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Chemokine Receptor Antagonists

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INTRODUCTION

Chemokines are small basic proteins that are defined by the number and position of the invariant cysteines in their protein sequence.[1] At the time this review was written more than 40 different chemokines clustered into two major (CC and CXC) and two minor (CX3C and C) classes have been identified.[1] Chemokines are potent chemoattractants and play an important role in host defense by mobilizing immune cells to combat invading microorganisms such as bacteria and viruses.[2] Under certain circumstances the immune system can turn rogue and destroy its own cells, leading to autoimmunity. Because of their role in the immune response, chemokines participate in this process; thus, they are important targets for treating autoimmune diseases.[3] Chemokines initiate immune cell activation by binding to cell surface receptors of the G-protein-coupled receptor (GPCR) superfamily.[4] At the last count, 19 functional chemokine receptors and two nonfunctional decoy receptors, Duffy antigen receptor for chemokines (DARC) and the protein D6, have been cloned.[5,6] There are several reviews that describe the biology of the chemokines and their receptors, and we refer the reader to these excellent monographs for more background than we can comfortably cover here.[2,3,5,6]

It is estimated that around 50 million Americans, especially women, suffer from autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, asthma, etc.;[7] therefore, the market for therapeutic approaches to treat these and other illnesses is huge[8] and could exceed $77 billion by the year 2017 according to a recent report by global industry analysts.[9] Consequently most of the major pharmaceutical companies have poured billions of dollars into research and development to identify safe and effective drugs to treat these diseases. Because of their important role in host defense, chemokine receptors have been closely scrutinized as drug targets by a number of companies, and a Pub Med search in 2012 with the term "chemokine receptor antagonists" lists over 4500 hits. However, despite the fact that numerous chemokine receptor antagonists have progressed from discovery into the clinic (Table 1), so far only two, the CCR5 inhibitor from Pfizer[10] and the CXCR4 antagonist from Anormed,[11,12] are registered drugs. These successes and failures will be discussed further below.

PROTEIN STRUCTURE AND REGULATION OF ACTIVITY

Chemokine receptors belong to the class A grouping of the GPCR superfamily, and technological breakthroughs in crystallization techniques over the past decade have led to a wealth of information regarding class A GPCR structure[13] exemplified by the recent structure of the chemokine receptor CXCR4 (a target recently reviewed in this journal[14]). GPCRs contain seven α-helices that span the membrane and are linked through three intracellular and three extracellular loops (Figure 1). The receptors are normally found in the inactive or ground state and, upon binding of ligand, adopt alternative active conformations that recruit signaling molecules to the intracellular face of the receptor and ultimately transduce a signal to the cytoplasm of the cell.

The first GPCR crystal structure obtained was that of ground state bovine rhodopsin[15] and showed that the highly conserved "DRY" (Asp-Arg-Tyr) motif at the extracellular end of transmembrane III (TM III) acts as an ionic lock, holding the receptor in the inactive state by means of salt bridges between the arginine residue and the aspartate and a neighboring glutamate residue in TM VI. Ionic locks were subsequently incorporated into several homology models based upon the rhodopsin structure. However, subsequent elucidation of crystal structures of other class A GPCRs in inactive conformations, notably the human β_2AR[16] and CXCR4,[17] indicated that despite the existence of the DRY motif, no ionic lock was evident. This may explain why some GPCRs are more prone to constitutive activity (signaling in the absence of ligand) and may account for the considerable technical difficulties that had to be overcome to obtain these crystal structures, typically by the introduction of stabilizing mutations.

Within class A GPCRs a major binding pocket (comprising TM helices IV, V, VI, and VII) and a minor binding pocket (TM helices II, III, and VII) form crevices into which small ligands such as peptides and amines can bind (Figure 1). This binding event triggers movement of the helices, resulting in coupling to heterotrimeric G proteins, although other effectors such as the arrestins can be activated independently.[18] Movement of TMVI is thought to be critical in the activation process, with the pivoting of the helix around a conserved proline residue, Pro 6.50.[19] This "rotomer toggle switch" involves a conserved tryptophan residue Trp 6.48.[19] Both residues play a role in the opening up of intracellular loop III, thereby providing access for G proteins and their subsequent activation. This results in the parting of the G protein α and $\beta\gamma$ subunits, leaving them free to activate a variety of effector molecules including phosphatidylinositide 3-kinase (PI3K), phospholipase Cβ, and Src family kinases.

The activation of chemokine receptors by their much larger protein ligands is, broadly speaking, a two-step process in which the typically basic chemokine is first tethered by interactions

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Table 1

receptor	company	phase	compound	generic name	affinity (nM)	MW	log *P*	indication	status
CCR1	Schering AG (Berlex)	II	BX471		1.0	434	2.1	MS, psoriasis, endometriosis	no efficacy
CCR1	Millennium	II	MLN 3701					MS, multiple myeloma	no longer reported
CCR1	Millennium	II	MLN 3897		2.3	518	5.1	RA, multiple myeloma	no efficacy in RA
CCR1	Pfizer	II	CP-481,715		64	482	2.3	RA	no efficacy
CCR1	AstraZeneca	II	AZD4818		5.0	461	1.9	COPD	no efficacy
CCR1	Chemocentryx	II	CCX354		1.5			RA	ongoing
CCR1	Merck	II	C-4462					RA	no efficacy
CCR1	Merck	II	C-6448					MS	no efficacy
CCR2	Millennium	II	MLN 1202[a]					RA	no efficacy
		II						atherosclerosis, MS	ongoing
CCR2	Incyte	I	INCB8696					MS, lupus	no longer reported
CCR2	Incyte	II	INCB32S4		3.7	520	1.9	RA, type II diabetes	no longer reported
CCR2	Chemocentryx	I	CCX915					MS	terminated
CCR2	Chemocentryx	II	CCX140		2.3			diabetic nephropathy	ongoing
CCR2	Merck	II	MK-0518		5.0	469	2.4	RA, MS	no efficacy
CCR2	Pfizer	II	PF-4136309					pain	no longer reported
CCR2	BMS	II	BMS-741672					diabetic neuropathy	ongoing
CCR2	J & J	II	JNJ-17166864		20.0	443	3.6	allergic rhinitis	no efficacy
CCR3	Pharmaxis	II	ASM8[b]					asthma	ongoing
CCR3	GSK	II	GSK766994		10.0	450	2.2	asthma and allergic rhinitis	no efficacy
CCR3	Dupont	I	DPC168		2.0	475	5.1	asthma	development halted
CCR3	BMS	I	BMS-639623		0.3	481	3.5	asthma	ongoing
CCR3	Novartis	I	QAP-642					allergic rhinits	development halted
CCR3	AstraZeneca	II	AZD3778		8.1	526	3.8	allergic rhinitis	no longer reported
CCR4	Amgen	II	KW-0761[a]					oncology	ongoing
CCR4	GSK	I	GSK2239633		10.0	506	27	asthma	ongoing
CCR5	Pfizer	II	UK-127,857	maraviroc	3.0	513	4.4	RA	no efficacy
		approved						AIDS	registered drug
CCR5	Schering-Plough	II	SCH-C		2.0	557	29	RA	no efficacy
		I						AIDS	development halted
CCR5	Schering-Plough	II	SCH-D	vicriviroc	0.45	533	3.6	AIDS	development halted
CCR5	GSK	III	GW2239633	aplaviroc	3.0	577	3.8	AIDS	development halted
CCR5	Incyte	II	INCB9471		3.1	559	3.9	AIDS	development halted
CCR5	Progenies	II	Pro 140[a]					AIDS	ongoing
CCR5	Tobira	II	TAK652	cenicriviroc	3.1	696	10.2	AIDS	ongoing
CCR5	AstraZeneca	II	AZD5672		0.26	672	3.1	RA	no efficacy
CCR5	Novartis	I	NIBR-6465		0.8			AIDS	ongoing
CCR5	Sangamo	II	SB-728[c]					AIDS	ongoing
CCR5	HGS	I	HGS004[a]					AIDS	ongoing
CCR9	Chemocentryx	III	CCX-282	vercimon	6.0	444	4.7	IBD, Crohn's	ongoing
CXCR1/	Schering-Plough	II	SCH 527123		3.9	415	1.0	COPD	ongoing
CXCR2					0.049				
CXCR1/	Damps	II	Repertaxin		1.0	283	2.5	pancreatic islet	ongoing
CXCR2					100			transplantation	
CXCR2	GSK	I	SB-656933		5.1	463	4.2	COPD, cystic fibrosis	ongoing
CXCR3	Amgen	II	AMG487		8.0	603	3.8	psoriasis	no efficacy
CXCR4	Genzyme	approved	AMD3100	plerixafor	74	502	−0.4	MM, non-Hodgkins lymphoma	registered drug
CXCR4	TaiGen	II	Burixafor					stem cell transplant	ongoing
CXCR4	Polyphor	II	POL6326					stem cell transplant	ongoing
CXCR4	Medarex	I	MDX-1338[a]					multiple myeloma	ongoing

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Table 1. continued

receptor	company	phase	compound	generic name	affinity (nM)	MW	log *P*	indication	status
CXCR4	Biokine	I	BKT140[d]					stem cell transplant	ongoing

[a]Neutralizing monoclonal antibodies. [b]Antisense oligonucleotide. [c]Zinc finger nuclease. [d]Peptide.

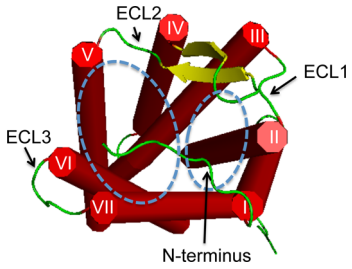

Figure 1. Agonist and antagonist binding pockets in GPCRs. Model of the crystal structure of the inactive state of CXCR4 (PDB code 3ODU) as viewed from the extracellular face. TM helices are numbered (Roman numerals) and the extracellular loops (ECLs) marked. Major and minor binding pockets are denoted by dashed lines.

with the acidic amino-terminal domain of the receptor[20,21] with additional charge contributions from sulfated tyrosine residues[22] and O-linked carbohydrate[23] within this region. This serves to orientate the chemokine, allowing the N-terminus of the chemokine to insert into the helical bundle (Figure 2A) and interact with residues in either the major or minor binding pockets.[24]

Truncation of the N-termini of many chemokines by the enzyme CD26/dipetidylpeptidase IV (DPP-IV) typically ablates a chemokine's agonist activity while leaving its capacity to bind to the chemokine receptor untouched. This leads to the generation of endogenous antagonists[25] and underscores the importance of access of this part of the chemokine to the binding pocket for effective agonism. These findings have been exploited to create chemokine analogues with potent antagonist activity and efficacy in vivo.[26] The requirements for the receptor N-terminus can be bypassed by synthetic small molecule agonists that insert themselves into the major or minor binding pockets of the chemokine receptor to directly induce receptor activation, independently of the extracellular domains (Figure 2B).[27] In the case of a recently described CXCR3 agonist, this small molecule successfully mimicked a region of a natural chemokine agonist to induce receptor activation.[28] Similarly, the tripeptide *N*-acetyl Pro-Gly-Pro (PGP), derived from the proteolysis of extracellular matrix, mimics a portion of CXCL8 and can activate CXCR2 in vivo and in vitro.[29]

A vast majority of small molecule antagonists described to date are allosteric modulators and interact with either the minor or minor intrahelical binding pockets distinct from the main chemokine binding sites found in the extracellular domains. In doing so, they stabilize inactive receptor conformations, which in many cases can still bind ligand but are unable to transduce a signal (Figure 2C). More recently a second class of allosteric modulator has been described[30] that requires access to the intracellular C-tail of the chemokine receptors CCR4 and CCR5 (Figure 2D). The binding site of these compounds is thought to include helix VIII, found within the C-tail of several class A GPCR crystal structures, which may play a key role in receptor conformation and signaling.[31] Notably this motif is absent from the CXCR4 crystal structure,[17] suggesting that this

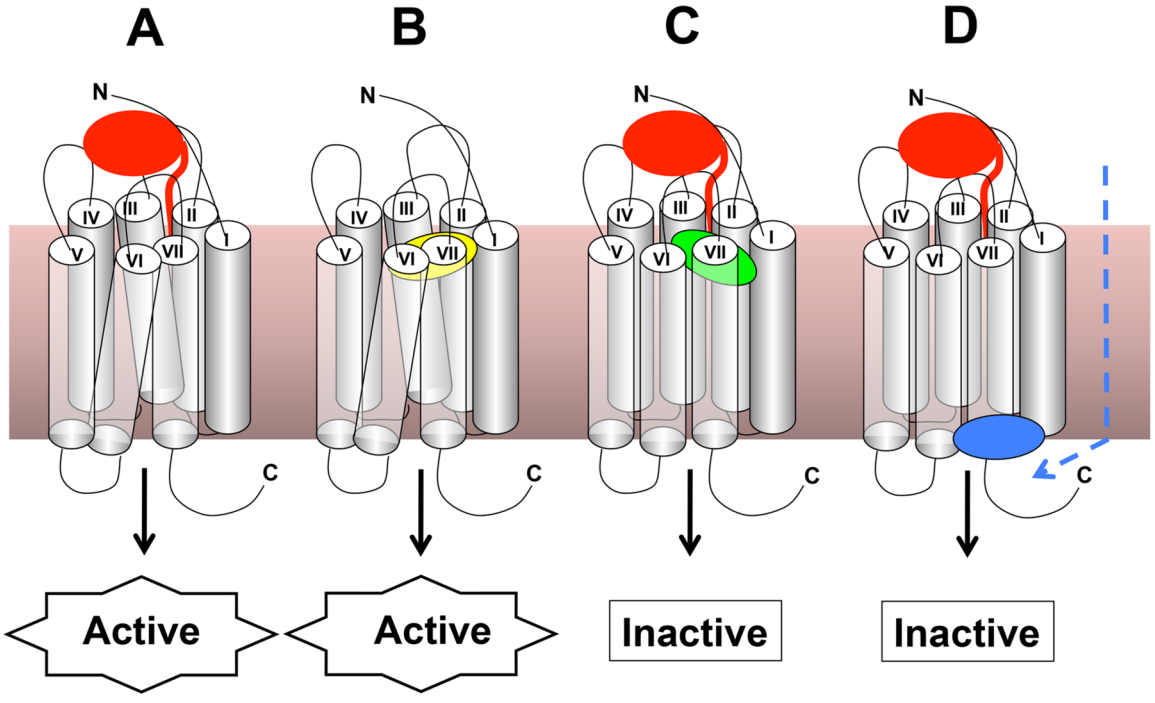

Figure 2. Chemokine receptor activation and inactivation. Panels A–D show cartoons of receptor activated by chemokines (red) or small molecule agonists (yellow) or inactivated by intrahelical small molecule antagonists (green) or intracellular antagonists (blue).

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class of intracellular antagonist may have limited activity among the chemokine receptor family.

SELECTED BIOLOGY AND PATHOPHYSIOLOGY

In a review of this size we can only hope to scratch at the surface of the role that chemokines and their receptors play in health and disease. Thus, we have restricted ourselves here to discussing the evidence that links chemokine receptors to the pathophysiology of multiple sclerosis, rheumatoid arthritis, and asthma. Indeed these autoimmune diseases represent those that have been the most targeted in the clinic by chemokine receptor antagonists (Table 1).

