CHEMOKINE RECEPTORS

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Chemokines mediate their multiple effects by binding to a variety of specific receptors, that comprise a subfamily of rhodopsin-like, 7-transmembrane domain receptors, coupled to G proteins. Some of these receptors serve as coreceptors for HIV, some of them could be expressed as markers for T lymphocyte functional differentiation. This review aims at summarising data on chemokine receptors, their function *in vivo,* their target cells and it also mentions association of chemokine receptor polymorphisms with human disease.

THE CHEMOKINE RECEPTOR FAMILY

Chemokines constitute a large family of small glycoproteins that regulate diverse biological processes, including leukocyte trafficking, angiogenesis, hematopoiesis, and organogenesis[1].

Chemokines mediate their activities by binding to the receptors that comprise a subfamily of rhodopsin-like, 7-transmembrane (7TM) domain receptors (also called serpentine receptors), G protein-coupled receptors (GPCR); they signal mainly through Gi-type proteins[1].

A general model for plasma membrane insertion of (7TM) proteins has been proposed, based on the distribution of conserved sequences and the known structure of rhodopsin. In this model, the receptor polypeptide is stitched into the membrane with the N-terminus extracellular domain, the C-terminus intracellular domain, and each of seven hydrophobic domains passing as α helices through the membrane[2]. This allows four extracellular, and four intracellular domains: a total of six loops (il-3 and el-3) connecting the seven (TM) domains, which form the (TM) core, and the free N- and C-termini[2]. The receptors have a single polypeptide chain and have 25–80 % of amino acid sequence identity to each other. These and other aspects of their structure indicate that chemokines and chemokine receptors each arose from common ancestors by repetitive gene duplication. Although chemokine receptors are similar to many GPCRs, they have unique structural motifs such as the amino acid sequence DRYLAIV in the second intracellular domain[3].

Chemokines have two main sites for interaction with their receptors: the first lies in the amino-terminal region and the second is located in the exposed loop of the backbone between the second and the third cysteine[4]. The receptor initially recognises the chemokine loop region, and this interaction is necessary for the correct presentation of the amino-terminal region, which is then essential for the receptor triggering.

The chemokine signalling network is astonishingly complex, however the primary task of each receptor is conceptually simple, to bind a chemokine and to relay its signal to a heterotrimetric guanine nucleotide-binding regulatory protein (Gαβγ protein). Agonist binding to the receptor catalyses the exchange of GTP for the GDP that is bound to the Gα subunit and induces a dissociation/reassociation cycle of the Gα and Gβγ subunits[5]. The GTP-bound Gα subunit and the Gβγ subunits then both independently activate downstream effectors such as adenylate cyclase, phospholipase Cβ, phosphatidylinositol-3-kinase and protein tyrosine kinases – allowing for cross-talk with the mitogen-activated protein kinase cascades (recently reviewed by Ward[6] et al., 1998).

At present, 17 receptors have been identified. In agreement with the current chemokine nomenclature[7], a name for a chemokine receptor starts with the abbreviation of its chemokine ligand subclass specificity (CC, CXC, XC, or CX3C), which is followed by "R" (for receptor) and then ends with a serial number. Thus, we have CCR1-10, CXCR1-5, XCR1 (the lymphotactin, XCL1, receptor), and CX3CR1 (the fractalkine, CX3CL1, receptor). The known chemokine receptors, together with their ligands and cell types for expression, are shown in the Table 1. The table also illustrates "shared binding specificity" between many ligands and receptors. This "promiscuity" is real because it involves high-affinity interactions. Thus, a single chemokine may bind to several receptors, whereas a single chemokine receptor may transduce signals for several chemokines. This is one of the most intriguing features of the chemokine superfamily and may reflect their ability to regulate many different leukocyte subpopulations, especially in complex microenvironments such as acute or chronic inflammatory responses.

Some of novel chemokine receptor belong to "orphan" receptors. These are proteins where the ligand had not been identified.

Table 1. Human chemokine receptors

Receptor (alternative names)	Ligands (alternative names)	Main cells expression
CC chemokine receptors family		
CCR1	MIP-1α, MCP-3, RANTES MIP-5/Lkn-1/HCC-2	Mo, T, NK, iDC, Neu
CCR2	MCP-1, MCP-2, MCP-3, MCP-4	Mo, T (act) NK (act.)
CCR3	MCP-2, MCP-3, MCP-4, RANTES, Eotaxin-1, Eotaxin-2/MPIF-2, Eotaxin-3, MIP-5/Lkn-1/HCC-2	Eo, Ba, T (Th2)
CCR4	TARC, MDC/STCP-1	T (Th2, Tc2), NK, iDC
CCR5	MIP-1α, MIP-1β, RANTES	Mo, T (Th1, Tc1), iDC
CCR6	MIP-3α/LARC/Exodus-1	T, iDC (CD34+)
CCR7	MIP-3β/ELC/Exodus-3, SLC/Exodus-2/6Ckine	T, Mo, mDC
CCR8	I-309, TARC, MIP-1β	T (Th2/Tc2), Mo
CCR9	TECK	Non-haematopoietic
CCR10	CTACK	T
CXC chemokine receptors family		
CXCR1 (IL8RA)	IL-8, GCP-2, GROα	Neu
CXCR2 (IL8RB)	IL-8, GROs, NAP-2, ENA-78, GCP-2	Neu
CXCR3	IP-10, MIG, I-TAC	T (Th1, Tc1)
CXCR4 (LESTR/fusin)	SDF-1α/β	Widely expressed receptor
CXCR5 (BLR-1)	BCA-1/BLC	B
C chemokine receptors family		
XCR1	Lymphotactin/SCM-1/ATAC	T, NK
CX3C chemokine receptor family		
CX3CR1 (V28)	Fractalkine/neurotactin	T (Th1), NK, Mo
Miscellaneous chemokine receptor		
DARC	IL-8, GROα, RANTES, MCP-1, TARC	Erythrocytes

