Topics in Medicinal Chemistry 14

Nuska Tschammer *Editor*

Chemokines

Chemokines and Their Receptors in Drug Discovery

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14
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Editor

Chemokines

Chemokines and Their Receptors in Drug Discovery

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Preface

Sophisticated homeostatic and inflammatory actions of our immune system are orchestrated by a myriad of different proteins and other signaling molecules. One of the crucial molecular components of the immune system is a complex network of small soluble proteins named chemokines and their G protein coupled receptors. The GPCR superfamily is the largest family of transmembrane receptors, which transmit signals from outside of the cell across the membrane to signaling pathways within the cell. A misbalance in the functions of chemokines and their receptors often leads to severe pathologies like autoimmune diseases (e.g., rheumatoid arthritis, psoriasis, and multiple sclerosis), asthma, and cancer. Furthermore, the chemokine receptors CCR5 and CXCR4 are also hijacked by HIV as co-receptors needed for the viral entry in the $CD4^+$ T cells. In accordance with the overall importance of chemokines and their receptors in pathologies, multiple pharmaceutical companies initiated screening campaigns dedicated to the development of chemokine receptor antagonists in a recent decade. After all, GPCRs are the site of action of about 30% current drugs, making the GPCR superfamily the largest and single most important family of drug targets. More than 40 antagonists of chemokine receptors entered the clinical trials that unfortunately largely failed. Only two candidates for the therapy of noninflammatory diseases progressed successfully on the market, one for the treatment of HIV (the CCR5 antagonist maraviroc, Selzentry®, Pfizer) and one for the hematopoietic stem cell transplantation in patients with lymphoma and multiple myeloma (the CXCR4 antagonist, perixafor, Mozobil®, Genzyme). The reasons for a tremendous failure of drug candidates in clinical trials were largely attributed to the redundancy of chemokine system, inappropriate target selection, suboptimal dosing regimen, off-target effects, and in some cases even poor drug-like properties of a drug candidate. Despite the higher-than-average failure rate, the quest for successful drug candidates, which would modulate the function of chemokine receptors, continues.

In this book the current development, opportunities, and challenges in the field of drug discovery related to chemokine receptors are presented and debated. As an example for the role of chemokines in autoimmunity and inflammation, their functions in the pathophysiology of asthma, multiple sclerosis, and rheumatoid arthritis are illustrated in the chapter "Chemokine Receptors in Allergy, Inflammation, and Infectious Disease", written by James Pease and Richard Horuk. The authors describe various strategies that pharmaceutical companies have come up

with to block the effect of chemokines in driving these disease processes, and how they have progressed in the clinic. Chapter "Role of 3D Structures in Understanding, Predicting, and Designing Molecular Interactions in the Chemokine Receptor Family", written by Irina Kufareva, Ruben Abagyan, and Tracy M. Handel, provides an excellent overview of pre- and post-structure efforts in understanding, predicting, and designing chemokine receptor interactions with small molecules and peptides, chemokines, and HIV gp120 proteins, as well as structure-guided insights regarding chemokine receptor dimerization and the impact of structures on rational molecular design initiatives. The efficient symbiosis of computational approaches with experimental structure determination is discussed in depth. The concept that GPCRs are natural allosteric proteins led to chapter "Allosteric Modulation of Chemokine Receptors", written by Arthur Christopoulos, Terry Kenakin, and myself, which discusses complex allosteric mechanisms by which the functions of chemokines and their receptors are fine-tuned and presents their impact on preclinical drug discovery. The opportunities and challenges of bench-to-clinic approaches are elucidated. Although allosteric modulation of chemokine receptors adds a level of complexity to analyses and approaches to drug discovery, it also introduces a tremendous capacity for pharmacologic control of this physiological system for therapeutic advantage. Chapter "Exploring the CXCR3 Chemokine Receptor with Small-Molecule Antagonists and Agonists", written by Rob Leurs and colleagues, illustrates on the example of the chemokine receptor CXCR3 nicely, how the combination of chemical, computational, and pharmacological tools and techniques increases our understanding of the molecular mechanisms by which small-molecule antagonists and agonists bind to the chemokine receptors compared to the relatively large chemokines. This knowledge potentially opens up novel therapeutic opportunities in the area of inflammation. In the last chapter " Selective and Dual Targeting of CCR2 and CCR5 Receptors: A Current Overview" Bernhard Wünsch and his colleagues present classical approaches in medicinal chemistry that fueled the development of antagonists for the chemokine receptors CCR2 and CCR5. These efforts led to the discovery of the CCR5 targeting drug that is used for the treatment of HIV-1 (maraviroc, Selzentry®, Pfizer). The reasons of failure of other promising clinical candidates are critically discussed.

I thank the authors for their valuable contributions to this volume. With their assistance this book provides profound insights into the failure-rich past, exciting present developments and promising future opportunities and challenges in the field of drug discovery dedicated to the manipulations of chemokine receptor network.

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Chemokine Receptors in Allergy, Inflammation, and Infectious Disease

James E. Pease and Richard Horuk

Abstract Chemokines play an important role in disease by virtue of their effects on immune cells. They mediate their biological effects by acting on G-protein-coupled receptors, which represent one of the most druggable classes of proteins. In this review we will examine the role of chemokines in autoimmunity and inflammation by concentrating on the part they play in the pathophysiology of several diseases including asthma, multiple sclerosis, and rheumatoid arthritis. We will describe the various strategies that pharmaceutical companies have come up with to block the effect of chemokines in driving these disease processes and how they have fared in the clinic. We will also briefly discuss the repurposing of chemokine receptor antagonists in new indications.