Multiple Sclerosis. Multiple sclerosis is the most common neurological disorder in young adults in the developed world. It is a chronic autoimmune disease that involves the central nervous system (CNS) and affects upward of 400 000 individuals in the U.S. alone. The total annual cost for patients with multiple sclerosis in Europe has recently been estimated at around 12.5 billion euros,[32] and this has attracted massive investment from the pharmaceutical industry in the development of new therapeutic approaches. A variety of approaches have been aimed at inhibiting chemokine receptors with the major rationale for targeting these proteins based on the pathophysiology of the disease and mainly provided by animal models. Multiple sclerosis appears to be induced when T helper 1 (Th1) cells 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 other than classical Th1 cells contribute to inflammation in multiple sclerosis 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.[33,34]

Demyelinating plaques from the brains of multiple sclerosis patients express a variety of inflammatory chemokines and their receptors[2] and notably contain macrophages and microglia that express CCR1 and its ligand CCL3.[34,35] In acute and chronic-active multiple sclerosis lesions, immunoreactivity for CCL7 (a CCR1 ligand) and CCL8 has also been described, with staining prominent throughout the lesion center.[36] CXCR3 is expressed by almost all the T cells recovered from the CSF of multiple sclerosis patients with active disease and is expressed at significantly higher levels on CD4+ T cells from the cerebrospinal fluid (CSF) compared with circulating cells.[35] Likewise, the CXCR3 ligands CXCL9 and CXCL10 are elevated in the CSF of patients with active multiple sclerosis.

The major driving force for therapeutic approaches that target chemokines in multiple sclerosis has mostly been provided by animal models of disease, particularly the experimental autoimmune encephalomyelitis (EAE) models carried out in rodents. Although these models have been extremely valuable in leading to an understanding of the pathogenesis of the human disease, they need to be interpreted with some caution particularly because they do not recapitulate the entire complex spectrum of the human disease. For example, animal studies revealed that blocking the TNF receptor was effective in ameliorating disease in an EAE model. However, 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. Also it is clear that many aspects of the human disease, in particular the contributions of B-lymphocytes and autoantibodies in disease pathology, are not captured by these models. Finally, as we shall see later in this review, 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. An excellent monograph on the role of translational animal studies for multiple sclerosis, particularly the EAE model, discusses this important issue further.[37]

Rheumatoid Arthritis. A hallmark of rheumatoid arthritis is an accumulation of leukocytes from the vasculature into the synovial tissue, notably monocytes and Th1 cells, where they initiate an autoimmune reaction leading to destruction of the cartilage. Consequently there has been much interest in the chemokines and receptors responsible for leukocyte trafficking to the synovium.

Among the CC chemokines, CCL3 and CCL5 are readily detectable in synovial fluid of rheumatoid arthritis patients with increased levels correlating with disease severity.[38,39] These clinical data have been supported by studies in murine models of rheumatoid arthritis. For example, in the collagen induced arthritis (CIA) model[40] and in a rat adjuvant-induced arthritis (AIA) model CCL3 levels are elevated.[41] Moreover, disease severity was significantly reduced by neutralizing anti-CCL3 and anti-CCL5 antibodies, establishing a proof of principle for antagonism of CCR1 signaling in rheumatoid arthritis.[42]

Several lines of evidence implicate CCR1 in the pathophysiology of rheumatoid arthritis. First, CCR1 is expressed in macrophages in rodent models of rheumatoid arthritis.[43] Second, CCR1 and its ligands showed significant expression in peripheral mononuclear cells obtained from biopsied synovial tissue from patients with rheumatoid arthritis.[44] Third, a non-peptide antagonist of murine CCR1 was efficacious in a collagen induced arthritis model in the mouse.[45] Finally, synovial biopsy specimens obtained in a small study of 16 patients with rheumatoid arthritis revealed a significant reduction in the number of macrophages in patients treated with a CCR1 antagonist compared with the placebo group[46] (see later).

The CCR2-speciifc chemokine CCL2 has also been reported to be up-regulated in the synovial tissue of rheumatoid arthritis patients[47] and to be produced by articular chondrocytes cultured ex vivo.[48] Studies of material from rheumatoid arthritis patients suggest that CD68+ macrophages that express CCR1, CCR2, and CCR5 are recruited to the synovium by these chemokines.[49]

Among CXC chemokines, the CXCR1/2 specific chemokines CXCL8 and CXCL1 are also abundantly expressed in the synovial tissues of rheumatoid arthritis patients[50,51] as are the CXCR3 specific chemokines CXCL9 and CXCL10.[52] Since most of the T cells within the synovium of rheumatoid arthritis patients express CXCR3, this axis may be worthy of targeting. Similarly, elevated CXCL16 levels are found in the synovial tissue of rheumatoid arthritis patients, which correlates with the recruitment of CXCR6+Th1/Tc1 effector cells[53] and suggests that CXCR6 might be a potential target.

Allergic Asthma. The allergic response results from an inappropriate immune response to an otherwise innocuous antigen and can be broken down into the early phase and late phase reactions. The early phase reaction peaks at around 15 min following allergen challenge and is mediated by the cross-linking of IgE by allergen. This activates mast cell degranulation and the release of preformed mediators, such as histamine, which is present in granules and rapidly synthesized mediators such as leukotriene C_4 (LTC_4) and prostaglandin D_2 (PGD_2). These mediators act in a paracrine fashion on other cells to induce airway smooth muscle contraction, increased microvascular permeability, and mucus production. The late phase asthmatic reaction follows the early phase reaction several hours later and is notable for an influx of eosinophils and lymphocytes to the inflamed tissue.

Eosinophils, in addition to being a source of leukotrienes (therefore promoting bronchoconstriction and mucus hypersecretion), are also a source of profibrotic cytokines such as transforming growth factor β1 (TGF-β1), which may promote an excessive repair process. This may result in increased airway smooth-muscle mass, collagen deposition, angiogenesis, and subsequent thickening of the airway wall, a process known as remodeling.[54] The recruitment of leukocytes in the late phase reaction is chiefly coordinated by chemokines that are released following the interaction of dendritic cells, T-lymphocytes, and structural cells. Consequently, blocking chemokine-mediated activation and trafficking of these cells is a potential therapeutic avenue for asthma treatment.

Co-stimulation of CCR1 and the high affinity IgE receptor FcεRI has been reported to enhance mast cell degranulation and conversely to decrease chemotaxis to CCL3,[55] which is thought to maintain mast cell numbers at sites of allergic inflammation. In keeping with this, antagonism of the CCR1/CCL3 axis results in a reduction in disease score following co-stimulation of CCR1 and FcεRI.[56] Several chemokine receptors have been reported to recruit immature dendritic cells (DCs) to allergen sensitized tissues, notably CCR6, CCR2, CCR5, and CXCR4.[57] Dendritic cell maturation is accompanied by the production of the CCR4 ligands CCL17 and CCL22. These two chemokines recruit Th2 cells which following polarization either in vitro or in vivo, express CCR3, CCR4, and CCR8 on their cell surface.[5] CCL22 and CCL17 are also up-regulated in the lung following allergen challenge[58] with CCR4 and CCR8 coexpressed on a significant number of T lymphocytes found following bronchioalveolar lavage (BAL) of asthmatics.[59] Similarly, increased numbers of CCR8+ cells have been reported in bronchial biopsies from asthmatics compared with controls which also correlated with 3-fold-higher levels of the CCR8 ligand CCL1 in the BAL fluid.[60]

The recruitment of eosinophils is characteristic of the late-phase allergic reaction and is predominantly mediated by the "eotaxins" CCL11, CCL24, and CCL26, which are selective agonists for CCR3, the principal chemokine receptor expressed by eosinophils.[5] CCL11 plasma levels are raised in acute compared with stable asthmatic humans, and increased expression of CCL11 and CCL24 is observed in the allergic lungs of asthmatics[61,62] and in their sputum.[63] CCR3 is also expressed on other cell types involved in the allergic response, notably Th2 lymphocytes;[64] therefore, targeting CCR3 may provide additional benefit, and several clinical trials have been carried out with CCR3 antagonists (Table 1).

LESSONS FROM ANIMAL KNOCKOUTS

Since the murine genome has orthologues for most of the human chemokines and receptors, the targeted deletion of these molecules in the mouse has been undertaken with the view of providing a greater understanding of the role of chemokine and receptor in vivo, allowing the role of each receptor/ligand axis to be probed in the disease setting. For the purposes of this review, we will focus upon a select group of murine chemokine receptors for which the human orthologue has been targeted in clinical studies. In the vast majority of cases, mice heterozygous for deletion of a particular chemokine receptor are viable, suggesting that there is apparent redundancy in the system.[65] However, closer scrutiny often reveals specific defects in some aspects of immune defense, suggesting a nonredundant role in vivo.

For example, deletion of CCR1 results not only in the predicted loss of neutrophil chemotaxis to the ligand CCL3 but also in a skewing of the Th1/Th2 cytokine balance.[66] In murine models of EAE, CCR1-deficent mice show significantly less severe disease scores in terms of both incidence and severity but maintain intact cutaneous hypersensitivity responses, suggesting that antagonism of CCR1 may be of therapeutic benefit in multiple sclerosis without the potential for immunosuppression.[67] Likewise, in models of cardiac transplant rejection, CCR1-deficent mice show a reduced risk of transplant rejection.[66] In stark contrast, adoptive transfer of CCR1-deficent bone marrow to atherosclerosis-prone ApoE-deficent mice results in enhanced plaque development, suggesting that in atherosclerosis, signaling via this receptor is anti-inflammatory.[68]

Deletion of CCR2 results in mice with impaired responses to intracellular pathogens,[69−71] suggesting that CCR2 is critical for an effective Th1 response and that its deletion results in defective trafficking of monocytes with Th1-polarizing potential. As might be expected with the reciprocal nature of Th1/Th2 responses, CCR2-deficient mice also exhibit enhanced Th2 responses in allergen challenge models.[72] However, all is not straightforward. Deletion of CCL2, the principal CCR2 ligand, results in a defect in Th2 polarization supported by data from studies in which depletion of CCL2 reduced airways hyper-responsivess (AHR) allergen challenge models.[73] There also appears to be a role for the CCR2/CCL2 axis in mast cell recruitment to allergic airways. Mast cell progenitor recruitment to the aiways following allergen challenge is reduced in both CCR2- and CCL2-deficient mice.[74] Deletion of CCR2 on the ApoE-deficient background results in reduced development of atherosclerotic lesions, implicating the receptor in the trafficking of monocytes to the plaque.[75]

Deletion of CCR3 results in mice with reduced numbers of eosinophils in the gut, suggesting an important role for this receptor in directing eosinophils to the gut where they can protect the host from helminth infection.[76] Likewise, the same CCR3-deficient mice reveal a role for CCR3 in mediating eosinophil recruitment to the allergic lung with decreased numbers of eosinophils observed following allergen challenge. Interestingly, in the same allergen challenge models, the eosinophils of CCR3-deficient mice are able to traffic from the circulation to the subendothelial space of the lung parenchyma but not beyond, suggesting that other chemoattractants cooperate in eosinophil recruitment.[76] In contrast, mast cell numbers in the skin are normal in CCR3-deficient

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mice following epicutaneous sensitization, suggesting that CCR3 does not play a role in mast cell homing to the sites of allergic inflammation in skin.[77] Intraperitoneal sensitization of CCR3-deficient mice results in increased numbers of intraepithelial mast cells in the trachea compared with that of wild type mice which is accompanied by increased AHR,[76] suggesting that CCR3 does not play a major role in mast cell homing to the lung. This is supported by an ex vivo study in which bone marrow derived mast cells were unresponsive to CCR3 ligands in chemotaxis assays.[78] Interestingly, intraperitoneal sensitization in the same CCR3-deficient background results in an absence of AHR development.[77]

The original description of CCR4-deficient mice found an unexpected role for the receptor in endotoxic shock, with CCR4-null mice significantly protected from the endotoxic effects of LPS.[79] In contrast and unexpectedly, CCR4-deficient mice showed no protection from airway inflammation following allergen challenge, despite CCR4 being a key receptor expressed by Th2 cells[79] and several earlier studies supporting a role for CCR4 ligands in T-cell trafficking to the allergic murine lung.[80] One complicating factor might be that CCR4 is also expressed by T regulatory cells (Tregs).[81] These cells have an anti-inflammatory phenotype; hence, CCR4 blockade may be detrimental. CCR4 is also expressed by innate natural killer cells, and the trafficking of these cells to the allergic lung is impaired in CCR4-null mice. In a model of inflammatory bowel disease (IBD), adoptively transferred CCR4-deficient Tregs failed to traffic to the mesenteric lymph nodes in the early stages of disease, resulting in a failure to suppress the generation of pathogenic T lymphocytes and the development of colitis.

CXCR1 and CXCR2 coordinate the trafficking of neutrophils to a variety of ELR+ CXC chemokines in humans, notably CXCL8 and related chemokines such as CXCL1, CXCL6, CXCL7 and also non-chemokine ligands such as macrophage inhibitory factor (MIF).[82] Curiously, there is no orthologue of human CXCL8 in the mouse. Initial studies of the murine genome by Southern blotting suggested that the mouse only harbored an orthologue of CXCR2,[83] although subsequent whole genome sequence revealed a CXCR1 orthologue in proximity on mouse chromosome 1, which responds to CXCL6 but not other murine CXC chemokines.[84] Deletion of CXCR2 results in outwardly healthy mice, although on closer examination, the mice exhibited lymphadenopathy and splenomegaly resulting from an increase in B lymphocyte and neutrophil numbers, suggesting that CXCR2 regulates both neutrophils and B lymphoyte development. CXCR2-null mice also display decreased numbers of mucosal mast cell progenitors within the intestine and the allergic lung, suggesting that CXCR2 is important for mast cell localization.[85] Neutrophils lacking CXCR2 have been reported to be preferentially retained in the bone marrow of mice, suggesting an additional role for CXCR2 in neutrophil retention.[86]

Unsurprisingly, mice deficient in CXCR2 display innate immune defects including increased susceptibility to *Toxoplasma gondii* infection.[87] Perhaps, more surprisingly, CXCR2 appears to play a role in the migration of oligodendrocyte precursors during the development of the spinal cord, with CXCR2 deficiency associated with reduced numbers of oligodendrocytes and abnormal distribution.[88] In a murine model of multiple sclerosis using cuprizone-induced demyelination, CXCR2 deficiency was associated with reduced oligodendrocyte loss, suggesting a role for CXCR2+ neutrophils in the disease process.[89] Similarly, a role for CXCR2-mediated macrophage recruitment to atherosclerotic plaques was supported by the finding that CXCR2-null mice on the LDL-deficient background have reduced plaque development when fed a high-fat diet.[90]

CHEMOKINE RECEPTOR ANTAGONISTS

The past decade has seen literally hundreds of chemokine receptor antagonist programs initiated against a broad range of the receptors by many of the major pharmaceutical and biotechnology companies. There have been a plethora of reviews that have discussed many of these programs, and thus, we will concentrate here only on those inhibitors, around 40 or so, that have at one time or another progressed into human clinical trials (Table 1). Most of these antagonists are small molecule allosteric receptor inhibitors, although neutralizing antibodies, peptides, and even nucleic acid approaches have also been described (Table 1).

