoinositide 3-kinases direct lymphocyte navigation? *Trends Immunol.* 25, 67–74
100 Ward, S.G. (2006) T lymphocytes on the move: chemokines, PI 3-kinase and beyond. *Trends Immunol.* 27, 80–87
101 Hata, A.N. *et al.* (2003) Expression and molecular pharmacology of the mouse CRTH2 receptor. *J. Pharmacol. Exp. Ther.* 306, 463–470
102 Li, Z. *et al.* (2000) Roles of PLC-beta2 and -beta3 and PI3Kgamma in chemoattractant-mediated signal transduction. *Science* 287, 1046–1049
103 Thomas, M.J. *et al.* (2005) Airway inflammation: chemokine-induced neutrophilia and the class I phosphoinositide 3-kinases. *Eur. J. Immunol.* 35, 1283–1291
104 Yum, H.K. *et al.* (2001) Involvement of phosphoinositide 3-kinases in neutrophil activation and the development of acute lung injury. *J. Immunol.* 167, 6601–6608
105 Camps, M. *et al.* (2005) Blockade of PI3Kgamma suppresses joint inflammation and damage in mouse models of rheumatoid arthritis. *Nat. Med.* 11, 936–943
106 Reif, K. *et al.* (2004) Cutting edge: differential roles for phosphoinositide 3-kinases, p110gamma and p110delta, in lymphocyte chemotaxis and homing. *J. Immunol.* 173, 2236–2240
107 Barber, D.F. *et al.* (2005) PI3Kgamma inhibition blocks glomerulonephritis and extends lifespan in a mouse model of systemic lupus. *Nat. Med.* 11, 933–935
108 Lee, K.S. *et al.* (2006) Inhibition of phosphoinositide 3-kinase delta attenuates allergic airway inflammation and hyperresponsiveness in murine asthma model. *FASEB J.* 20, 455–465
109 Maffucci, T. *et al.* (2005) Class II phosphoinositide 3-kinase defines a novel signaling pathway in cell migration. *J. Cell Biol.* 169, 789–799
110 Domin, J. *et al.* (2005) The class II phosphoinositide 3-kinase PI3K-C2beta regulates cell migration by a PtdIns3P dependent mechanism. *J. Cell. Physiol.* 205, 452–462
111 Volkov, Y. *et al.* (2001) Crucial importance of PKC-beta(I) in LFA-1-mediated locomotion of activated T cells. *Nat. Immunol.* 2, 508–514
112 Carnevale, K.A. and Cathcart, M.K. (2003) Protein kinase C beta is required for human monocyte chemotaxis to MCP-1. *J. Biol. Chem.* 278, 25317–25322
113 Cronshaw, D.G. *et al.* (2006) Evidence that phospholipase C-dependent, calcium-independent mechanisms are required for directional migration of T-lymphocytes in response to the CCR4 ligands CCL17 and CCL22. *J. Leukoc. Biol.* 79, 1360–1380
114 Petit, I. *et al.* (2005) Atypical PKC-zeta regulates SDF-1-mediated migration and development of human CD34+ progenitor cells. *J. Clin. Invest.* 115, 168–176
115 Giagulli, C. *et al.* (2004) RhoA and zeta PKC control distinct modalities of LFA-1 activation by chemokines: critical role of LFA-1 affinity triggering in lymphocyte *in vivo* homing. *Immunity* 20, 25–35
116 Hartwig, J.H. *et al.* (1992) MARCKS is an actin filament crosslinking protein regulated by protein kinase C and calcium-calmodulin. *Nature* 356, 618–622
117 Iwabu, A. *et al.* (2004) Epidermal growth factor induces fibroblast contractility and motility via a protein kinase C delta-dependent pathway. *J. Biol. Chem.* 279, 14551–14560
118 Tan, S.L. and Parker, P.J. (2003) Emerging and diverse roles of protein kinase C in immune cell signaling. *Biochem. J.* 376, 545–552
119 Evans, D.J. *et al.* (1999) Expression and activation of protein kinase C-zeta in eosinophils after allergen challenge. *Am. J. Physiol.* 277, L233–L239