Keywords Allergy, Antagonists, Chemokine receptors, Chemokines, Infection, Inflammation

Contents

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1 Introduction

Chemokines belong to a small family of chemoattractant proteins that orchestrate the directed trafficking of immune cells; thus, they play an important role in host defense. Inappropriate activation of the immune response by chemokines can also lead to autoimmunity, giving rise to a number of devastating diseases that include asthma, multiple sclerosis, and rheumatoid arthritis. These and other autoimmune diseases pose an ever-increasing health burden on our society and affect millions of individuals each year. Consequently, pharmaceutical companies have poured billions of dollars into research and development to identify safe and effective drugs to treat these diseases. The chemokines, because of their central role in coordinating the immune system have provided an enticing target for pharmacological intervention. In this chapter we will describe some of the key chemokines that are believed to be responsible for the leukocyte recruitment and underlying pathology in asthma, multiple sclerosis, and rheumatoid arthritis. In addition, we will describe strategies directed at inhibiting specific chemokine receptors that have been identified as being important in driving disease processes. Finally, we will discuss the progress that chemokine receptor antagonists have made in the clinic and we will conclude by looking at potential new therapeutic uses for them.

2 The Role of Chemokines in Asthma

Asthma is a complex, multifactorial, heterogeneous disease, which has reached epidemic levels in the Western world, with over 5 million people in the UK alone receiving some type of asthma treatment. Asthma broadly describes a variety of patients with hyperactive airways, which when trigged by antigen results in compromised lung function. This so-called early phase asthmatic reaction is triggered by antigen crosslinking the high affinity IgE receptor on mast cells and results

in their degranulation and the release of preformed mediators such as histamine and newly synthesized lipid mediators such as the leukotriene LTB_4 and the prostaglandin PGD_2. These molecules exert a variety of actions on the airways including bronchoconstriction, increased microvascular permeability and increased mucus production, all of which contribute to the asthmatic phenotype of breathlessness [1]. The mediators also influence the recruitment of leukocytes to the allergic lung, either by directly serving as leukocyte chemoattractants themselves (LTB_4 and PGD_2) or directing the structural cells of the lung to produce chemokines. This chemokine production is highly dependent upon the interplay of structural and immune cells, notably dendritic cells and T_H2 cells and results in the late-phase reaction. This typically occurs several hours after the early phase reaction and is notable for the recruitment of a variety of leukocytes to the lung. Originally thought of as a chiefly eosinophilic disease, the identification of different subgroups or asthma phenotypes over recent years suggests that novel strategies to target the trafficking of disparate cell types may provide additional therapeutic benefit [2].

3 Chemokine-Driven Eosinophil Recruitment in Asthma

The recruitment of eosinophils to the respiratory system is considered a characteristic hallmark of asthma. As long ago as the late 1800s, Paul Ehrlich, who is credited with the discovery of the eosinophil, postulated that eosinophil recruitment to specific tissue sites required a stimulus that induced their "chemotactic irritability" [3]. A little over a century later, the group of Tim Williams at Imperial College London proved this hypothesis, with the discovery of a CC chemokine that was produced in the guinea pig lung following allergen challenge [4]. They named this chemokine "eotaxin," and the identification of the mouse orthologue and the eotaxin receptor, CCR3, quickly followed [5]. Subsequent identification of the additional CCR3 ligands, Eotaxin-2/CCL24, and Eotaxin-3/CCL26 suggested further means by which eosinophils could be recruited. In mouse models of allergic airways disease, CCL11 expression is induced following allergen challenge [6]. In humans, both CCL11 mRNA and CCL11 protein levels have been observed indirectly in the allergic lung tissue of both atopic asthmatics (individuals suffering from allergic conditions, e.g., hay fever or allergic dermatitis) and also that of non-atopic asthmatics [7]. Similarly, CCL11 levels have been reported to be elevated in the plasma of acute asthmatics compared with stable asthmatic patients [8]. Histamine release from degranulating mast cells can trigger the localized production of CCL11 by endothelial cells [9], as can the action of TNF-α and IL-4 on lung fibroblasts and human airway epithelial cells [10].

More recently, the role of the type 2 innate lymphoid cell in eosinophil homeostasis has begun to be appreciated. These are long-lived tissue resident cells and co-express IL-13 at sites of allergic inflammation, resulting in the expression of CCL11 and the recruitment of eosinophils [11]. At present, little is known about how these cells traffic to the lungs, but targeting this process may provide an alternative angle for intervention in asthma. Likewise, the specific roles of the

other two eotaxins, CCL24 and CCL26, in asthma pathogenesis are not fully appreciated. Mice differ from humans in lacking a functional orthologue of CCL26, although CCL11 and CCL24 both seem to be important for eosinophil recruitment to the allergic lung, with deletion of both chemokines needed for ablation recruitment in an ovalbumin sensitization model [12]. A recent study by Provost and coworkers suggested that CCL26 was a particularly potent in vitro recruiter of eosinophils from asthmatic individuals and that complete blockade was not achieved with a CCR3-specific antibody leading to the notion that an additional receptor for CCL26 may exist in humans [13]. Further work is needed to test this intriguing finding.

4 Targeting Eosinophil Chemokine Receptors

CCR3 is the principal chemokine receptor expressed by human eosinophil and was therefore an obvious target for eosinophil-directed drug development. Blockade of CCR3 by a specific monoclonal antibody by Heath and colleagues showed that the majority of eosinophil responses to CC chemokines could be inhibited by targeting of CCR3, establishing it as a key therapeutic target for the treatment of asthma [14]. A mouse CCR3-specific antibody was also developed by scientists at DNAX to validate CCR3 in vivo. The antibody, which was functional, had the unexpected property of depleting eosinophils from the circulation [15]. Other protein-based therapeutics aimed at the blockade of CCR3 on eosinophils were chemokine based such as Met-RANTES, a modified version of CCL5/RANTES with an N-terminal methionine extension which can bind but not activate CCR3 [16]. Likewise, a modified version of the chemokine CCL18 similarly extended at the N-terminus by a single methionine residue was shown to act as an antagonist [17].