CCR1 Antagonists. As far as we are aware, eight CCR1 antagonists have been in human clinical trials (Table 1 and Figure 3). One of the first was a compound from Berlex, BX 471 (**1**, Figure 3), which is a potent diacylpiperazine that is more than 1000-fold selective for CCR1.[91] The antagonist has a reported K_D of 1.0 nM for human CCR1 calculated from radiolabeled binding studies and was poorly cross-reactive with rat and mouse, CCR1 (K_D of 121, and 200 nM respectively). Nevertheless, the antagonist **1** had sufficient affinity that it could be tested in animal models and it was efficacious in an acute rat EAE model of multiple sclerosis, a rat heart transplant model, a mouse model of renal fibrosis, and a mouse model of multiple myeloma.[91] On the basis of these data, the antagonist entered phase I clinical trials. Although the safety profile of compound **1** was excellent, it had a relatively short biological half-life in humans ($t_{1/2} \approx 2.3$ h) and an extended release tablet had to be formulated that allowed three times a day dosing.[91] The CCR1 antagonist entered phase II clinical trials for multiple sclerosis in early 2004. Although the drug was well tolerated and showed no safety concerns, its development was stopped after the clinical phase II study failed to show a reduction in the number of new inflammatory CNS lesions (detected by magnetic resonance imaging).[91] In addition the CCR1 antagonist **1** was tested in phase II clinical trials for psoriasis and endometriosis but was not efficacious in either.[92]

Millennium has reported a number of CCR1 antagonists including MLN3701 (structure not disclosed) which was co-developed with Sanofi-Aventis (AVE9897) for multiple sclerosis, but no structures or data were ever published.[93] The most advanced CCR1 program from the company is MLN3897 (**2**, Figure 3).[94] This antagonist is a substituted pyridylbenzoxepine of a series previously described by this group and optimized from a tricyclic nonspecific CCR1 antagonist originally described by Berlex researchers.[95] It demonstrated high affinity binding for CCR1 (K_i = 2.3 nM) and had an IC_{50} of 3.4 nM for the inhibition of chemotaxis induced by CCL3. Compound **2** was effective in vivo and demonstrated an EC_{50} of 0.03 mg/kg in inhibiting CCL3 induced immune cell recruitment in a guinea pig skin sensitization model (pharmacokinetic studies revealed that it had a half-life of 3 h in rat and oral bioavailability of 35% in rat and 100% in dog). Recently compound **2** was shown to be able to impair osteoclastogenesis and to inhibit the interaction of multiple myeloma cells with osteoclasts, thus demonstrating a potential utility in treating multiple myeloma.[96] In 2004

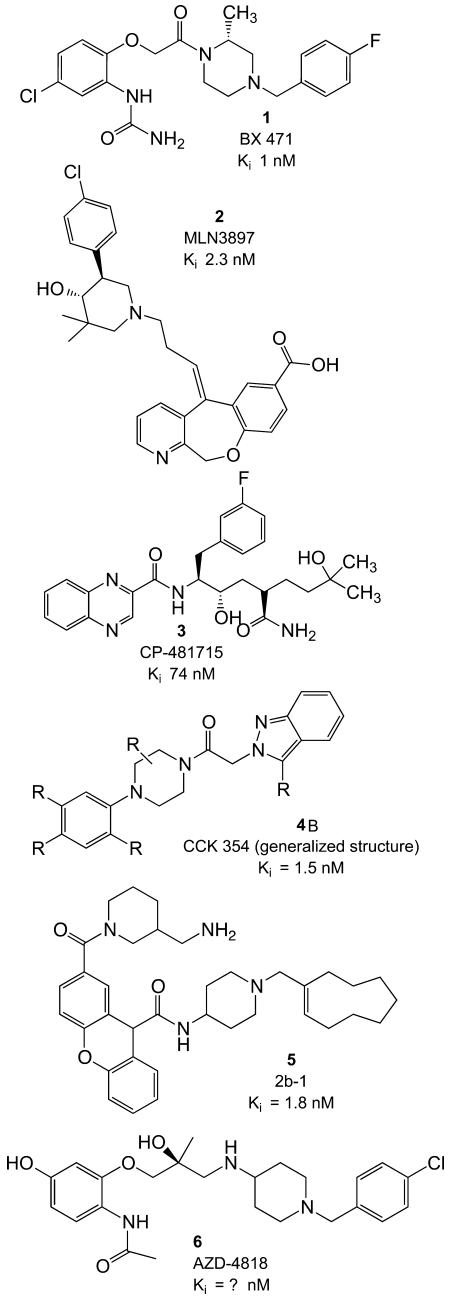

Figure 3. CCR1 antagonists.

Millennium announced that they were in phase I clinical trials with compound **2** and the major indications appeared to be rheumatoid arthritis and multiple myeloma. However, in November 2007 Millennium announced that they were terminating the development of compound **2** for rheumatoid arthritis because it had failed to reach its clinical end point in a phase II trial.[97]

Pfizer scientists discovered that a quinoline carboxamide containing a hydroxyethylene peptide isostere was a weak 2.3 μM CCR1 inhibitor.[98] Optimization of this weak hit included replacing the cyclohexyl ring with a phenyl ring and adding an extra nitrogen to the quinoline ring that yielded a 34-fold improvement in potency to 64 nM.[98] This template underwent further optimization to increase its metabolic stability and pharmacokinetic properties finally yielding CP-481715, a quinoxaline-2-carboxylic acid derivative (**3**, Figure 3).[98] The CCR1 inhibitor is a competitive and reversible antagonist and is more than 100-fold selective for CCR1 as compared to a panel of G-protein-coupled receptors.[98] Unfortunately, compound **3** is species specific for human CCR1, precluding its evaluation in classical animal models of disease. To circumvent this problem, Pfizer researchers generated transgenic mice expressing human CCR1 and demonstrated efficacy in models of inflammation in these animals.[99] Compound **3** successfully completed phase I safety studies and demonstrated efficacy in a 16-patient phase Ib clinical trial.[98] On the basis of these data, compound **3** entered phase II studies, but the trial was stopped after 6 weeks because the compound did not demonstrate any efficacy.[98]

Chemocentryx has disclosed a number of CCR1 inhibitors in patent applications including a series of azaindazole compounds.[100] Presumably CCX354 **4A** (structure not disclosed), which is their lead compound and is currently in phase II clinical trials,[101] is a member of this class of compounds, and a generalized structure is shown (**4B**, Figure 3). Compound **4A** blocked radiolabeled binding of CCL15 to human monocytes with a K_i of 1.5 nM and was a fully functional antagonist inhibiting CCL15 mediated chemotaxis in THP-1 cells (IC_{50} of 1.4 nM), even in the presence of 100% human serum.[101] In addition, compound **4A** also blocked the chemotaxis of THP-1 cells that were induced with synovial fluid from rheumatoid arthritis patients. The antagonist was specific for CCR1 and had no effect on the induction of chemotaxis through 13 other chemokine receptors at concentrations of up to 10 μM. The antagonist was active in vivo, since it was able to block leukocyte trafficking in two animal models (thioglycollate-induced peritonitis in rats and LPS-induced synovitis in rabbits). On the basis of these favorable animal data, compound **4A** was evaluated in a human phase I clinical trial for safety. The antagonist appeared to be well tolerated, and no serious adverse events were reported. It displayed linear dose−response kinetics at all doses up to 300 mg ($t_{1/2}$ = 7 h), and steady state plasma levels fluctuated from 0.5 to 1.3 uM. Phase II clinical trial data for compound **4A** reported at the recent American College of Rheumatology meetings showed that at a once daily dose of 200 mg the antagonist was safe and well tolerated by patients with rheumatoid arthritis. Furthermore the compound reached its clinical end points in the study (reduction in disease score and in the levels of proinflammatory markers).[102]

Merck entered phase II studies with two CCR1 antagonists, C-6448 (structure not disclosed) for multiple sclerosis and C-4462 (structure not disclosed) for rheumatoid arthritis.[103] Although little has been reported about these programs, it is possible the compounds are xanthene carboxamides from the CCR1 antagonist program developed by Banyu (Merck's subsidiary in Japan),[104] and a prototypical structure is shown (**5**, Figure 3). Both programs were listed in the Merck annual report for 2004 as being in phase II clinical trials;[103] however, neither compound was listed in the Merck annual report for 2005 and no further mention of these compounds has since appeared.

AstraZeneca identified CCR1 antagonists from its screening of an in-house library, and optimization yielded the clinical candidate AZD4818 a spirocyclic piperidine derivative (**6**, Figure 3).[105] The drug inhibited CCL3 binding to human mouse and rat CCR1 receptors (no affinity reported) and blocked chemotaxis of human monocytes.[105] On the basis of these and other nonreported data, the CCR1 antagonist entered clinical trials for the treatment of chronic obstructive pulmonary disease (COPD).[105] The CCR1 antagonist was

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given by inhalation at a dose of 300 μg twice daily for 4 weeks to patients with COPD. Although the drug was well tolerated, it failed to meet its clinical end points and there was no benefit to COPD patients treated with the antagonist compared to patients that were given placebo.[105]

CCR2 Antagonists. Seven CCR2 inhibitors including small molecule receptor antagonists and neutralizing antibodies to the receptor have been evaluated in clinical trials (Table 1 and Figure 4).

Merck has disclosed a variety of CCR2 antagonists[106−108] including the structure of MK-0812, a potent CCR2 antagonist (IC_{50} of 5.0 nM) with four chiral centers featuring a tetrahydro-3-trifluoromethyl-1,6-naphthyridine nucleus (**7**, Figure 4).[108] The molecule was their clinical candidate in phase II clinical trials for both rheumatoid arthritis and multiple sclerosis.[91] The CCR2 antagonist failed to show any significant improvement compared to placebo for any of the end points studied.[91] 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 new GD-enhancing lesions by MRI.[109] Merck has so far not reported any data for this study, and all reference to the program has been removed from the company's Web site and from its pipeline. However, it is possible that there is still some interest in the program, perhaps in the area of pain relief. In support of this are the findings that CCR2 knockout mice show a marked attenuation of pain responses[110] and that compounds belonging to this series of antagonists are effective in rat models of pain.[111]

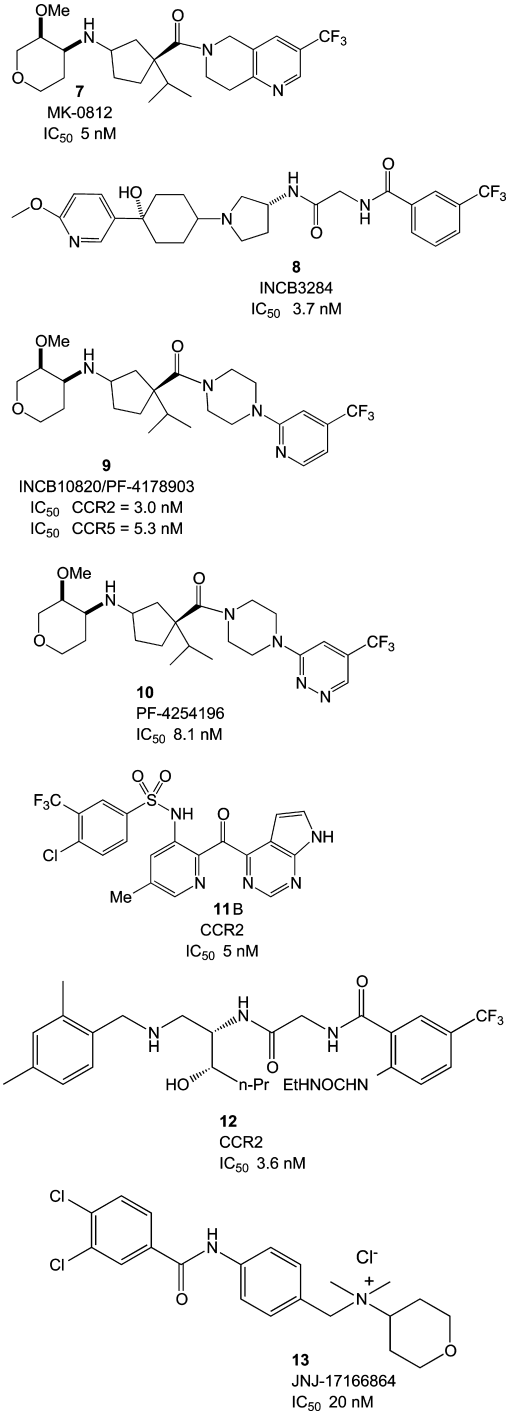

Figure 4. CCR2 antagonists.

Incyte has disclosed a number of CCR2 antagonists,[112−116] some of which have been developed jointly with Pfizer.[114−116] The lead series are 3-aminopyrrolidine derivatives exemplified by INCB3284 (**8**, Figure 4), which was one of their clinical compounds in phase II clinical studies for multiple sclerosis and lupus.[113] Previously Incyte had reported that INCB3344 (*N*-[2-[[(3*S*,4*S*)-1-[4-(1,3-benzodioxol-5-yl)-4-hydroxycyclohexyl]-4-ethoxypyrrolidin-3-yl]amino]-2-oxoethyl]-3-(trifluoromethyl)-benzamide), another potent 3-aminopyrrolidine derivative, was efficacious in an EAE model of multiple sclerosis in mice and reduced inflammation in a rat adjuvant induced model of arthritis.[112] Neither compound **8** nor INCB8696[117] (structure not disclosed), another clinical CCR2 antagonist, is currently reported in the company's pipeline, suggesting either that the clinical trials were not successful or that the company is no longer pursuing these projects for business reasons.

In addition to these compounds Incyte reported the joint development of CCR2 antagonists with Pfizer. One of the lead candidates PF-4136309 (structure not disclosed) has been reported to be in phase II clinical trials for neurophathic pain;[118] however, no structure was ever revealed and the compound is no longer reported in Pfizer's pipeline. Incyte also recently disclosed INCB10820/PF-4178903, which was jointly developed with Pfizer and is a dual CCR2/CCR5 antagonist with an IC_{50} for inhibition of CCR2 and CCR5 binding of 3.0 and 5.3 nM, respectively (**9**, Figure 4).[116] This compound is structurally identical to the Merck compound **7** except that it has a trifluoromethylpyridinepiperazine ring that replaces the trifluoromethylnaphthyridine group in **7** (**7** and **9**, Figure 4). Compound **9** was initially selected as a clinical candidate based on its potency and favorable pharmacokinetic properties; however, it had some cardiovascular liabilities, an affinity of 1.7 μM for hERG (an ion channel associated with cardiovascular adverse events) that precluded further clinical development.[114] Further optimization of this template by replacement of the pyridine ring on the right-hand side of the compound (**9**, Figure 4) with a pyradizine ring resulted in PF-4254196, which had no cardiovascular liabilities (IC_{50} = 31.3 μM for hERG) and was potent for CCR2 (IC_{50} for inhibition of CCR2 binding of 8.1 nM) (**10**, Figure 4).[114]

Millennium has long had an interest in chemokines and chemokine receptors as drug targets and has developed blocking antibodies to these proteins as potential therapeutics. CCR2 neutralizing antibodies as a therapeutic to treat autoimmune diseases such as rheumatoid arthritis represent one of the company's most advanced programs in its portfolio.