120 Marsland, B.J. *et al.* (2004) Protein kinase C theta is critical for the development of *in vivo* T helper (Th)2 cell but not Th1 cell responses. *J. Exp. Med.* 200, 181–189
121 Moroi, Y. *et al.* (2004) Effects of MAPK inhibitors on CCR4-mediated chemotaxis against thymus and activation-regulated chemokine (TARC/CCL17). *J. Dermatol. Sci.* 36, 186–188
122 Ashida, N. *et al.* (2001) Distinct signaling pathways for MCP-1-dependent integrin activation and chemotaxis. *J. Biol. Chem.* 276, 16555–16560
123 Fuhler, G.M. *et al.* (2005) Impaired interleukin-8- and GRO{alpha}-induced phosphorylation of extracellular signal-regulated kinase result in decreased migration of neutrophils from patients with myelodysplasia. *J. Leukoc. Biol.* 77, 257–266
124 Liu-Bryan, R. *et al.* (2005) The CXCR1 tail mediates beta1 integrin-dependent cell migration via MAP kinase signaling. *Biochem. Biophys. Res. Commun.* 332, 117–125
125 Heit, B. *et al.* (2002) An intracellular signaling hierarchy determines direction of migration in opposing chemotactic gradients. *J. Cell Biol.* 159, 91–102
126 Underwood, D.C. *et al.* (2000) SB 239063, a potent p38 MAP kinase inhibitor, reduces inflammatory cytokine production, airways eosinophil infiltration, and persistence. *J. Pharmacol. Exp. Ther.* 293, 281–288
127 Duan, W. *et al.* (2005) Inhaled p38alpha mitogen-activated protein kinase antisense oligonucleotide attenuates asthma in mice. *Am. J. Respir. Crit. Care Med.* 171, 571–578
128 Eynott, P.R. *et al.* (2003) Allergen-induced inflammation and airway epithelial and smooth muscle cell proliferation: role of Jun N-terminal kinase. *Br. J. Pharmacol.* 140, 1373–1380
129 Dent, G. *et al.* (2004) Contribution of eotaxin-1 to eosinophil chemotactic activity of moderate and severe asthmatic sputum. *Am. J. Respir. Crit. Care Med.* 169, 1110–1117
130 Sabroe, I. *et al.* (2000) A small molecule antagonist of chemokine receptors CCR1 and CCR3. Potent inhibition of eosinophil function and CCR3-mediated HIV-1 entry. *J. Biol. Chem.* 275, 25985–25992
131 Elsner, J. *et al.* (1997) The CC chemokine antagonist Met-RANTES inhibits eosinophil effector functions through the chemokine receptors CCR1 and CCR3. *Eur. J. Immunol.* 27, 2892–2898
132 Kuppens, T. *et al.* (2006) Elucidation of the absolute configuration of JNJ-27553292, a CCR2 receptor antagonist, by vibrational circular dichroism analysis of two precursors. *Chirality* 18, 609–622
133 White, J.R. *et al.* (2000) Identification of potent, selective non-peptide CC chemokine receptor-3 antagonist that inhibits eotaxin-, eotaxin-2-, and monocyte chemotactic protein-4-induced eosinophil migration. *J. Biol. Chem.* 275, 36626–36631
134 Morokata, T. *et al.* (2006) A novel, selective, and orally available antagonist for CC chemokine receptor 3. *J. Pharmacol. Exp. Ther.* 317, 244–250
135 Purandare, A.V. *et al.* (2005) Identification of chemokine receptor CCR4 antagonist. *Bioorg. Med. Chem. Lett.* 15, 2669–2672
136 Nicholson, G.C. *et al.* (2006) A novel flow cytometric assay of human whole blood neutrophil and monocyte CD11b levels: Upregulation by chemokines is related to receptor expression, comparison with neutrophil shape change, and effects of a chemokine receptor (CXCR2) antagonist. *Pulm. Pharmacol. Ther.* (in press)
137 Liston, T.E. *et al.* (1998) Pharmacokinetics and pharmacodynamics of the leukotriene B4 receptor antagonist CP-105,696 in man following single oral administration. *Br. J. Clin. Pharmacol.* 45, 115–121