The first description of a small-molecule antagonist of CCR3 came from one of our own groups, with the compound UCB 35625 shown to block CCR3 at low nanomolar concentrations (Table 1 and **1** Fig. 1) [25]. Intriguingly, despite blocking CCR3 in a number of different assays, UCB 35625 did not displace radiolabeled CCL11 from CCR3-transfectants in contrast to small-molecule antagonists of other chemokine receptors described at the time. This led to what was then a controversial hypothesis; that is, these compounds did not antagonize the chemokine-binding site directly, but instead altered the receptor conformation such that signaling could not take place. Since the compound also had significant activity at CCR1 (which shares excellent homology with CCR3 in the transmembrane helices), we postulated that the compound resided in the intrahelical bundle of either receptor, which was formally proven in subsequent studies [26, 27].

Since these initial studies, several different CCR3 antagonists have been described in the literature with typically low nanomolar affinity despite quite diverse chemical structures [18–20, 22–24, 28–30]. Some notable examples and their effects in vitro and in vivo are summarized in Table 1. However despite often demonstrating quite

Table 1 Preclinical CCR3 antagonists to treat asthma

Compound	In vitro activity	In vivo activity	References
A-122057 A-122058 (Abbott Laboratories)	Inhibition of CCL11 binding (IC_{50} values of 600 and 975 nM)	Reduction in CCL11-induced peritoneal eosinophilia in mice (10 mg/kg)	[18]
GW701897B (GlaxoSmithKline)	None published	Prevention of antigen-induced clustering of eosinophils along the vagus nerves and hyperresponsiveness to vagal stimulation following antigen inhalation in guinea pigs	[19]
14n (Schering-Plough)	Inhibitor of CCL11 binding ($K_i = 4$ nM) and chemotaxis ($IC_{50} = 160$ nM) of human eosinophils		[20]
LH31407 (Boehringer Mannheim)	Blockade of CCL11 binding to human eosinophils ($K_i = 14$ nM). Inhibition of CCL11-induced Ca^{2+} influx in human eosinophils ($IC_{50} = 11$ nM)	Diminished infiltration of eosinophils into the airway lumen at 30 mg/kg	[21]
YM-344031 (Yamanouchi Pharmaceutical Co.)	Inhibition of chemotaxis of human CCR3-expressing cells ($IC_{50} = 20$ nM)	Oral administration to macaques (1−10 mg/kg) significantly inhibited CCL11-induced eosinophil shape change in whole blood. Oral administration to mice (100 mg/kg) prevented both immediate- and late-phase allergic skin reactions	[22]
YM-355179 (Yamanouchi Pharmaceutical Co.)	Inhibition of intracellular Ca^{2+} influx, chemotaxis, and eosinophil degranulation (respective IC_{50} values of 8, 24, and 29 nM)	Oral administration (1 mg/kg) inhibited CCL11-induced shape change of eosinophils in macaques. Intravenous injection (1 mg/kg) also inhibited eosinophil infiltration into macaque airways following segmental bronchoprovocation with CCL11	[23]
Ki19003 (Gifu Pharmaceutical University)	Inhibition of CCL11-induced murine eosinophil migration ($IC_{50} = 200$ nM)	Blockade of eosinophil levels in mouse BALF at 3 or 10 mg/kg	[24]

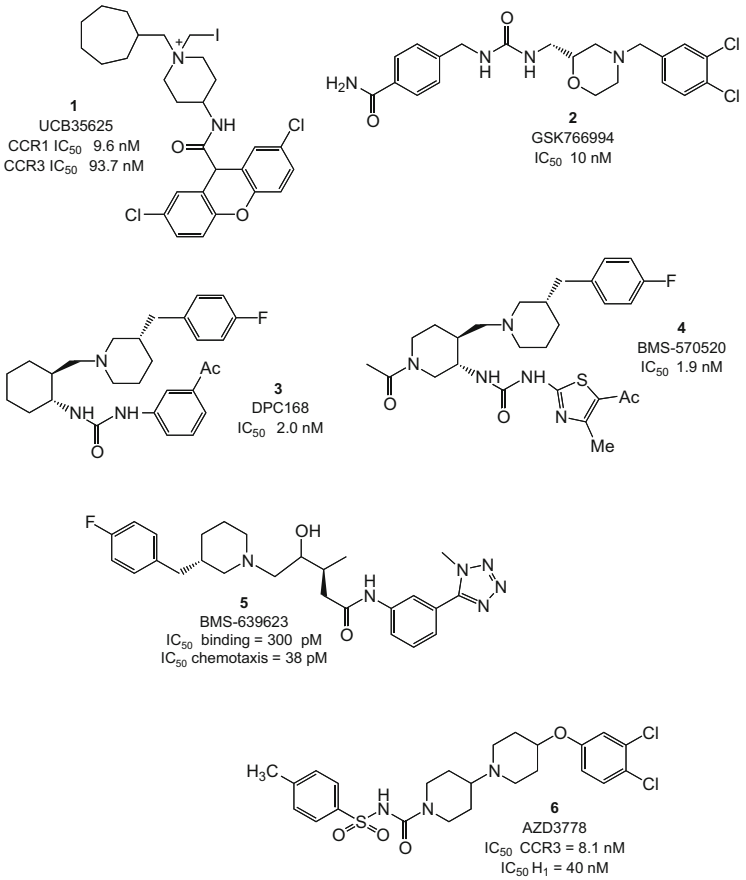


Fig. 1 CCR3 antagonists in asthma and allergic disease (unless otherwise noted all kinetic data are inhibition of receptor binding)

convincing in vitro and in vivo data, none have so far progressed beyond phase II clinical trials (Table 2).