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This approach has had mixed success with positive results in phase II trials for atherosclerosis[119] and multiple sclerosis[120] but negative results in a phase II trial for rheumatoid arthritis.[121] In a double-blind placebo controlled study in patients at risk for atherosclerotic cardiovascular disease, subjects were given a single infusion of 10 mg/kg MLN1202. The drug was well tolerated and fully met its primary end point of a significant reduction in C-reactive protein levels, an inflammatory biomarker associated with atherosclerosis, after a single dose of MLN1202. These results were statistically and clinically significant relative to the placebo control arm. No serious adverse events were observed in patients exposed to MLN1202. These data indicate that blockade of CCR2 could be of potential benefit in atherosclerosis. At the American Neurological Association meeting in 2007 Millennium announced that their CCR2 neutralizing antibody, 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.[120] These two positive clinical trials have been somewhat tempered by results from a phase IIa trial with MLN1202 in patients with active rheumatoid arthritis.[97,121] Thirty-two patients received three infusions over a period of 6 weeks with either placebo or MLN1202 at three doses. Treatment with MLN1202 reduced the levels of free CCR2 on CD14 monocytes, demonstrating the biologic activity of the compound. However, there was no reduction in the levels or expression of any of the synovial biomarkers and no clinical improvement was observed.

Chemocentryx has reported an interest in CCR2 antagonists in several patent applications,[122,123] and two candidate molecules have progressed to clinical trials (Table 1). The development of their first clinical candidate CCX915 (structure not disclosed) was terminated because of its poor pharmacokinetic properties in phase I clinical trials.[124] Their current clinical candidate is CCX140 (**11 A**, structure not disclosed). Although the structure of **11A** has not been reported, it is likely that it belongs to the triazolylpyridylbenzenesulfonamide class of compounds exemplified by **11B** (Figure 4), which is reported to have a potency of 5 nM in an inhibition of chemotaxis assay, excellent pharmacokinetic properties, and no cytochrome p450 issues.[123] Recently the pharmacological characterization of **11A** was reported.[125] It has an affinity of 1.5 nM measured by direct binding of radiolabeled compound to human monocytes, inhibited CCL-2 induced chemotaxis to human monocytes with an IC_{50} of 17 nM, and had no activity against a selected panel of 142 other receptors tested. **11A** was also tested at 10 μM against the major CYP isoforms and was found to lack any inhibitory activity in enzymatic assays. Assessment of inhibition of the hERG potassium channel activity by patch clamp technique showed that **11A** lacked inhibition of hERG at concentrations up to 100 μM. The compound had favorable pharmacokinetics in rodents and in dogs with $t_{1/2}$ of 2.8 and 8.7 h, respectively, and was highly bioavailable. Although the compound had poor crossreactivity in rodents, it was effective in reducing hyperglycemia and insulin sensitivity in obese human CCR2 knock-in mice. The compound is currently being evaluated in clinical trials for the treatment of type II diabetes, and favorable results from a phase II trial were recently reported.[126] In this trial a total of 159 subjects, on stable doses of the antidiabetes drug metformin, were randomized to receive placebo, 5 mg or 10 mg of **11A**, or 30 mg of pioglitazone hydrochloride once daily for 4 weeks. Fasting plasma glucose decreased dose-dependently with **11A** treatment, and hemoglobin A1c (a marker of diabetes) was significantly reduced by the higher dose of **11A**. Plasma CCL2 and circulating monocyte levels were unchanged by **11A** treatment, and the compound was generally well tolerated and safe in this study.

Bristol-Myers Squibb has been very active in the CCR2 antagonist field and has disclosed capped diaminopropionamidoglycine dipeptides, di- and trisubstituted cyclohexanes, spiroindenes, and piperidines as potent CCR2 inhibitors.[127] The company reported clinical trials for BMS-741672 (structure not disclosed), which presumably belongs to one of the class of compounds reported above. A prototypical CCR2 antagonist from this group is shown as **12** (Figure 4).[128] Two phase II clinical trials have been described for BMS-741672; one was a double blind study for the treatment of neuropathic pain associated with type II diabetes, and the clinical end points were a reduction in pain score.[129] The second trial was a 12-week randomized double blind study for the treatment of type II diabetes in which patients were treated with placebo or with 50 mg of CCR2 antagonist given once a day.[130] The clinical end point was the reduction of glycated hemoglobins (a marker of diabetes). Both clinical studies ended in 2009; however, no reports were ever issued.

A number of CCR2 antagonists have been disclosed by Johnson & Johnson including indole substituted dipiperidines and azetidinylcyclohexanes.[131−133] But perhaps their most interesting approach was the extensive SAR carried out around the Takeda dual CCR2/CCR5 antagonist to eliminate the CCR5 activity and discover a specific CCR2 antagonist.[131] This led to the identification of JNJ-17166864, which was highly selective for CCR2 and had a binding affinity of affinity of 20 nM (**13**, Figure 4).[131] The compound had a 100-fold reduced affinity for rodent CCR2 (IC_{50} for mouse of 2 μM) but was still able to demonstrate efficacy in two mouse models of inflammation.[131] In line with its structure as a quaternary, compound **13** demonstrated poor oral bioavailability but dosing by nasal spray allowed it to enter human clinical trials for allergic rhinitis.[134] No clinical data from this trial have ever been revealed, but it is clear from the recent flurry of activity describing CCR2 antagonists that the company is still interested in this receptor as a drug target.

CCR3 Antagonists. Several pharmaceutical companies have disclosed CCR3 antagonists, and six have been described in human clinical trials (Table 1 and Figure 5).

Dupont Merck described a series of 4-benzylpiperidine analogues with good potency for CCR3 but with limited selectivity. Subsequent SAR resulted in several compounds with midpicomolar to high picomolar range antagonism in both calcium mobilization and chemotaxis assays.[135] Replacement of the 4-benzylpiperidine with a 3-benzylpiperidine and the phenyl linker with a cyclohexyl linker resulted in the discovery of DPC168 with inhibition of CCR3 binding of 2.0 nM and picomolar activity in CCL11-induced chemotaxis assays (**14**, Figure 5).[136] Compound **14** was found to have limited oral bioavailability in mice and reduced eosinophil recruitment into the lungs in a dose-dependent manner following allergen challenge. Further development of compound **14** was discontinued however because of potent cytochrome P450 2D6 and hERG activity (IC_{50} of 40 and 400 nM, respectively).

Dupont sold its pharmaceutical business in 2004, and the CCR3 program was acquired by BMS who took over development of this project. They made substantial progress

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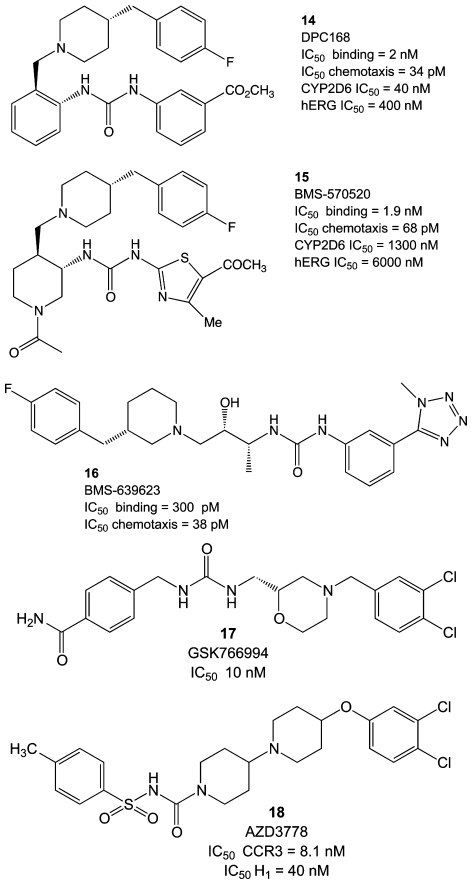

Figure 5. CCR3 antagonists.

in overcoming the p450 and hERG channel activity by replacing the cyclohexyl central ring of **14** with saturated heterocycles, which maintained potency for CCR3 with improved selectivity against CYP2D6 and hERG activities. BMS-570520 emerged as the analogue with the best overall balance of selectivity and predicted pharmacokinetic characteristics (**15**, Figure 5).[137] Further development of **15** led to the subnanomolar compound BMS-639623 (**16**, Figure 5) that is in phase I clinical development for asthma.[138]

GlaxoSmithKline reported that it was in clinical trials with GSK766994, a potent CCR3 antagonist (**17**, Figure 5). In preclinical studies this compound had good pharmacokinetics in dogs with a reported 89% bioavailability and a half-life of 2.8 h. This compound was also orally active in a Brown Norway rat model of asthma and had a potency of 10 nM for CCR3. The drug has been reported as failing to show efficacy in a phase III clinical trial for the treatment of allergic rhinitis.[139] However, development still appears to be active, since the company reported that it was conducting phase II studies in patients with asthma in early 2011, but no data have been reported as yet.[140]

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 (**18**, Figure 5).[141] A phase II clinical trial in patients with allergic rhinitis revealed that **18** exerted moderate antieosinophilic and symptom-reducing effects thought to be through inhibition of CCR3 rather than through its effects on the histaminergic receptor.[141] Since the effects of **18** were only modest, no further development of the compound has been reported (see also the section Promiscuous Chemokine Receptor Antagonists later).

Novartis had a CCR3 antagonist program and identified the compound QAP 642 (structure not disclosed) as the clinical lead[142] but has 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.[142] 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.[143]

A novel approach to inhibiting CCR3, ASM8, has been recently described by scientists at Pharmaxis.[144] ASM8 contains two modified phosphorothioate antisense oligonucleotides designed to inhibit allergic inflammation by down-regulating human CCR3 and the common β 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.[144,145] 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.

CCR4 Antagonists. In addition to its role in allergic inflammation, CCR4 is expressed on tumor cells from patients with adult T-cell leukemia (ATL),[146] an aggressive peripheral T-cell cancer caused by the human T-cell lymphotropic virus type I (HTLV-1). The disease does not respond well to conventional chemotherapeutics and thus has a poor prognosis. A number of small molecule CCR4 antagonists have been described, several of which are still in preclinical development.[147] Two CCR4 antagonists have been described in clinical studies: an antibody to the receptor and a small molecule inhibitor (Table 1).

A humanized monoclonal antibody, KW-0761, directed against CCR4 with potential anti-inflammatory and antineoplastic activities is being developed by Amgen (Table 1). KW-0761 selectively binds to and blocks the activity of CCR4, which may inhibit CCR4-mediated signal transduction pathways and therefore chemokine-mediated cellular migration and proliferation of T cells and chemokine-mediated angiogenesis. In addition, this agent may induce antibody-dependent cell-mediated cytotoxicity against CCR4-positive T cells. Initial phase I studies were carried out in relapsed patients with CCR4 positive adult T-cell leukemia-lymphoma and peripheral T-cell lymphoma. KW-0761 was tolerated at all of the dose levels tested, demonstrating potential efficacy against relapsed CCR4-positive ATL.[148] On the basis of the positive phase I study, a phase II study of KW-0761 in CCR4-positive ATL patients was carried out to evaluate the efficacy of the drug. Twenty-eight patients were enrolled in a multicenter study and received intravenous infusions of KW-0761 once per week for 8 weeks at a dose of 1.0 mg/kg. KW-0761 demonstrated clinically meaningful antitumor activity in patients with relapsed ATL.[149] The most common adverse events were infusion reactions and skin rashes, which were manageable and reversible in all cases.

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A number of companies have described CCR4 antagonists, and these have been recently reviewed.[147] All of these molecules are still preclinical, but GSK recently disclosed a series of indazolesulfonamides[150] and identified a clinical development compound, (GSK2239633) (**19**, Figure 6) that

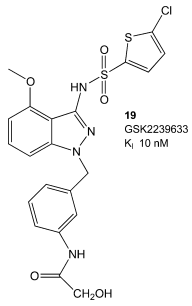

Figure 6. CCR4 antagonists.

has entered phase I clinical trials presumably for allergy and asthma.[151] The pharmacokinetics of the compound were tested in rats and in beagle dogs with a half-life of 2.5 and 2.6 h, respectively, and oral bioavailability of 85% and 97%, respectively. The authors in the study pointed out that they did not generate any in vivo pharmacodynamic data for compound **19** in animals, citing differences in immunology between animals and humans and the complexity of chemokine systems across species. They did not reveal whether the antagonist cross-reacted with rodent CCR4 receptors. In addition they noted that in their opinion the lack of convincing evidence that CCR4 can drive inflammation in animal models of asthma rendered such studies moot. Instead the authors regarded data from in vitro studies using target human CD4+ CCR4+ and CD45RO+ T cells and biopsies taken from atopic asthmatics, supernatants from which were used to induce chemotaxis of autologous memory T-lymphocytes, as more relevant to confirm the role of CCR4 in asthma.

CCR5 Antagonists. Although CCR5 was initially viewed as a target for autoimmune diseases such as rheumatoid arthritis and multiple sclerosis (discussed earlier), it quickly attracted attention from the pharmaceutical industry because of its role as an entry factor for macrophage-tropic strains of HIV-1.[152] Indeed all of the CCR5 small molecule antagonists currently in the clinic are antiviral inhibitors of CCR5 (Table 1 and Figure 7).

To date, only one small molecule antagonist of CCR5, maraviroc, originally known as UK-427,857 (**20**, Figure 7), has made it as an approved drug.[153] The discovery of the drug came at the end of a long screening and optimization process. This initially involved high throughput screening of the Pfizer compound library with a chemokine radioligand binding assay. Initial hits from this screen included compounds such as the imidazopyridine UK107,543; however, these compounds were not ideal because although they inhibited CCL4 binding to CCR5, they had no measurable antiviral activity.[154] Subsequent work was directed at optimizing these compounds to produce novel, selective ligands with enhanced ligand efficiency.[154] This necessitated the replacement of the imidazopyridine moiety to avoid inhibition of cytochrome p450 2D6. Further optimization was focused upon reducing the lipophilicity of the series by introducing a variety of amide substituents. Among the resulting series of analogues, benzamide, isopropylamide, and cyclobutylamide were identified with potency in ligand binding

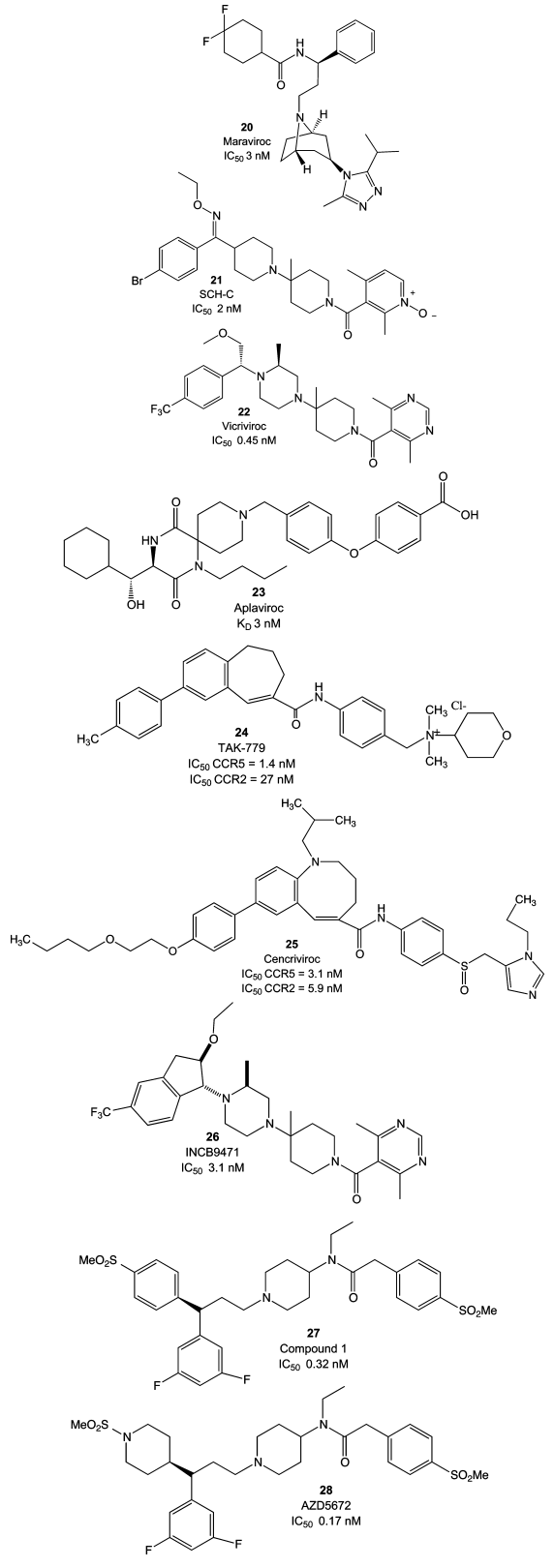

Figure 7. CCR5 antagonists.

assays; however, this did not necessarily correlate with antiviral activity.[154] After SAR analysis of over 1000 compounds, compound **20** was discovered.

Compound **20** is potent against all known CCR5-trophic HIV-1 strain, with a mean IC_{90} of 2 nM in antiviral assays.[155] It

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was also active against 200 clinically derived HIV-1 envelope pseudoviruses, many of which were from viruses that were resistant to other HIV treatments. Unlike the earlier analogues in the series, compound **20** had no detectable in vitro issues, notably a lack of activity for the hERG ion channel. Subsequent phase IIb/III studies found the antagonist to be efficacious in reducing the viral load at 48 weeks in CCR5-trophic HIV-1 infected patients.[156] Once bound to CCR5, compound **20** appears to have a long dissociation time because in monotherapy studies over 60% of receptors remained occupied 5 days after the discontinuation of treatment.[156] The CCR5 antagonist is currently approved by the U.S. Food and Drug Administration for use in combination with other antiretroviral agents in patients infected with multidrug-resistant, CCR5-tropic HIV- 1.[157]

To search for CCR5 antagonists, scientists at Schering-Plough screened for hits with high-throughput CCL5 competition binding assays and then tested interesting compounds in viral entry assays. Screening of the compound library led to the identification of a CCR5 antagonist, compound **2**, which had modest affinity for the receptor (K_i = 1.0 μM) but was also a potent muscarinic M_2 receptor antagonist (M_2 K_i = 1.3 nM).[158] Extensive SAR that involved replacing the unsymmetrical nicotinamide *N*-oxide moiety with a 4,6-dimethylpyrimidine-5-carboxamide gave a molecule that had improved potency for CCR5 and reduced affinity for the muscarinic receptors.[159] Subsequent SAR investigations using the knowledge gained from this approach yielded a low molecular weight early lead, compound **3** (CCR5 K_i = 66 nM; M2 K_i = 1323 nM). Further SAR development of oximinopiperidinopiperidineamides derived from this series led to the discovery of the clinical candidate SCH 351125 also known as SCH-C (**21**, Figure 7). This compound had potent activity in CCL5 displacement assays (K_i of 2 nM), subnanomolar activity in the blockade of viral entry (IC_{50} of 0.6 nM against the HIV-1 reporter virus ADA), and good oral bioavailability in rats, dogs, and monkeys.[158] Unfortunately phase I studies were suspended in part because of the affinity of the antagonist for the hERG ion channel. Inhibition of this channel in human phase I studies led to QTc prolongation in patients, increasing their risk of developing ventricular arrhythmias and potentially resulting in sudden death.[160]

Parallel SAR studies of an earlier lead compound resulted in the discovery of SCH-417690, also known as SCH-D (vicriviroc), a methoxymethyl analogue, which had improved receptor selectivity and notably a reduced affinity for hERG (**22**, Figure 7).[161] On the basis of these favorable data, compound **22** entered clinical trials for treatment of AIDS. Unfortunately the development of the drug experienced multiple problems; initially a phase II study in treatment-naive individuals was halted in 2005 because individuals who first experienced viral suppression when taking the drug quickly experienced viral rebound.[162] Although a recent study indicated that the antagonist demonstrated potent virologic suppression in HIV-1-infected, treatment-experienced patients, increased liver malignancies were observed in the drug treated group.[163] Subsequent to these data Merck acquired Schering-Plough in 2009 and a recent communication from the company stated that all further development of compound **22** had ceased and the program was terminated.[164]

In common with other major pharmaceutical companies GSK also had an interest in CCR5 antagonists. Their most advanced compound GW873410 (aplaviroc) is a spiroketopiperazine obtained from collaboration with Ono and originally designated as ONO-4128 (**23**, Figure 7) discovered from their spiroketopiperazine program.[165] The compound was a potent CCR5 inhibitor and blocked the binding of CCL3 and CCL4 to CCR5 (K_D of 3.0 nM) but interestingly had almost no effect on inhibiting the binding of the CCR5 ligand CCL5.[165] In addition compound **23** potently blocked the binding of a wide spectrum of laboratory and primary R5 HIV isolates (50% inhibitory concentration values of 0.1−0.6 nM). Compound **23** demonstrates a slow receptor off rate with a half-life of receptor dissociation exceeding 150 h in vitro.[166] Pharmacokinetic studies revealed favorable oral bioavailability in monkeys of 30%.[166] On the basis of these and other favorable preclinical data, it entered clinical trials as a viral entry inhibitor.

Compound **23** completed phase I safety studies, and single and repeat doses of 50−800 mg were well tolerated, with no serious adverse events. No specific trends in laboratory parameters or clinically significant ECG changes were noted. The pharmacokinetic and safety profile supported the continued investigation of 873140 with HIV-infected subjects. Unfortunately, however, phase II trials for the antagonist were halted in 2005 because of serious liver toxicity.[167] GSK tried to salvage the drug by continuing phase III trials with treatment-experienced patients and monitoring for liver toxicity; however, these trials were recently terminated because of poor efficacy and GSK stopped the further development of compound **23** and abandoned the program.[168]

Takeda has disclosed a number of CCR5 antagonists obtained from HTS screening of an inhibition of CCL5 binding assay of the Takeda compound library. One of these compounds, TAK-779, an anilide derivative with a quaternary ammonium group, had good in vitro activity in an inhibition of HIV entry assay and displayed nanomolar affinity for CCR5 as well as CCR2 in binding assays (**24**, Figure 7).[169,170] Unfortunately **24** had poor oral bioavailability in part due to the quaternary ammonium moiety.[171] Extensive chemical optimization included replacing the quaternary ammonium group of the compound with a polar sulfoxide moiety, replacement of the [6,7]-fused 1-benzazepine nucleus to a (6,8)-fused nuclei, and substitution of a 4-(2-butoxyethoxy) group for the methyl group.[171−173] These changes led to the identification of the clinical candidate TAK-652 (cenciviroc) (**25**, Figure 7), which was a potent, metabolically stable, and orally bioavailable CCR5 antagonist.[171] Compound **25** blocked the binding of CCR5 chemokines with an IC_{50} of 3.0 nM for CCL3. It also inhibited CCL2 binding to CCR2 with an IC_{50} of 5.9 nM, demonstrating that it was a dual CCR2/CCR5 antagonist.[171] The compound was active against HIV-1 clinical isolates with an EC_{50} of 61 pM.[171]

Phase I clinical studies in healthy volunteers have shown the drug to be safe, with a mean half-life of 35−40 h, supporting once a day dosing.[171] Currently compound **25** is in phase II clinical trials to treat AIDS and Tobira Therapeutics has obtained the exclusive worldwide rights to develop, manufacture, and commercialize this anti-HIV drug.[174] A dose-escalating study to assess the antiviral activity, pharmacokinetics, safety, and tolerability of an oral once-daily monotherapy of the drug in HIV-1-infected, antiretroviral-treatment-experienced, CCR5-antagonist-naive subjects has just been reported.[175] The study found that compound **25** caused significant reductions in HIV-1 RNA at all doses tested. The drug was well tolerated with no dose-limiting adverse events and demonstrated potential as a once-daily oral CCR5

antagonist. Interestingly **25** also appears to have clinical potential as a CCR2 receptor antagonist, since it demonstrated strong CCR2 antagonism manifested as significant CCL2 increases in all subjects tested. The increases in CCL2 would have the effect of down-regulating CCR2 by inducing receptor internalization.

Incyte has disclosed CCR5 antagonists for the treatment of AIDS.[176,177] Its approach included rational drug design around existing CCR5 literature compounds especially the inhibitors designed by Schering-Plough, which are potent antiviral CCR5 inhibitors with good pharmacokinetic properties (see **21** and **22**, Figure 7). On the basis of the idea that the phenyl ring on the left-hand side of compound **22** is a critical component of its activity, they carried out extensive SAR on this region of the molecule and discovered that a benzene-fused bicyclic system such as an indane could be beneficial to the molecular properties of the antagonist if it was connected to the piperazine nitrogen. This molecule constituted the starting point of further optimization that resulted in the discovery of INCB9471 (**26**, Figure 7).[176] The antagonist demonstrated fast on rates and slow off rates for CCR5 with an overall K_D of 3.1 nM in human peripheral blood mononuclear cells. It was a potent CCR5 antagonist and inhibited calcium transients induced by CCR5 agonists with an IC_{50} of 16 nM. Compound **26** was highly selective for CCR5 and did not cross-react with any other chemokine receptors or GPCRs tested. Kinetic studies indicated that **26** is an allosteric noncompetitive CCR5 inhibitor and has highly potent anti-HIV-1 activity with an IC_{90} of 9.0 nM for all M5 viruses, whereas it is inactive against cells infected with X4 HIV-1 strains. Furthermore **26** was found to be a potent inhibitor of mutant HIV-1 variants that are resistant to other drugs. The antagonist inhibited hERG with an IC_{50} of 4.5 μM, which is 500 times above its mean antiviral IC_{90} value. The compound is not a cytochrome p450 inhibitor, with IC_{50} values greater than 25 μM against the five major cytochrome p450 isozymes tested. In vivo, the antagonist exhibited good oral bioavailability in rats (F = 100%) and in dogs (F = 95%), and this coupled with its excellent half-life suggested the possibility of once a day dosing in humans. On the basis of these studies together with its safety in toxicology studies in rodents and primates, the compound was advanced into human clinical trials.

Results from a 14-day placebo-controlled phase IIa study, which involved a total of 23 HIV-infected patients, demonstrated that compound **26** was safe and well tolerated with no clinically significant chemistry, hematology, or ECG changes compared to placebo patients. Once a day oral dosing with 200 mg of compound **26** had prolonged antiviral effects in HIV patients infected with R5-tropic virus. Consistent with the long half-life of the molecule ($T_{1/2}$ = 58−60 h), treated patients continued to show evidence of viral suppression 2 weeks after their last dose of drug.[178] Despite these promising data, Incyte announced its decision to halt all further internal development of its CCR5 antagonist to allow for focus on their JAK kinase inhibitors for the treatment of cancer.[179]

AstraZeneca has disclosed a number of CCR5 antagonists including a series of substituted 1-(3,3-diphenylpropyl)-piperidinephenylacetamides.[180−182] Optimization of this series yielded compound **1** (**27**, Figure 7) that had excellent CCR5 potency (binding IC_{50} = 0.32 nM) and selectivity together with good oral pharmacokinetic profiles in rat and dog. However, **27** showed moderate activity against the hERG ion channel, an indicator of cardiotoxicity risk, and its cardiac safety margin (250-fold) was judged to be insufficient for further development. However, further optimization of compound **27** revealed that replacing the upper phenyl ring in the diphenylpropyl group with a C-linked piperidine reduced hERG activity and gave a much improved cardiac safety margin (6400-fold).[183] The resulting compound AZD5672 (**28** Figure 7) had excellent CCR5 potency (binding K_i = 0.17 nM) and excellent pharmacokinetic properties that merited its further development as a clinical compound. Compound **28** was subsequently tested in a phase IIb study in patients with rheumatoid arthritis.[184] Although the compound had excellent, once daily oral pharmacokinetic properties and exhibited high levels of receptor occupancy and maximal inhibition of CCR5 as confirmed by an ex vivo pharmacodynamic assay (% CCR5 internalized following ex vivo stimulation with 100 nM CCL4), it had no efficacy.[184]

Two companies, Progenics and Human Genome Sciences, have described monoclonal antibodies as inhibitors of CCR5 function. Progenics is developing a humanized monoclonal antibody to CCR5 (PRO 140) as a potent inhibitor of viral entry for the treatment of X5 HIV-1 infected individuals. A small phase IIa clinical trial evaluated the pharmacokinetics, antiviral activity, and tolerability of PRO 140 in 31 HIV positive adults randomly assigned to receive single 5 or 10 mg/kg iv doses or placebo. A single intravenous dose of PRO 140 reduced virus by nearly 100-fold for up to 10 days, and the drug was found to be safe and generally well tolerated with no serious drug-related adverse events or dose-limiting toxicities.[185] In further studies, the investigators demonstrated that 10 mg/kg iv and 324 mg subcutaneous forms of PRO 140 had similar efficacy. Given its antiviral equivalence, Progenics has chosen the subcutaneous formulation of PRO 140 for further development because it can potentially be self-administered by patients. The drug is continuing its development in further clinical trials.

Human Genome Sciences has described a human IgG4 monoclonal antibody against CCR5, HGS004, that is a potent inhibitor of all three of the CCR5 ligands CCL3, CCL4, and CCL5, and in addition the antibody has potent HIV co-receptor activity[186] Phase 1 clinical studies demonstrated that HGS004 was safe and well tolerated by patients.[186] However, the nonlinear pharmacokinetics exhibited by HGS004 indicated that its anti-HIV potency as a single agent might be suboptimal. Of further concern was the finding that some patients treated with high doses of HGS004 showed a switch from CCR5- to CXCR4-trophism. This change in chemokine coreceptor usage by the virus could be deleterious in the treatment of HIV patients because when it occurs during the natural course of HIV infection, it usually correlates with subsequent disease progression and a poorer prognosis.

Novartis is in phase I clinical trials with a CCR5 antagonist (Table 1).[187] This inhibitor is a dual CCR2/CCR5 antagonist and will be covered later in the section under promiscuous antagonists.