GlaxoSmithKline (GSK) have been active in the search for CCR3 antagonists and have identified several clinical candidates (Table 2). Although very little published data on the potency of these compounds is available, the company has published a number of patents claiming various acyl and urea derivatives of 2-aminomethyl-4-benzylmorpholine [21]. One of the compounds GSK766994 (Table 2 and **2** Fig. 1) demonstrated excellent pharmacokinetics in preclinical studies [31] and also showed efficacy in a mouse model of age-related macular degeneration [32]. Although the drug showed no safety concerns, it failed to show efficacy in a phase II clinical trial for the treatment of allergic rhinitis [31]. Despite this setback the development of the antagonist was continued and it was tested in a phase II clinical trial for asthma in 53 patients [33]. Unfortunately, the compound

Table 2 Summary of clinical development of chemokine receptor antagonists to treat asthma, allergic rhinitis, multiple sclerosis, and rheumatoid arthritis

Receptor	Company	Compound	Affinity (nM)	Indication	Clinical phase	Status
CCR1	Schering AG (Berlex)	BX 471	1.0	MS, psoriasis, endometriosis	II	No efficacy
CCR1	Millennium	MLN 3701		MS, multiple myeloma	II	Not reported
CCR1	Millennium	MLN 3897	2.3	RA	II	No efficacy
CCR1	Pfizer	CP-481,715	64	RA	II	No efficacy
CCR1	GSK	CCX354	1.5	RA	II	Ongoing
CCR1	BMS	BMS-817399		RA	II	Ongoing
CCR2	Millennium	MLN 1202[a]		RA	II	No efficacy
				Atherosclerosis, MS	II	Ongoing
					II	Uncertain
CCR2	CCX	CCX915		MS	I	Terminated
CCR2	Merck	MK-0812	5.0	RA, MS	II	No efficacy
CCR2	Incyte	INCB8696		MS, lupus	I	Not reported
CCR2	Incyte	INCB3284	3.7	RA, type II diabetes	II	Not reported
CCR3	Pharmaxis	ASM8[b]		Asthma	II	Ongoing
CCR3	GSK	GSK766994	10.0	Asthma and allergic rhinitis	II	No efficacy
CCR3	GSK	GSK766904		Asthma	II	Ongoing
CCR3	GSK	GW824575		Asthma	I	Terminated
CCR3	DuPont	DPC168	2.0	Asthma	I	Terminated
CCR3	BMS	BMS-639623	0.3	Asthma	I	Ongoing
CCR3	Novartis	QAP-642		Allergic rhinitis	I	Terminated
CCR3	AstraZeneca	AZD3778	8.1	Allergic rhinitis	II	Not reported
CCR4	Amgen	KW-0761[a]/Mogamulizumab		Oncology	II	Ongoing
				Asthma	I	Ongoing
CCR4	GSK	GSK2239633	10.0	Asthma	I	Terminated
CCR5	Pfizer	UK-427,857 (maraviroc)	3.0	RA	II	No efficacy
				AIDS	Approved	Registered drug

(continued)

Table 2 (continued)

Receptor	Company	Compound	Affinity (nM)	Indication	Clinical phase	Status
CCR5	Schering-Plough	SCH-C	2.0	AIDS	I	Terminated
				RA	II	No efficacy
CCR5	GSK	GSK-873140 (aplaviroc)		AIDS	II	Terminated
CCR5	AstraZeneca	AZD5672	0.26	RA	II	No efficacy

[a]Neutralizing monoclonal antibodies

[b]Antisense oligonucleotide

BMS, Bristol–Myers Squibb, *GSK* GlaxoSmithKline, *CCX* ChemoCentryx, *MS* multiple sclerosis, *RA* rheumatoid arthritis

failed to meet its clinical end points ([34], and so its future development is uncertain.

Bristol–Myers Squibb (BMS) took a lead compound from DuPont-Merck, DPC168 (Table 2 and **3** Fig. 1), which was a potent CCR3 antagonist, but had cytochrome P450 and cardiovascular liabilities [30], as their starting point for the development of CCR3 antagonists. Extensive structural modifications allowed them to overcome the cytochrome P450 and cardiovascular liabilities with BMS-570520 (**4** Fig. 1) [35] which after further optimization gave rise to their clinical development compound BMS-639623 that was reported to be in phase I clinical development for asthma (Table 2 and **5** Fig. 1) [36].

AZD3778 is a novel low molecular weight dual CCR3 and histamine H_1 receptor antagonist developed by AstraZeneca. The compound has an IC_{50} of 8.1 nM for the inhibition of eotaxin binding to CCR3 and an IC_{50} of 40 nM for the inhibition of binding to the H_1 histamine receptor (Table 2 and **6** Fig. 1) [37]. A phase II clinical trial in patients with allergic rhinitis revealed that AZD3778 exerted moderately anti-eosinophilic and symptom-reducing effects thought to be through inhibition of CCR3 rather than through its effects on the histaminergic receptor [37]. Since the effects of the compound were only modest, no further development of has been reported.

Novartis had a CCR3 antagonist program and identified the compound QAP 642 (structure not disclosed) as the clinical lead, but have not disclosed any structural or potency data. A human clinical pharmacodynamic study reporting the effects of QAP642 on cutaneous eosinophil migration in the skin following subcutaneous injection of eotaxin in human volunteers has been reported [38]. At the highest dose the compound caused a modest increase in the QTc prolongation. The compound was able to inhibit eosinophil migration in this human pharmacodynamic study; however, it subsequently failed in clinical trials for asthma, and its development was discontinued [39].