A novel approach to target CCR5 is that taken by Sangamo Science which uses zinc finger nuclease technology to cut DNA sequences.[188] They are in phase II clinical trials to treat AIDS with SB-728 a zinc finger nuclease that modifies the gene encoding CCR5, thus knocking out the chemokine receptor and recapitulating the HIV resistance manifested by individuals that express the naturally occurring CCR5-Δ32 mutation.[189]

CCR9 Antagonists. CCR9 is selectively expressed by thymocytes, small intestinal lamina propria lymphocytes, and

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intraepithelial lymphocytes and binds the ligand CCL25/TECK.[5] CCR9+ peripheral blood T lymphocytes have been reported to be markedly elevated in patients with diseases of the small bowel that correlates with increased expression of CCL25, suggestive of a role for the CCR9/CCL25 axis in Crohn's disease.[190] These data prompted Chemocentryx to develop CCR9 antagonists for the treatment of Crohn's disease, and Chemocentryx has filed a number of patents claiming CCR9 antagonists including arylsulfonamides.[191] The clinical candidate CCX282 from this program (**29**, Figure 8)[192] has

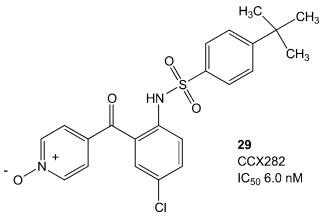

Figure 8. CCR9 antagonists.

been reported to have an IC_{50} of 6 nM in direct binding experiments with radiolabeled compound.[193] The compound inhibits CCL25 induced chemotaxis in cells expressing CCR9 with an IC_{50} of 2.8 nM and has clear selectivity against a panel of 25 other GPCRs tested. Oral administration of **29** was efficacious in murine models of ileal Crohn's disease and ulcerative colitis.[193] In a 4-week phase II clinical trial of patients with moderate-to-severe Crohn's disease, a single daily dose of 250 mg of compound **29** was well tolerated and displayed clear signs of clinical activity as recorded by a reduction in blood levels of C-reactive protein.[194] In 2006, a phase II/III clinical trial to further assess the safety and efficacy of the antagonist in patients with moderate to severe Crohn's disease was initiated. The study revealed that the 500 mg once daily dose of **29** in patients with small bowel and/or colonic Crohn's disease was consistently superior to placebo across multiple efficacy end points. Recently GlaxoSmithKline exercised its option to obtain an exclusive license to compound **29** and is now solely responsible for all further clinical development of the drug (renamed vercirnon) that is currently in four pivotal phase III clinical trials.

CXCR1 and CXCR2 Antagonists. The pathophysiological role played by CXCL8 receptors has generated considerable interest by pharmaceutical companies, and several small molecule inhibitors of the receptors have been described (Figure 9).

A phenol containing diarylurea SB 225002 was the first small molecule chemokine receptor antagonist to be described in the literature (**30**, Figure 9).[195] It is an antagonist of CXCL8 binding to CXCR2 with an IC_{50} of 22 nM and showed >150-fold selectivity over other GPCRs tested. In vitro, **30** potently inhibited human and rabbit neutrophil chemotaxis induced by both CXCL8 and CXCL1. In vivo, SB 225002 selectively blocked CXCL8-induced neutrophil migration in rabbits. Early SAR evaluation indicated that substitution at both the 3 and 4 positions on the phenol ring was well tolerated.[196] However, this compound and some others from this series were not developed further because of undesirable pharmacokinetics.[196] Extensive SAR around this issue revealed that insertion of a sulfonamide group on the phenol ring greatly reduced metabolic clearance.[197] Substitution of a 3-piperidinesulfonamide moiety on the phenol ring yielded the compound SB

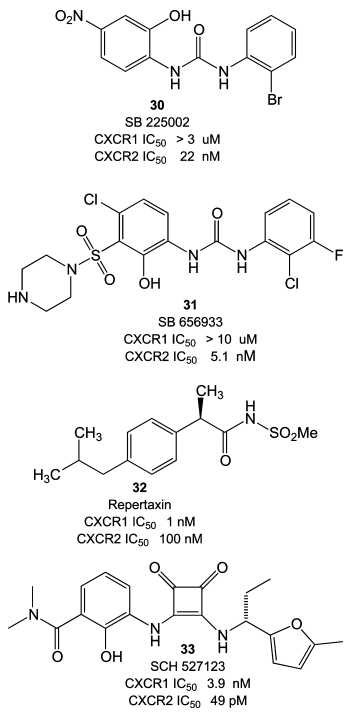

Figure 9. CXCR1 and CXCR2 antagonists.

656933 (**31**, Figure 9) with an IC_{50} of 22 nM for binding to CXCR2. Compound **31** was found to inhibit neutrophil CD11b up-regulation (IC_{50} of 260.7 nM) and shape change (IC_{50} of 310.5 nM) in patients with chronic obstructive pulmonary disease and entered clinical trials for cystic fibrosis and chronic obstructive pulmonary disease.[198]

Phase I clinical studies assessed the safety, pharmacokinetics, and pharmacodynamics of single escalating doses of **31** in healthy subjects, as well as the effect of CXCR2-selective antagonism on airway inflammation in healthy humans using an inhalation challenge model of ozone-induced airway inflammation. These two studies demonstrated that the antagonist was safe and well tolerated at all doses (2−100 mg). In addition single doses of **31** reduced ozone-induced airway inflammation in a dose-dependent manner.[198]

The observation that 2-arylpropionic acids such as ibuprofen were able to potently inhibit CXCL8-induced chemotaxis in neutrophils prompted scientists at Dompé to screen for novel potent inhibitors of CXCL8-induced chemotaxis.[199] A class of derivatives of 2-arylphenylpropionic acids was extensively investigated leading to the selection of an acylmethanesulfonamide derivative, repertaxin (**32**, Figure 9), as the lead compound.[199] Compound **32** was able to inhibit CXCL8-induced neutrophil chemotaxis with an IC_{50} of 1 nM but interestingly did not inhibit chemokine binding;[199] thus, its mechanism of action is unclear. Compound **32** is reported to be in phase II clinical trials for ischemia and reperfusion injury that is responsible for poor graft function after organ transplantation, but no published data have been forthcoming so far. In addition a recent report suggests that it may have some utility in certain forms of breast cancer.[200] Apparently the drug targets breast cancer stem cells in an NOD/SCID mouse model by inhibiting tumor growth and metastasis formation. The same group has recently begun a clinical trial combining repertaxin with chemotherapy in women with advanced breast cancer.[201]

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Structure−activity studies of a lead cyclobutenedione compound enabled scientists at Schering-Plough to identify SCH-527123 (**33**, Figure 9) as a potent, orally bioavailable dual CXCR1/CXCR2 receptor antagonist.[202] The compound had good pharmacokinetic properties and oral bioavailability in the rat. The antagonist inhibited neutrophil chemotaxis and myeloperoxidase release in response to CXCL1 and CXCL8 but had no effect on irrelevant ligands. The antagonist displayed saturable and reversible binding kinetics to both CXCR1 (K_D of 3.9 nM) and CXCR2 (K_D of 40 pM).[203] In a lipopolysaccharide-induced model of pulmonary inflammation in the mouse, oral treatment with **33** blocked both pulmonary neutrophilia and goblet cell hyperplasia.[204] In a similar model in the rat, **33** was equally as efficacious and suppressed both pulmonary neutrophilia and the increase in BAL induced by intratracheal administration of lipopolysaccharide.[204] In cynomolgus monkeys, **33** reduced the pulmonary neutrophilia induced by repeat bronchoscopy and lavage.[204]

On the basis of these data, compound **33** was recently tested in an ozone-induced airway neutrophilia clinical study in healthy subjects.[205] The drug significantly lowered sputum neutrophil counts compared with prednisolone or placebo. Comparable results were obtained for total cell count, percentage of sputum neutrophils, and interleukin-8 and myeloperoxidase in sputum supernatant. All treatments were safe and well tolerated. Further evaluation in a large trial of patients with pulmonary disorders is planned.[205]

CXCR3 Antagonists. As mentioned earlier, the chemokine receptor CXCR3 is highly expressed in activated T cells of the Th1 phenotype and is implicated in autoimmune diseases such as RA. In line with these data, a CXCR3 antagonist SCH 546738 (**34**, Figure 10) has shown utility in a mouse collagen induced arthritis model of arthritis as well as in rat and mouse experimental autoimmune encephalomyelitis models of multiple sclerosis.[206]

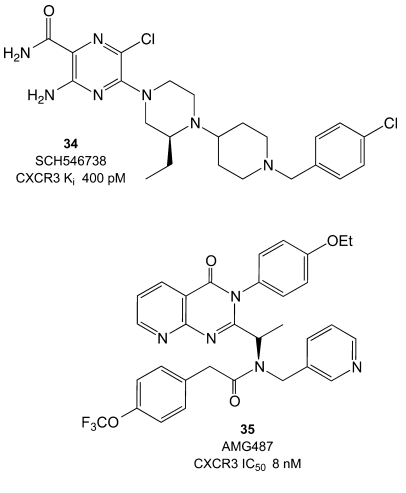

Figure 10. CXCR3 antagonists.

Chemocentryx has disclosed a number of CXCR3 antagonists including a series of dihydroquinazoline analogues.[207] Amgen exercised its option to obtain an exclusive license to the lead compound from this series and renamed it AMG 487 (**35**, Figure 10). In preclinical studies, **35** blocked immune cell migration and demonstrated excellent potency, high selectivity, and good oral bioavailability.[207] The drug dose-dependently inhibited cellular infiltration of immune cells into the lungs in a bleomycin-induced model of inflammation in mice. A twice daily dose of 3 mg/kg of compound **35** (given subcutaneously) was as effective in inhibiting immune cell migration into the lungs as that observed for CXCR3 deficient mice. The evaluation of **35** in the clinic was complicated by the discovery of significant circulating levels of a pyridine *N*-oxide active metabolite.[208] Nevertheless the compound entered phase II clinical trials for the treatment of psoriasis but failed to demonstrate any signs of efficacy, and the trial was terminated.[91]

CXCR4 Antagonists. CXCR4 is expressed by most of the leukocyte subsets, including naive T lymphocytes, B lymphocytes, monocytes, and neutrophils[5] and binds the chemokine CXCL12/SDF-1.[5] Notably, CXCR4 is used as a portal for the entry of T-tropic HIV-1 strains,[5] a finding that has greatly accelerated research in this area. CXCR4 has recently been the subject of an excellent review in this journal,[14] so our treatment here will be brief.

The first CXCR4 antagonist to be described was the bicyclam-containing small molecule AMD3100 (plerixafor) (**36**, Figure 11).[11] The antagonist was initially selected for

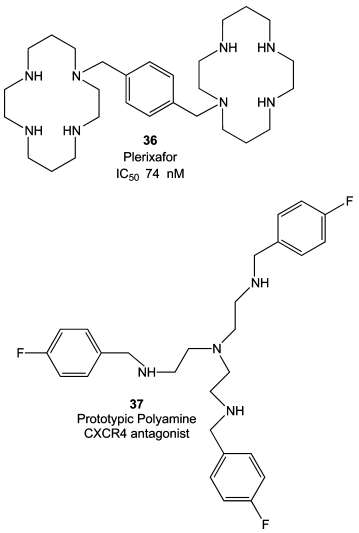

Figure 11. CXCR4 antagonists.

clinical trials for the treatment of AIDS, but although effective in vitro, it suffered from a lack of oral bioavailability.[11] Interestingly, however, the development of **36** took on an entirely different direction based on the finding that the CXCR4/CXCL12 axis plays a major role in the retention of hematopoetic stem cells and their progenitors in the bone marrow.[209] By inhibiting CXCR4, the antagonist was shown to be able to rapidly mobilize stem cells from the bone marrow, increasing their numbers in the circulation. These cells can then be harvested and given back to patients suffering from white blood cell cancers such as non-Hodgkin lymphoma.[210] Because **36** also mobilizes cancer cells in the bone marrow and these cells are a major cause of relapse in multiple myeloma, the drug could be a useful treatment of this cancer. The drug entered the clinic for both diseases and, based on strong positive data for the drug in phase III,[211] was approved by the FDA in 2008 to treat non-Hodgkin lymphoma and multiple myeloma.[12]

The success of **36** has paved the way for a number of companies to target CXCR4 for the indications above (Table 1). Foremost among these is TaiGen with their CXCR4

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antagonist TG-0054 (Burixafor). Although the structure of the drug has not yet been disclosed, TaiGen has disclosed polyamine and pyrimidines as CXCR4 antagonists in patent applications.[212] Receptor binding studies revealed potencies for CXCR4 ranging from 4 to 100 nM, and a prototypical structure is shown (**37**, Figure 11). Phase I clinical trials revealed that single-dose administration of the antagonist more than increased the number of circulating stem cells to the numbers required for a successful transplant.[213] Phase 1 results also indicated that TG-0054 may be given as a monotherapy in contrast to **36** that is given with granulocyte macrophage colony stimulating factor (GM-CSF). Currently the antagonist is undergoing several phase II clinical trials for the treatment of multiple myeloma and non-Hodgkin's lymphoma.

The CXCR4 antagonist described by Polyphor, POL6326, is derived from their proprietary technology, known as Protein Epitope Mimetics. These molecules are peptidomimetics of 0.7−2 kDa that are potent CXCR4 antagonists.[214] According to the company, POL6326 has successfully completed phase I clinical trials in the U.K. with 74 healthy volunteers. The drug was safe and well tolerated and is currently being investigated in a phase II clinical trial for safety and efficacy in transplantation of autologous hematopoetic stem cells in multiple myeloma patients after chemotherapy.

Two other CXCR4 approaches in the clinic are worth mentioning. The first is a neutralizing antibody to the receptor (MDX-1338) that shows promise in treating multiple myeloma.[215] This is currently being evaluated in phase I clinical trials as a monotherapy with chemotherapy to treat patients with relapsed or refractory acute myelogenous leukemia. Also in phase I clinical trials is a peptide (BKT140) that is reported to block CXCR4[216] (Table 1).

Potential Reasons for the Failures of Chemokine Receptor Antagonists in the Clinic. Clinical trials of chemokine receptor antagonists for the treatment of autoimmune diseases have been disappointingly unsuccessful. Out of the more than 40 development candidates for which clinical trials have been reported, only two have become registered drugs and those are in indications that are totally unrelated to inflammation (Table 1). There are numerous explanations to account for these clinical failures, including chemokine and chemokine receptor promiscuity, disease heterogeneity, insufficient receptor occupancy or blockade, incorrect clinical indications, misleading data from animal models, etc., and they have been discussed in several recent reviews.[91,98,217] The first two of these options, chemokine and chemokine receptor redundancy and disease heterogeneity, could well account for some of these clinical failures. For example, more than 40 chemokines and 19 chemokine receptors have been identified and the complexity of this vast system of immunological molecules might make it extremely difficult to demonstrate clinical efficacy with a specific antagonist to a single receptor. Consider CCR1 antagonists for the treatment of multiple sclerosis, for example; three separate clinical trials have failed to demonstrate efficacy (Table 1). The evidence for a role of CCR1 in the pathophysiology of multiple sclerosis is 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.[218] 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.[67] Third, CCR1 is expressed in human multiple sclerosis lesions associated with hematogenous macrophages usually coexpressed with CCR5.[34] Finally, a non-peptide antagonist of CCR1, **1**, was efficacious in a dose responsive manner in an acute rat EAE model of multiple sclerosis.[219]

At first glance then the idea that CCR1 plays a role in multiple sclerosis would appear to be quite strong. However, each of the studies described above has some potential caveats. For example, while it is quite true that neutralizing antibodies to the CCR1 ligand CCL3 blocked disease, this chemokine is also a ligand for CCR5[5] and we cannot rule out that the beneficial effects seen in this model could be accounted for by blocking the activity of both receptors. This idea is supported by the fact that in contrast to the almost complete inhibition of disease by neutralizing antibodies to CCL3,[218] the deletion of CCR1 only inhibited disease by 50% in the MOG model.[67] Interestingly CCR1 is almost always coexpressed with CCR5 in human multiple sclerosis lesions especially in early lesions;[34] thus, it remains a formal possibility that both CCR1 and CCR5 and potentially other chemokine receptors might play a role in the pathophysiology of the disease. Furthermore CCR1 is constitutively expressed on neutrophils in rodents but mainly on monocytes and activated T lymphocytes in humans. This clearly reflects cell type differences that could also influence the outcome of animal models of disease, and this has to be taken into consideration when extrapolating successful studies showing efficacy of a compound in an animal model of disease to the clinical disease in humans. For example, the hyperacute EAE model of multiple sclerosis in Lewis rats is characterized by a short incubation period, severe paralysis, high mortality, and abundant neutrophil infiltrates. Therefore, it remains a possibility that the positive response observed for the Berlex CCR1 antagonist in this model of multiple sclerosis could be mediated by the suppression of CCR1 expressing neutrophils, which are not the driving force of the human form of the disease.

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 also account for the observed clinical failures. The mechanisms of action of two of the clinically approved multiple sclerosis treatments target adhesion molecules that block the migration of all activated T cells (natiluzumab) or cause the retention of activated T cells in the lymph nodes where they accumulate (fingolomid), thus strongly intervening in the pathophysiology of disease.[220] In contrast blocking individual chemokine receptors on cells that can respond to more than one receptor will clearly not be as effective as either of these two therapies. Thus, the failure of chemokine receptor antagonists in clinical trials for multiple sclerosis can probably be ascribed to some combination of the issues discussed above.

A striking example of the difficulty in selecting an appropriate chemokine receptor as a target for disease intervention is provided by the failures of the CCR5 antagonists **20**, **21**, and **28** in clinical trials for rheumatoid arthritis.[184,221] Since the costs of running human clinical trials can run into hundreds of millions of dollars, pharmaceutical companies do not generally enter into them lightly. So what led three major pharmaceutical companies, Astra-Zeneca, Pfizer, and Schering-Plough, to pursue CCR5 antagonists as a potential therapy for rheumatoid arthritis?

There are several lines of evidence for a role of CCR5 in rheumatoid arthritis. First, synovial tissue from patients with rheumatoid arthritis shows abundant expression of CCR5 and its ligands.[38,39] In addition, CCR5 expression has been shown to be significantly increased on macrophages in the synovial fluid and synovial tissue of patients with rheumatoid arthritis.[49] Second, Met-RANTES, which blocks both CCR1 and CCR5, caused the amelioration of adjuvant-induced arthritis in Lewis rats.[222] Third, the small molecule CCR5 inhibitors SCH-X (structure not disclosed) and **24** were efficacious in CIA models of disease in rhesus monkeys and in mice, respectively.[223,224] Finally, individuals who have a 32 base pair deletion in the gene for CCR5 (CCR5-Δ32 allele), which abolishes receptor expression in homozygotes, appear to be protected from developing rheumatoid arthritis because this gene mutation was significantly lower in rheumatoid arthritis patients than in healthy individuals.[225]

Thus, on the basis of the studies presented above, it would appear that the evidence to target CCR5 in rheumatoid arthritis was pretty solid. However, the following points need to be considered. First, expression of CCR5 on inflamed synovial tissue is not by itself evidence for a role of the protein in the disease process, especially since a variety of other chemokine receptors such as CCR1, CCR2, and CXCR3 have all been shown to be expressed in rheumatoid tissue and could all play a role in disease pathology. Second, the therapeutic effect of the antagonist Met RANTES in the AIA-model of rheumatoid arthritis could equally well have been due to the inhibition of CCR1. Third, the CIA studies with the CCR5 inhibitors SCH-X and **24** involved small numbers of animals, 5 in the monkey study and 10 in the mouse study, and the antagonists were given prophylactically and not therapeutically. In addition the induction of arthritis in the mouse CIA model resulted in a massive leukocyte infiltration into the joints consisting mainly of neutrophils, which is clearly not consistent with the pathophysiology of the human disease. Finally, part of the rationale for treating rheumatoid arthritis patients with a CCR5 inhibitor is based on the finding that individuals expressing the Δ32 mutation of CCR5 appear to be protected from the disease. However, it is possible that the genetic deletion of CCR5 has quite different effects on the immune response than that induced by simply blocking the receptor with a small molecule inhibitor, and this may account for the failure of the antagonist in treating rheumatoid arthritis patients.

Thus, it is obvious from this discussion that the preclinical evidence for a role of CCR5 in the pathophysiology of rheumatoid arthritis is not as strong as first indicated. It is of course possible that CCR5 may not be a clinically relevant target for the treatment of rheumatoid arthritis; however, another reason to consider for the failure of these programs is the possible deleterious effect of CCR5 antagonists on Tregs (the $CD4^+CD25^+$ regulatory T cell population). Tregs express CCR5 and are important mediators of peripheral tolerance. It is known that deficiency of these cells is associated with autoimmune inflammation in some animal models of autoimmune disease. Thus, blocking CCR5 on Tregs might counterbalance the beneficial effects of inhibiting the receptor on autoreactive Th1 cells involved in the inflammation associated with rheumatoid arthritis. This topic is further discussed in the context of the dual CCR2/CCR5 inhibitors later.

A key question in any drug discovery program targeting chemokine receptors is what degree of receptor blockade is required to achieve a therapeutically beneficial effect. This is quite difficult to answer with any precision, but it seems that it needs to be greater than 90%.[98] The degree of receptor blockade for three different CCR1 antagonists in clinical trials for rheumatoid arthritis was recently compared. The three drugs were **2** and **3**, which failed to show efficacy in phase II trials,[97,98] and **4** which has demonstrated clinical efficacy in phase II trials.[102] An estimate of the degree of CCR1 coverage achieved on blood monocytes with the various CCR1 antagonists tested clinically was assessed with a monocyte chemotaxis assay. A comparison of the blood plasma levels achieved in the clinic with oral doses of these compounds revealed that the level of unoccupied CCR1 was around 17−26% for **2**, 3−6% for **3**, and 1−4% for **4**.[101] On the basis of these data, it is highly likely that the failure of **2** was partly due to an inability of the drug to block sufficient receptors to prevent CCR1 activation. The data presented suggest that almost complete antagonism is required for 24 h a day for clinical efficacy. Thus, successful agents will necessarily require excellent human pharmacokinetics and/or a very slow receptor off rate to achieve complete pharmacodynamic blockade of the receptor system being antagonized.

The failure of the Pfizer CCR1 antagonist, **3**, in clinical trials does not, however, appear to be a result of receptor occupancy. In contrast it has been speculated that it might be ascribed to the design of the clinical trial.[98] It was observed that patients in the placebo arm of this 12-week clinical trial had a higher than usual ACR20 score (an American College of Rheumatology criterion used to assess efficacy of a drug in an arthritis trial). This might have made it more difficult to get a statistically significant response in patients treated with the CCR1 antagonist compared to patients given placebo. In addition the dosing regimen and the formulation of the drug used in the failed phase II trial differed from that used in the much smaller 1b trial,[46] which had previously demonstrated efficacy of the drug in rheumatoid arthritis.[97]

Another possibility to account for the failure of chemokine receptors in clinical trials might be because the clinical indication that the drug was tested in was not therapeutically appropriate (as discussed above for CCR5 in rheumatoid arthritis). A good example of this is provided by the three failed clinical trials targeting CCR2 for the treatment of rheumatoid arthritis: two small molecule antagonists **7** and **8** and a neutralizing monoclonal antibody MLN1202 (Table 1). It is possible that these failures reflect the likelihood that CCR2 does not represent an appropriate target for rheumatoid arthritis. Indeed the evidence for a role of CCR2 in the pathophysiology of rheumatoid arthritis is somewhat contradictory. For example, CCR2 gene deletion studies in mice show that the disease score is exacerbated in a type II collagen-induced arthritis model in CCR2 knockout mice compared to their wild-type littermates.[226] In contrast a potent and selective small molecule antagonist of the mouse CCR2 receptor significantly attenuated the disease score in a rat model of adjuvant-induced arthritis.[112] Furthermore recent studies demonstrate that peripheral blood monocyte migration from rheumatoid arthritis patients in response to synovial fluid from these patients cannot be effectively blocked by targeting CCR2 or CCR5 or both but can be effectively blocked with CCR1, suggesting that blocking CCR2 is therapeutically not important for the treatment of rheumatoid arthritis.[227]

Another example of pursuing a clinically irrelevant target with a chemokine receptor antagonist is provided by the failure

of the CXCR3 antagonist **35** in clinical trials for psoriasis (Table 1). Evidence for a role of CXCR3 in the pathophysiology of psoriasis is mainly provided by the fact that CXCR3 shows strong expression in activated T cells of the Th1 phenotype. Skin biopsies from patients with psoriasis showed increased expression of the CXCR3 ligands CXCL10, CXCL9, and CXCL11, which correlated with CXCR3 expression by infiltrating T-cells, suggesting a functional interaction between locally produced chemokines and CXCR3-expressing T cells.[228] Beyond these "guilt by association studies", evidence for a role of CXCR3 in psoriasis was provided by a number of indirect studies. First, blocking activated T cells of the Th1 phenotype in an adjuvant induced peritonitis model[229] or in an allograft transplant survival model,[230] with a neutralizing CXCR3 antibody, demonstrated efficacy. Second, the CXCR3 antagonist **35** was able to inhibit cellular recruitment of immune cells in a bleomycin induced lung injury model;[207] however, this might not be enough to make a difference in psoriasis, which is driven by cell types other than CXCR3-expressing T lymphocytes.[231] Thus, in conclusion none of these indirect studies have really confirmed a role for CXCR3 in psoriasis.

Promiscuous Chemokine Receptor Antagonists. Promiscuous small molecules that are able to target several GPCRs are well-known in the literature, and this topic has been discussed in a number of excellent reviews.[232,233] The classic example of a promiscuous drug is Zyprexa, a tricyclic benzodiazepine that binds with high potency to 14 different GPCRs and that is used to treat schizophrenia and bipolar disorder.[234] Promiscuity of small molecule antagonists can, however, be somewhat of a double edged sword. On the one hand it can give rise to undesirable side effects. On the other it might be beneficial in treating complex diseases where more than one receptor plays a role. We will give examples of the pros and cons of promiscuous antagonists in drug development in this review.

A number of dual chemokine receptor antagonists have been described in the literature.[91] These are mostly to the highly related receptors CCR1 and CCR3, which share around 59% sequence identity, or to the CCR2 and CCR5 receptors which share around 72% sequence identity, but others have also been described.

Three examples of CCR1/CCR3 dual antagonists have been described in the literature. The first is a 2-(benzothiazolylthio)-acetamide compound from Takeda (**38**, Figure 12) which binds both receptors with IC_{50} of 450 and 32 nM, respectively.[235] Similarly the antagonist UCB 35625 (**39**, Figure 12) is a potent antagonist for both receptors inhibiting chemotaxis by CCL3 in CCR1 with an IC_{50} of 9.6 nM and CCL11 in CCR3 with an IC_{50} of 93.7 nM.[236] Banyu have identified several potent CCR1/CCR3 dual antagonists exemplified by compound 2q-1 (**40**, Figure 12), which is a quaternary ammonium compound with IC_{50} of 0.9 and 0.58 nM, respectively.[237]

For the CCR2/CCR5 receptors two compounds from Takeda, **24** and **25**, are potent inhibitors of this receptor pair.[169,238] Of interest here also is the recent disclosure by Incyte of compound **9**, which is a dual CCR2/CCR5 antagonist[116] and structurally similar to the Merck CCR2 antagonist compound **7**, a tetrahydro-3-trifluoromethyl-1,6-naphthyridine, which not surprisingly is also a potent CCR5 antagonist.[217]

In a recent patent application Novartis disclosed dual CCR2/CCR5 inhibitors.[239] Lead optimization using a high throughput

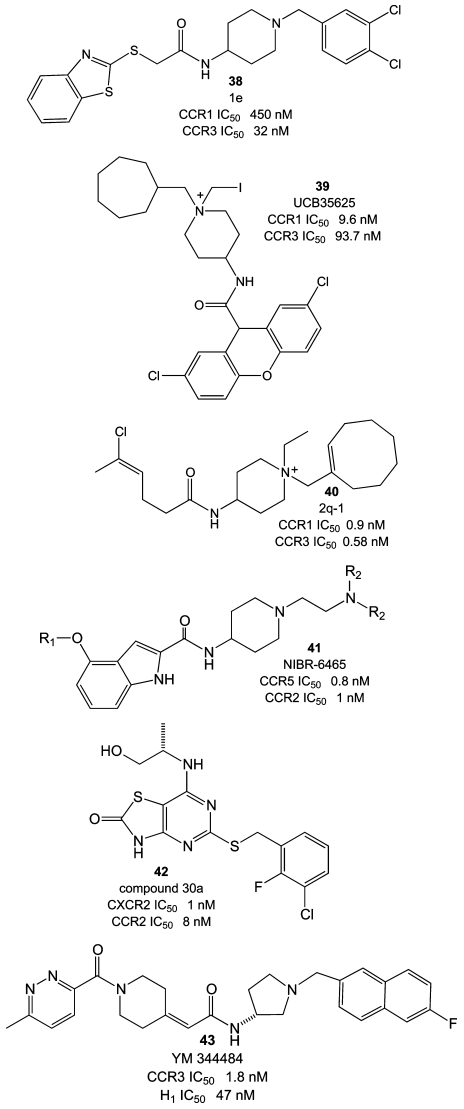

Figure 12. Promiscuous chemokine receptor antagonists.

scintillation proximity binding assay of their compound library identified a number of structures as CCR2 antagonists. One of these, a highly lipophillic benzothiophene with modest CCR2 affinity, underwent optimization to generate a series of benzoxepines that were less lipophilic and more potent CCR2 receptor antagonists. These molecules were then further optimized to generate a series of 4-substituted indoles of which NIBR-6145 was the lead compound (**41**, Figure 12).[187] This compound had high potency on human and rodent CCR2/CCR5, no cardiovascular problems (the absence of any QT prolongation in a monkey telemetry study), no interactions with any cytochrome p450 subtypes examined, and good pharmacokinetic properties with a long terminal half-life. The compound was efficacious in an in vivo peritoneal monocyte migration assay in the rat and in a mouse dextran sodium sulfate (DSS) colitis model as well as in an acute mouse EAE model of multiple sclerosis. At a dose of 3 mg/kg given orally in monkeys it easily achieved 90% or greater blockade of both CCR2 and CCR5. Recently Novartis disclosed that they had initiated clinical trials with **41** for the treatment of AIDS.[240] The phase I studies involved 56 normal subjects who were given seven ascending doses of drug. The drug was well tolerated, and there were no significant safety concerns. A

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receptor occupancy of greater than 90% was achieved for both CCR2 and CCR5 receptors. On the basis of this positive outcome, the drug is continuing its clinical development.