A novel approach to inhibiting CCR3, ASM8, has been recently described by scientists at Pharmaxis (Table 2) [40]. ASM8 contains two modified phosphorothioate antisense oligonucleotides designed to inhibit allergic inflammation by downregulating human CCR3 and the common beta chain of the IL-3, IL-5, and GMCSF receptors. In a small clinical study with patients with mild asthma, the drug was safe and well tolerated. It attenuated the allergen-induced increase in target gene mRNA, allergen-induced sputum eosinophils, and the early and late asthmatic responses [40, 41]. It also reduced the number of CD34(+) CCR3(+) cells and CD34(+) IL-5Rα(+) cells and the proportion of CD34(+) cells expressing IL-5Rα. Currently ASM8 is being evaluated in larger phase II clinical trials for asthma.

5 Chemokine-Driven Lymphocyte Recruitment in Asthma

As mentioned previously, the recruitment of the T_H2 type lymphocyte in asthma is thought to be pivotal to asthma pathogenesis and strategies to block T_H2 cell recruitment are of great interest in asthma. T_H2 cells are notable for their expression of a number of chemokine receptors including CCR3, CCR4, and CCR8

[42–44]. CCR4 is activated by the chemokines CCL22 and CCL17 [45] which are produced by dendritic cells in response to allergen [46, 47].

Several studies have identified CCR4 as being preferentially expressed by T_H2 cells [42, 44], regulatory T cells [48], and mast cells [49] suggestive of a role in allergic disease. High levels of CCR4 expression on specific subpopulations of T cells, including skin-homing cutaneous lymphocyte antigen $(CLA)^+$ T cells [50], implicate the receptor in the pathology of atopic dermatitis (AD) [51]. In vivo studies suggest that CCR4 is expressed by the majority of murine T_H2 lymphocytes and facilitates CCL17- and CCL22-mediated chemotaxis [52]. While deletion of CCR4 has no effect on either T_H2 lymphocyte differentiation in vitro or on a T_H2-dependent model of allergic airway inflammation [53], the CCR4/CCL17/CCL22 axes have been shown to play a pivotal role in the late phase of allergic airway inflammation, in studies employing treatment with blocking antibodies specific for the murine orthologues of CCL22 and CCL17 [6, 54]. Moreover, in clinical studies of allergen-challenged atopic asthmatics and rhinitis, the majority of T lymphocytes present in bronchial biopsies were found to be CCR4 positive [55]. Consequently, CCR4 arouses much interest as a potential therapeutic target for the treatment of allergic disease [1].

As stated earlier, CCR8 is also expressed on lymphocytes of the T_H2 lineage and is therefore perceived to play a role in adaptive immunity. CCR8 is best known as the sole receptor for the chemokine CCL1 [56, 57] that has been reported to be upregulated in the allergic lung [55, 58, 59]. The level of infiltrating CCR8-expressing T_H2 cells has been shown to correlate with the severity of asthmatic responses following allergen challenge [55]. More recently, CCL18 has been identified as an additional CCR8 ligand [60]. CCL18 is highly expressed in the human lung [61] and has been reported to be upregulated in the BAL of allergic asthmatics [62] and to be chemotactic for T_H2 cells [60]. Interestingly, no direct equivalent of CCL18 exists in the mouse; instead, CCL8 acts as a functional orthologue of CCL18, activating mouse CCR8 despite sharing little identity with CCL18 [63].

6 Targeting Lymphocyte Chemokine Receptors in Asthma

The role of CCR4 in T-cell trafficking to the allergic lung was initially supported by studies in mice in which the CCR4 ligands CCL22 and CCL17 were neutralized by antibody [54, 64] and also adoptive studies in which T_H2 cells from CCR4-deficient were introduced into wild-type allergic mice [65]. In each case, perturbation of CCR4 signaling resulted in reduced T_H2 recruitment and associated inflammation. Likewise, blockade of human CCR4 by a monoclonal antibody was reported to abolish many of the features of inflammation in a mouse model in which human peripheral blood mononuclear cells were used to reconstitute a SCID mouse [66]. Consequently, many pharmaceutical companies pursued small-molecule antagonists of CCR4.

Table 3 Preclinical CCR4 antagonists in allergic disease

Compound	In vitro activity	In vivo activity	References
22 (Bristol–Myers Squibb)	Inhibition of CCL22-induced chemotaxis (IC_{50} = 3 nM)	A 30 mg/kg dose effective in reducing eosinophil numbers into murine BALF	[67]
8c (Astellas Pharma Inc)	Inhibition of CCL22-induced chemotaxis (IC_{50} = 23 nM)	A 30 mg/kg dose resulted in inhibition of ear swelling in a murine contact hypersensitivity model	[69]
Compound 1 (AstraZeneca)	Inhibition of CCL22-induced Ca^{2+} influx (K_i = 10 nM)		[70]
RS-1154 (Daiichi Sankyo Co.)	Inhibition of CCL17-induced chemotaxis (IC_{50} = 5.5 nM)	Effective at reducing ovalbumin-induced ear swelling at 30 mg/kg in mice	[76]
RS-1748 (Daiichi Sankyo Co.)	IC_{50} of 60nM in CCL17 binding assay and ^{35}S-GTPγS	Effective at ovalbumin lung inflammation at 10 mg/kg in a guinea pigs	[73]
K327 (Kyowa Hakko Kirin Co.)	Inhibition of CCL17 binding (IC_{50} = 72 nM)	Inhibited the of $CCR4^+$ $CD4^+$ T-cell recruitment to the murine lung in an ovalbumin-challenge model (44 mg/kg, twice daily)	[77]
K777 (Kyowa Hakko Kirin Co.)	Inhibition of CCL17 binding (IC_{50}: 57 nM) and CCL17-induced chemotaxis (9 nM)		[72]

The high level of conservation between human and rodent CCR4 species has meant that many of the compounds developed against human CCR4 generally possess good potency at the murine counterpart. Table 3 lists some published data regarding the in vitro and in vivo activities of preclinical CCR4 antagonists [67, 69, 70, 72, 73, 76, 77]. AstraZeneca has been active in the CCR4 antagonist area and has identified *N*-pyrazin-2-yl-arylsulphonamides as potent CCR4 antagonists [70]. One of these molecules is AstraZeneca compound 1 (Table 3 and **7** Fig. 2) which appears to have a different mode of action compared to other chemokine receptor antagonists. Instead of binding to an intrahelical site composed of the transmembrane regions, compound 1 appears to require access to an intracellular site within the CCR4 C-terminus to exert its antagonistic effects [70]. Another CCR4 antagonist (Table 3 and **8** Fig. 2) from this series was able to dose-responsively inhibit CCR4 ligand-induced actin polymerization in T lymphocytes, which is a measure of T-cell function [68].

To date, only one small-molecule CCR4 antagonist, the GSK compound GSK2239633, has been reported in clinical trials (Table 2 and **9** Fig. 2). Although generally well tolerated, less than 80% receptor occupancy was achieved at doses of 1,500 mg 1 h following administration, which dropped to 50% occupancy by 4 h

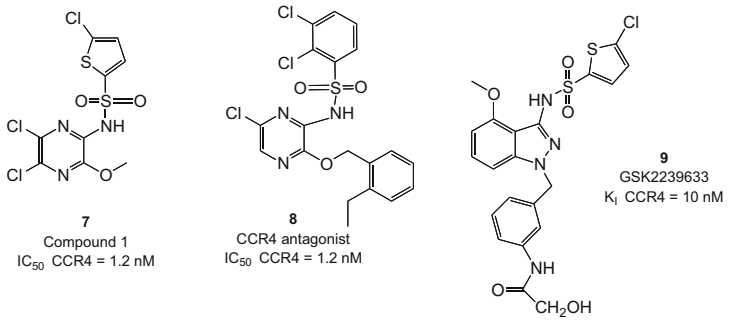


Fig. 2 CCR4 antagonists in asthma and allergic disease (unless otherwise noted all kinetic data are inhibition of receptor binding)

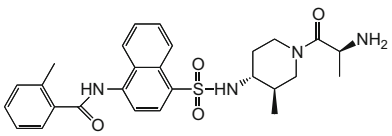
post-dose. As a consequence, GSK2239633 is not being developed further at this time [71]. The antibody Mogamulizumab (KW-0761; AMG-761) a defucosylated humanized IgG1 mAb specific for CCR4 is being developed by Kyowa Hakko Kirin and Amgen for the intravenous treatment of T-cell lymphoma. At the time of writing, Mogamulizumab has been approved in Japan for the treatment of relapsed or refractory adult T-cell leukemia-lymphoma, while Amgen is currently conducting a phase I asthma trial for the use of Mogamulizumab in asthma (Table 2) [78].

In terms of targeting CCR8, supportive data have been slow in emerging, with the reporting of small-molecule CCR8 antagonists slower still (Table 4). An initial study of allergen-challenged CCR8-deficient mice [85] appeared to support a role in allergic airway disease, although proved controversial, with subsequent in vivo studies failing to support such a role [86, 87]. In humans, the lack of reliable CCR8-specifc antibodies has been one obstacle [88] although the generation of the 433 H mAb, by ICOS scientists, provided a work-around [89]. Using this antibody, Mutalithas and colleagues were able to show that greater percentages of $CCR8^+$ T cells were found in PBMCs isolated from the venous blood of asthmatics compared with those of control subjects (4.7% c.f. 3.0%), suggesting a role for CCR8 in asthma and the use of CCR8 as a biomarker of disease progression. A handful of small-molecule CCR8 antagonists have subsequently been described in the literature (Table 4) with both in vitro and in vivo efficacy.

Against this backdrop, scientists from Medlmmune recently reported the results of a study using the CCR8 antagonist ML604086 (Table 4 and **10** Fig. 3), in an *Ascaris suum* airway challenge model in cynomolgus monkeys [83]. Despite almost complete occupancy of CCR8 with the drug during the study, no significant effects on any marker of airway inflammation were observed, leading the authors to conclude that CCR8 plays a dispensable role in asthma, certainly in the primate model employed. One potential caveat of targeting both CCR4 and CCR8 is their expression on regulatory T cells [48], which may have undesired proinflammatory effects.

Table 4 Preclinical CCR8 antagonists in allergic disease

Compound	In vitro activity	In vivo activity	References
AZ6 (AstraZeneca)	Inhibition of CCL1-induced chemotaxis (IC_{50} = 300 nM) and Ca^{2+} (IC_{50} = 630 nM)		[80]
AZ084 AstraZeneca)	Inhibition of CCL1-induced chemotaxis (IC_{50} = 1.3 nM)	Well tolerated in rats and dogs exposed for 7 consecutive days at doses up to 650 mg/kg/day and 8.7 mg/kg/day, respectively	[82]
ML604086 (Medlmmune)	Inhibition of CCL1-induced chemotaxis (IC_{50} = 1.3 μM) and Ca^{2+} IC_{50} = 1 μM)	In a primate model of asthma, no significant effect on Ag-induced BAL eosinophilia, mucus production, or T_H2 cytokine production, despite >98% coverage on T cells	[83]



10
ML604086
IC_{50} CCR8 in whole blood = 1.0 uM

Fig. 3 CCR8 antagonists in asthma and allergic disease (unless otherwise noted all kinetic data are inhibition of receptor binding)

7 Multiple Sclerosis

Multiple sclerosis is a chronic autoimmune disease in which the immune system attacks and destroys the myelin sheath that surrounds nerve cells. Although the cause of the disease is unknown, it is thought to involve a combination of toxicological, viral, bacterial, and genetic factors. Multiple sclerosis affects close to half a million individuals in the USA alone and is the most common form of paralysis in young adults in the developed world. A conservative annual cost for patients with multiple sclerosis in the USA has recently been estimated at anywhere from 3 to 8 billion dollars [90] and this has attracted massive investment from the pharmaceutical industry in the development of new therapeutic approaches. Chemokine receptors have been considered an attractive target for the treatment of multiple sclerosis. The major rationale for targeting these proteins has been based both on the pathophysiology of the disease and also from animal models of disease.