The promiscuous antagonists discussed so far all bind to highly related chemokine receptors such as the CCR1/CCR3 and CCR2/CCR5 type; however, there are examples of antagonists that bind totally unrelated chemokine receptors illustrated by the recent disclosure of a dual CXCR2/CCR2 receptor antagonist.[241] The chemokine receptors CXCR2 and CCR2 belong to two different classes and have less than 20% sequence homology; however, scientists at AstraZeneca disclosed a series of thiazolo[4,5-*d*]pyrimidines that were potent dual chemokine receptor antagonists. One of these compounds, **30a**, inhibited calcium transients in cells expressing CXCR2 and CCR2 with IC_{50} values of 1 and 8 nM, respectively (**42**, Figure 12).

Even more interesting is the dual antagonist of the CCR3 and H_1 histamine receptors YM-344484 (**43**, Figure 12).[242] These two receptors have limited homology; less than 14% are activated by totally unrelated ligands, a protein, and a biogenic amine, and yet both are potently inhibited by a single non-peptide. This compound inhibits both the CCL11-induced Ca^{2+} influx in human CCR3-expressing cells (K_i of 1.8 nM) and histamine-induced Ca^{2+} influx in histamine H_1 receptor-expressing PC3 cells (K_i of 47 nM).[242] It is likely that compound **43** inhibits both CCR3 and the H_1 receptor by binding to a site in the minor or major binding pockets as do most chemokine receptor antagonists.[243] Given the fact that several small molecule chemokine receptor antagonists have tertiary amine groups, it is likely that some of these compounds will have activity at aminergic GPCRs. Furthermore it also emphasizes that it is possible to obtain dual antagonists for GPCRs that have highly dissimilar ligands. Since these receptors play an important role in atopic diseases such as asthma, then antagonism of this receptor pair by a single drug might prove to be a more effective therapeutic to treat these inflammatory diseases than targeting each receptor separately (see also compound **18**, a dual antagonist of these two receptors that showed a modest effect in clinical trials for treating allergic rhinitis).

Our discussion so far has centered only on a description of the molecular properties of dual chemokine receptor antagonists. We have not yet touched upon their utility or possible deleterious side effects. The potential therapeutic benefits of blocking multiple chemokine receptors are illustrated by two recent studies. In the first example two totally unrelated chemokine receptors, CCR5 and CXCR3, both of which are important proinflammatory receptors in autoimmunity, were targeted using knockout mice and receptor neutralizing antibodies.[244] Both CCR5 and CXCR3 have previously been shown to play a role in organ transplant rejection mainly by the induction of infiltrating T cells into the transplanted organs.[230,245] A heterotopic heart transplantation model in BALB/c to B6/129 mice deficient in CCR5 was carried out in the absence and presence of neutralizing antibodies to CXCR3.[244] Recipient mice were then assessed daily for allograft function. The donor hearts in the CCR5 deficient control group were all rejected at 6 days after transplantation. The survival of the donor hearts in the CCR5 deficient mice receiving control antibody and in the wild-type mice receiving anti-CXCR3 antibodies was prolonged to 29 and 34 days, respectively. However, the animals receiving a combined blockade of CXCR3 and CCR5 had a greater than 15-fold prolonged allograft survival compared to the control group; all of the allografts survived for greater than 100 days, after which the study was terminated. In addition, the donor hearts did not display any of the signs that are characteristic of chronic rejection. In summary these studies demonstrate that blocking the chemokine receptors CCR5 and CXCR3 with dual antagonists could be beneficial in acute organ transplantation rejection.

In the second example the unrelated chemokine receptors CCR2 and CX3CR1, which have been independently shown to be important in the development of coronary artery disease,[246] were targeted by using a small molecule antagonist in association with a receptor gene deletion. MRL-677 is a small molecule CCR2 antagonist (structure not disclosed) (IC_{50} = 1.8 nM) that is able to block macrophage trafficking in a mouse peritoneal thioglycollate model.[247] The drug was used in an intimal hyperplasia model in both wild type and CXCR3-deficient mice. Blocking CCR2 with the antagonist resulted in a 56% decrease in the vascular injury response in normal animals. Mice in which both CCR2 and CX3CR1 pathways were targeted had an 88% decrease in the injury response.[247] Thus, this study demonstrates that CCR2 and CXCR3 play nonredundant roles in vascular inflammation and further suggests that dual chemokine receptor antagonists could be therapeutically beneficial.

On the basis of the examples above, it is clear that chemokine receptor antagonists that block multiple receptors might be useful in treating certain diseases. For example, a small molecule antagonist that would block CCR5 and CXCR4, the major co-receptors for HIV-1 infection of human cells, could be useful therapeutically as a fusion inhibitor to treat AIDS patients. Although the idea is quite attractive, it remains to be seen whether the design of dual GPCR inhibitors is possible. Two recent studies demonstrate that in principle this might be feasible.

The first of these involves the unrelated angiotensin II (AT_1) and endothelin (ET_A) receptors. The ligands for these receptors, angiotensin II and endothelin, are potent vasoconstrictors, and AT_1 antagonists have already taken their place alongside ACE inhibitors as successful treatments for hypertension. Thus, it is expected that dual antagonists of these two receptors could be of greater benefit in the treatment of pulmonary hypertension, congestive heart failure, and arteriosclerosis. On the basis of this premise, scientists at Pharmacopiea designed a dual AT_1/ET_A receptor antagonist using known antagonists of each of these receptors as a starting point (**44** and **45**, Figure 13). The resulting dual antagonist PS433540 (**46**, Figure 13) has successfully completed phase II clinical trials and was recently licensed to Retrophin who intends to develop it for orphan indications of severe kidney diseases.[248]

The second example is provided by histamine and thromboxane, which play an important role in the pathogenesis of asthma. It is possible that a dual antagonist of their receptors, H_1 and TxA_2, might be therapeutically useful in treating this disease. On the basis of this rationale, Oshima et al.[249] utilized a approach similar to that described above for the AT_1 and ET_A receptors to design dual histamine H_1 and thromboxane TxA_2 receptors for potential use as potent anti-inflammatory compounds to treat asthma. Their approach was based on the observation that compounds that had been discovered to be potent antagonists to both of these receptors contained a common benzoxepin core (**47** and **48**, Figure 13). The

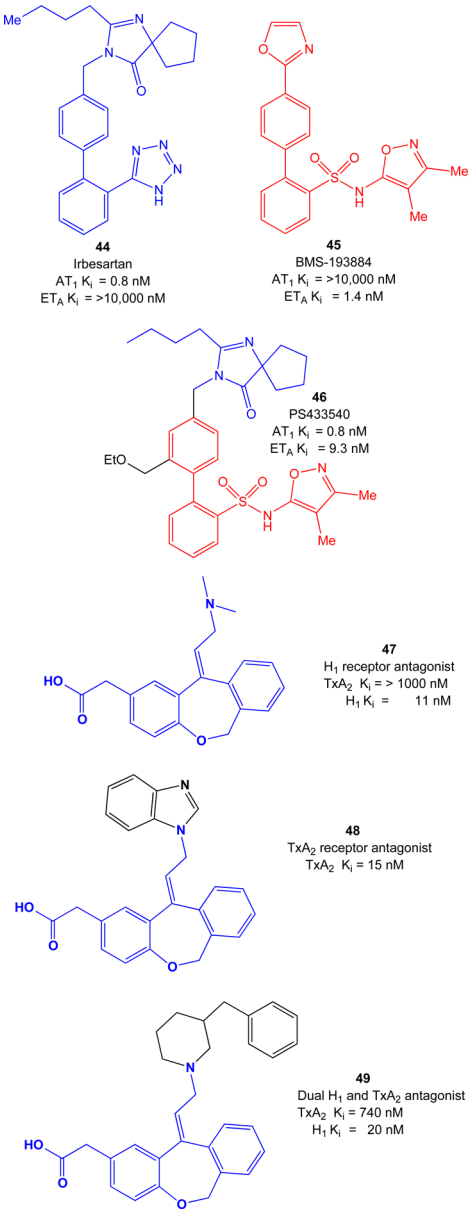

Figure 13. Design of dual receptor antagonists.

benzimidazole group is a key element that is crucial for the TxA_2 activity of **47**, and replacement with a tertiary amine (**49** Figure 13) mimicked this activity, resulting in a dual antagonist that was active at both of these receptors. Clearly this approach and the one just discussed above worked well because highly potent templates to both receptors that had common core structures existed and these were tractable enough that a hybrid molecule that could interact with the binding pockets of both receptors could be designed. In such cases it is not really even necessary that the two receptors should share a similar binding domain.

In addition to the beneficial effects of dual receptor antagonists that we have discussed, however, are their potential for side effects. This is illustrated by the CCR2 antagonists, many of which, as we have seen, are also potent CCR5 antagonists. The failure of the CCR2 antagonist compound **7** in phase II clinical trials for rheumatoid arthritis has been ascribed to the possibility that it also inhibits CCR5 which is expressed on regulatory T cells in the inflamed synovium.[217] This off-target effect of the drug could, by blocking CCR5, dampen the normally anti-inflammatory effects of regulatory T cells. This might offset any benefit gained from blocking CCR2 in rheumatoid arthritis and account in part for the failure of the drug in the clinic.

CONCLUSIONS AND FUTURE DIRECTIONS

The historical success of pharmaceutical companies in targeting GPCRs has perhaps been a millstone around the necks of the various programs around the globe targeting chemokine receptors by small molecules. Compound libraries were screened with success, and lead compounds against several chemokine receptors were progressed into clinical development at great pace. When most of these drugs displayed little efficacy in the treatment of inflammatory disorders, there was much gnashing of teeth and a consensus emerged that the redundancy in the chemokine system meant that targeting single receptors was probably futile. Out of several multimillion dollar programs, only two drugs have obtained FDA approval, these from noninflammatory indications, where a single receptor appears to be responsible for the clinical symptoms (CCR5 in macrophagetrophic HIV-1 entry and CXCR4 in stem cell mobilization).

However, after several false starts, there is now growing optimism that some of the chemokine receptor antagonists currently progressing through the clinic may become useful therapeutics in the treatment of inflammatory disease. As we come to realize the levels of receptor occupancy that are required from a compound to translate into in vivo efficacy, then it has been possible to successfully target a single chemokine receptor and achieve a positive clinical outcome, notably in the ongoing CCR1 and CCR9 antagonist trials for the treatment of rheumatoid arthritis and Crohn's disease discussed earlier. Modulation of previously identified compounds to increase their levels of receptor occupancy or simply modulating the dosage of existing compounds may be fruitful.[250] Similarly, several antagonist programs have unearthed small molecule agonists of chemokine receptors that may be useful therapeutically by desensitizing responses to endogenous agonists. A small molecule agonist of CXCR3 was recently shown to mimic the endogenous ligand CXCL10[28] and showed efficacy in a murine model of rheumatoid arthritis.[251]

A more detailed understanding of the biology of chemokines and their receptors in homeostasis and disease is clearly required to enable their targeting with greater efficacy and to uncover additional therapeutic avenues for such drugs. For example, the chemokine eotaxin, originally identified as an eosinophil chemoattractant implicated in allergic inflammation, has recently been implicated in the pathogenesis of age-related macular degeneration (AMD)[252] and cognitive dysfunction associated with aging[253] in the absence of any eosinophilic involvement. A comprehensive understanding of tissue chemokine receptor expression in humans and rodents is highly desirable, and coupled with the use of conditional mouse models in which receptors are selectively deleted from discrete populations of cells, we may be able to unpack some of the complexities of chemokine signaling in the disease process.

For some chemokines with apparently complex in vivo roles, the generation of transgenic mice in which distinct chemokine functions are disabled may be required. For example, the CXCR6/CXCL16 axis appears to be important in atherosclerosis, where the chemokine CXCL16 can function as both a

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scavenger receptor for OxLDL[254] and a ligand for CXCR6+ Th1, T-cytotoxic 1, and NKT cells.[53] However, individual deletion of ligand and receptor results in opposing pro- and antiatherosclerotic phenotypes (reviewed in ref 255). Likewise, for some receptors, targeting accessory proteins that are critical for function may be beneficial. For CCR2 and CCR5, binding of the nucleoporin known as FROUNT to the receptor C-terminus appears to be essential for directed migration,[256,257] adding another level of complexity to receptor signaling and suggesting alternative ways of targeting both receptors. Targeting of the receptor C-terminus may also be successful in the selective inhibition of nascent chemokine receptor trafficking, a poorly understood process clearly in need of more basic scientific research.

In summary, chemokine receptor antagonists still represent an extremely fruitful intervention therapy in the treatment of inflammatory and autoimmune diseases and several agents are still in late stage clinical trials. Despite the clinical failures discussed above, we remain cautiously optimistic that chemokine receptor antagonists can be of benefit in man. Their ultimate success, however, will depend on our ability to more clearly understand the role of chemokine receptors in driving the pathophysiology of complex autoimmune diseases than we currently do. This coupled with a parallel understanding of the animal models used, as predictors of the human disease, will also need to be more appreciated. Taken together, this information should help to target the correct receptors in treating human disease. Finally, much better clinical markers of the disease process in man will also be required not only to set up clinical trials more intelligently but also to ultimately monitor their progress. If we can make real progress in coming to grips with the issues discussed above, we might finally realize the promise of delivering chemokine receptor antagonists as registered drugs.

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Notes

The authors declare no competing financial interest.

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James Pease obtained a Ph.D. in Biochemistry from the University of Sheffield, U.K. He was a Postdoctoral Fellow at the NIH in the laboratory of Dr. Philip Murphy, where he gained valuable expertise in the biochemistry of chemokine receptors. Currently he is a Reader in the Leukocyte Biology Section at the National Heart and Lung Institute, Imperial College London and is a PI in the MRC & Asthma UK Centre in Allergic Mechanisms of Asthma. At Imperial he has established a group working various aspects of chemokine biology. His group was the first to publish the characterization of a small molecule antagonist of CCR3 and has published several papers describing the activation and inactivation of chemokine receptors by a variety of agonists and antagonists.

Richard Horuk obtained a Ph.D. from the University of London under Professor Sir Thomas Blundell. He was a Postdoctoral Fellow in the laboratory of Dr. Martin Rodbell at the NIH. From there he joined Dupont where his research centered on interleukin-1 receptors. Then he joined Genentech where his research on chemokine receptors led to the discovery that the Duffy antigen, a portal of entry for the malarial parasite *Plasmodium vivax*, was also a chemokine receptor. These findings set the stage for the discovery that chemokine receptors were HIV-1 co-receptors. Horuk worked at Berlex BioSciences for 13 years where his research interests were centered on GPCRs. Currently he is a Visiting Professor in the Department of Pharmacology at the University of California—Davis.

ABBREVIATIONS USED

DPP-IV, dipetidylpeptidase-IV; Src, sarcoma; EAE, experimental autoimmune encephalomyelitis; CIA, collagen induced arthritis; AIA, adjuvant induced arthritis; LTC_4, leukotriene C_4; PGD_2, prostaglandin D_2; DC, dendritic cell; BAL, bronchioalveolar lavage; ApoE, apolipoprotein E; Th1, T helper 1; Th2, T helper 2; Tregs, T regulatory cells; THP-1, human acute monocytic leukemia cell line; hERG, human ether-a-go-go-related gene; ATL, adult T-cell leukemia; MOG, myelin oligodendrocyte glycoprotein; AMD, age-related macular degeneration; NKT, natural killer T (cell); ECL, extracellular loop; TM, transmembrane

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