Multiple sclerosis appears to be induced when T Helper 1 cells (T_H1) recognize components of the myelin sheath. Activated, autoreactive T cells within the lesions

are believed to drive the chronic inflammatory process and activate local or hematogenous macrophages that destroy myelin. This inflammatory cascade leads to large focal lesions of primary demyelination with relative axonal preservation. Recent research suggests that the pathogenetic scheme described above is oversimplified and cannot explain lesion formation. It is known that T-cell populations like T_H17 cells can also contribute to inflammation in multiple sclerosis [81] and that amplification of demyelination in a chronic inflammatory reaction in the brain requires additional factors. Furthermore, the patterns of demyelination are different between different subgroups of multiple sclerosis patients, which suggests that the disease is heterogeneous [79, 84].

Evidence from a variety of studies has implicated chemokines in the pathophysiology of multiple sclerosis. Early studies by Godiska demonstrated that mRNAs encoding a variety of chemokines including CCL3 and CCL5 were induced in the spinal cord 1–2 days before the clinical signs of disease were apparent [91]. This was followed by studies that showed that a neutralizing antibody to CCL3 ameliorated disease in an animal model of multiple sclerosis in the mouse [92]. The link to the human disease was soon established from studies in which demyelinating plaques from the brains of multiple sclerosis patients were shown to express a variety of inflammatory chemokines and their receptors [93]. Microglial activation is thought to contribute directly to myelin destruction in multiple sclerosis through mechanisms that include the production of proinflammatory cytokines and chemokines [94]. In chronic active human multiple sclerosis lesions, the chemokine receptors CCR2, CCR3, and CCR5 have been shown to be present on infiltrating macrophages and activated microglia, while CCR2 and CCR5 were also present on large numbers of infiltrating lymphocytes [95]. In addition, macrophages derived from blood-borne monocytes and microglia have been shown to express CCR1 and its ligand CCL3 [79]. T cells expressing the chemokine receptors CCR5 and CXCR3 and their ligands CCL3 and CXCL10 are expressed in demyelinating brain lesions [96]. Interestingly the cytokine interferon beta which is used to treat multiple sclerosis reduced the expression of CXCR3 on $CD4^+$ and $CD8^+$ T cells [97]. The authors concluded that since CXCR3 cells are enriched in cerebrospinal fluid and are detected in lesion material in multiple sclerosis, this might represent one important means of interferon-beta action in treating multiple sclerosis.

As outlined above animal models of disease, particularly the experimental autoimmune encephalomyelitis (EAE) models carried out in rodents, have provided valuable insight into the role of chemokines in the human disease [98]. However, although these models have led to an understanding of the pathogenesis of the human disease, they need to be interpreted with some caution especially because they do not recapitulate the complex spectrum of the human disease. A prime example is the animal studies that showed that blocking the TNF receptor was effective in decreasing disease in a rodent model [99]. In contrast, when this approach was translated to human clinical trials in patients suffering from multiple sclerosis, the trials had to be halted because the TNF receptor blockers actually made the disease worse [100]. Also it is clear that many aspects of the human disease, in particular, the contributions of B lymphocytes and $CD8^+$ cells in disease pathology, are not captured by these models. Finally, selection of the appropriate

EAE model is important in determining the validity of a disease target. For example, the acute EAE model in rats is driven by a cell type, neutrophils, that does not really figure in the human disease [101].

8 Targeting Chemokine Receptors in Multiple Sclerosis

CCR1 and CCR2 are the major chemokine receptors that have been targeted pharmacologically in human clinical trials for the treatment of multiple sclerosis (Table 2). These include two CCR1 receptor antagonists BX 471 and MLN 3701 and four CCR2 antagonists MLN1202, INCB8696, CCX915, and MK-0812.

The evidence for a role of CCR1 in the pathophysiology of multiple sclerosis was based on a number of studies. First, neutralizing antibodies to one of the CCR1 ligands, CCL3, prevented the development of both acute and relapsing paralytic disease as well as infiltration of mononuclear cells into the CNS initiated by the transfer of activated T cells [102]. Second, deletion of CCR1 was protective in a myelin oligodendrocyte glycoprotein (MOG) model of multiple sclerosis in mice decreasing the disease score by around half compared to their wild-type littermates [103]. Finally, CCR1 is expressed in human multiple sclerosis lesions associated with hematogenous macrophages usually coexpressed with CCR5 [79].

Based on these data Berlex initiated a CCR1 antagonist program and identified BX 471 (Table 2 and **11** Fig. 4), a potent diacyl piperazine, as its clinical candidate. The antagonist had a reported K_D of 1.0 nM for human CCR1 and was more than 1,000-fold selective for CCR1 [104]. Although the antagonist was poorly cross-reactive with rat and mouse, CCR1, it had sufficient affinity to be tested in animal models and it was efficacious in an acute rat EAE model of multiple sclerosis [104]. Based on these data the antagonist entered human clinical trials. The drug was well tolerated and had no safety issues in phase I; however, its development was stopped after the phase II study failed to demonstrate a positive clinical end point, a reduction in the number of new inflammatory CNS lesions [105].

Millennium has reported a CCR1 antagonist MLN3701 in phase II clinical trials for multiple sclerosis. This compound was being codeveloped with its partner Sanofi-Aventis (AVE9897), but no structures or data were ever published [106].

The evidence for a role of CCR2 in the pathophysiology of multiple sclerosis was based on a number of studies.

First, CCL2 is one of the major chemokines responsible for the recruitment and activation of monocytes in the blood and macrophages in the tissues [107]. These cells play a central role in the disease pathology involved in multiple sclerosis and thus their modulation might be of benefit in treating multiple sclerosis.

Second, Karpus [92] demonstrated that the production of the CCR2 ligand CCL2 correlated with the relapse induced in an EAE model of multiple sclerosis in the mouse. Furthermore, neutralizing antibodies to CCL2 significantly reduced the severity of relapsing EAE and significantly inhibited the adoptive transfer of EAE when included in in vitro activation cultures, suggesting a regulatory anti-inflammatory property.

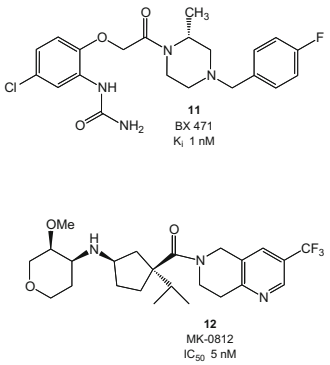


Fig. 4 Chemokine receptor antagonists in multiple sclerosis (unless otherwise noted all kinetic data are inhibition of receptor binding)

Finally, animals genetically deficient in the receptor for CCL2 (CCR2) were found to be resistant to disease induction in an EAE model of disease [108]. These animals failed to develop mononuclear cell inflammatory infiltrates in the CNS and failed to increase CNS levels of the chemokines CCL5, CCL2, and CXCL10 as well as the chemokine receptors CCR1, CCR2, and CCR5.

Based on these studies a number of companies felt encouraged to pursue CCR2 antagonists as therapeutics for treating multiple sclerosis. One of the first to be described was Merck's MK-0812, a pyridine-substituted piperidine (Table 2 and **12** Fig. 4) [109]. MK-812 is a potent CCR2 antagonist, IC_{50} of 5.0 nM, and was tested in phase II clinical trials for multiple sclerosis and rheumatoid arthritis (see later) [105]. The multiple sclerosis trial was a randomized, double-blind, placebo-controlled study with a 12-week protocol and 120 patients. The primary end point was for the compound to decrease the presence of new gadolinium-enhancing lesions as measured by MRI [110]. Unfortunately, the compound increased rather than decreased the presence of gadolinium-enhancing lesions and the development of the compound for multiple sclerosis was terminated by Merck [111].

Millennium has also described the development of MLN 1202, which is a blocking antibody to CCR2, as a potential therapeutic for multiple sclerosis and rheumatoid arthritis (see later). The antibody was reported to have positive results in a phase II trial for multiple sclerosis [112]. Millennium announced at the American Neurological Association meeting in 2007 that MLN1202 reduced gadolinium-enhancing lesions on magnetic resonance images of the brain in a multicenter phase II clinical trial involving 50 patients with relapsing-remitting multiple sclerosis [111, 112]. However beyond these data there have been no further reports of activity in multiple sclerosis and currently this molecule is reported to be in a phase II clinical trial for the treatment of bone metastases [113].

Incyte had reported that INCB3344 a tool compound that they developed for target validation was efficacious in a mouse model of multiple sclerosis [114]. Based on these studies they developed a clinical CCR2 receptor antagonist INCB8696 (structure not known) that they reported was in phase I clinical trial for multiple sclerosis [115]. However beyond this initial communication by the

company in 2007 there have been no further reports of any activity of this molecule in multiple sclerosis and we are left to conclude that the program was discontinued.

ChemoCentryx identified a CCR2 antagonist CCX915 (structure not disclosed) as a clinical candidate for the treatment of multiple sclerosis [116, 117]. Unfortunately the development of CCX915 was terminated due to its poor pharmacokinetic properties in phase I clinical trials.

9 Reasons for the Clinical Failure of Chemokine Receptor Antagonists in Multiple Sclerosis

The data from the chemokine receptor antagonists in the various clinical trials for multiple sclerosis has been hugely disappointing. None of the compounds have advanced further than phase II clinical trials and the failures have cost the pharmaceutical companies developing these compounds multiple millions of dollars. It is difficult to determine the reasons for the failures of these drugs as therapeutics for multiple sclerosis, because in the majority of cases the companies developing these compounds have chosen not to reveal any clinical data. One can guess that the primary reason for companies to take such a negative approach is that by doing this they will not provide any potential advantage to their competitors. However, this approach certainly does a major disservice to patients suffering from multiple sclerosis, since valuable insights into the potential mechanisms of the disease that could provide new approaches for the development of potentially useful novel drugs are lost.

Although there has been very little clinical data published to help explain the failures of chemokine receptor antagonists in treating multiple sclerosis, one obvious reason that comes to mind is that simply blocking one receptor to treat such a heterogeneous disease is way too simplistic an approach. Recall that multiple sclerosis is a complex disease; not only are there four clinical subtypes – relapsing remitting, secondary progressive, primary progressive, and progressive relapsing (of which relapsing remitting is the most common form of the disease) – but also recent work by several groups have revealed a further level of complexity based on the patterns of demyelination that exists between patients [84]. These data suggest that the disease is even more heterogeneous than simply classifying it according to clinical subtypes [79].

Since we do not yet have specific clinical markers to be able to stratify patients into chemokine receptor-specific subpopulations, then the selection of specific responders in a clinical trial is exceedingly difficult and could account for some of the observed clinical failures. In support of this idea is that the mechanism of action of most of the clinically approved multiple sclerosis treatments is relatively broad mechanistically. For example, the antibody Natiluzumab targets adhesion molecules that block the migration of all activated T cells [118], while the small-molecule SIP-1 receptor agonist Fingolomid causes the retention of activated