Abbreviations for cells: act., activated; Ba, basophils; DC, dendritic cells; iDC, immature DC; mDC, mature DC; DC (CD34+), DC derived from CD34+ cells *in vitro*; Eo, eosinophils; Mo, monocytes; Neu, neutrophils; NK, natural killer; Th, T helper; Tc, T cytotoxic

Abbreviations for chemokines: BCA-1, B cell-attracting chemokine 1; BLC, B-lymphocyte chemoattractant; 6Ckine, six-Cysteine chemokine; ENA-78, epithelial-cell derived neutrophil activating protein 78; ELC, EBI-1 ligand chemokine; GCP-2, granulocyte chemoattractant protein 2; GRO, growth-related oncogene; HCC-2, hemofiltrate CC chemokine 2; I-309, inducible 309; IL-8, interleukin 8; IP-10, interferon-inducible protein 10; I-TAC, interferon-inducible T-cell α chemoattractant; LARC, liver and activation-regulated chemokine; MCP, monocyte chemotactic protein; MDC, macrophage-derived chemokine; MIG, monokine induced by interferon gamma; MIP, macrophage inflammatory protein; NAP-2, neutrophil-activating peptide 2; SDF-1, stromal cell-derived factor 1; SLC, secondary lymphoid tissue chemokine; STCP-1, stimulated T cell chemotactic protein; RANTES, regulated on activation of normal T cell expressed and secreted; TARC, thymus and activation-regulated chemokine; TECK, thymus-expressed chemokine

Adapted from the ref. 7–9 (index x^2)

CHEMOKINE RECEPTOR – LIGAND INTERACTIONS: THE FOUR GENERAL CATEGORIES

Promiscuous receptors

The first class of chemokine receptors is that of the promiscuous receptor (Figure 1). It is defined here as a receptor which will bind chemokines of either CXC or CC classes. There is, however, only one example of this receptor to date, the erythrocyte chemokine receptor, name DARC (Duffy antigen receptor for chemokines). This receptor is expressed on erythrocytes and endothelial cells, where it was originally defined serologically as the Duffy blood group antigen and later pathologically as the invasin for the malaria-causing protozoan *Plasmodium vivax*[10, 11]. Although DARC is structurally related to chemokine receptors it lacks the DRYLAIV motif in the second cytoplasmic loop, it is not coupled to heterotrimetric G-proteins and importantly does not elicit any detectable signal-transduction events[10, 11]. The biological role for DARC is, therefore, unclear; it has been proposed to act as a "sink" for chemokines, which keeps their circulating levels low[10, 11].

Shared receptors

The second category is represented by the shared receptors, defined here as receptors which will bind to more than one chemokine within either the CXC or the CC class (Figure 1). There are three CXCR receptors (CXCR1-3), which bind to many of the CXC chemokines, and all CCR1-10 receptors, which bind to several of the CC chemokines.

Specific receptors

The third category is that of specific receptors, which seem to bind only one chemokine (Figure 1). These are the receptors such as XCR1 for lymphotactin (XCL1), CX3CR1 for fractalkine (CX3CL1), CXCR5 for BCA-1 (CXCL13), and CXCR4 for SDF-1 (CXCL12).

Virally encoded receptors

The fourth category of receptors are the virally encoded chemokine receptors (Figure 1). Although chemokines and chemokine receptors probably evolved as antimicrobial factors, many are exploited by infectious agents to facilitate the infection. Certain herpesviruses encode pirated chemokine receptors, including US28 (used by human cytomegalovirus), and ECRF3 (used by *Herpesvirus saimiri*)[12, 13]. US28 binds the CC chemokine MIP-1α (CCL3), MIP-1β (CCL4), RANTES (CCL5) and MCP-1 (CCL2) with similar affinity[12]. ECRF3 is selective for the CXC chemokines IL-8 (CXCL8), GROα (CXCL1) and NAP-2 (CXCL7)[13]. US28 and ECRF3 functions have not been identified yet. Human cytomegalovirus and *Herpesvirus saimiri* could use chemokine receptors to control viral replication by regulating cell cycle progression of the host cell, or by inhibition apoptosis of the host cell[12, 13].

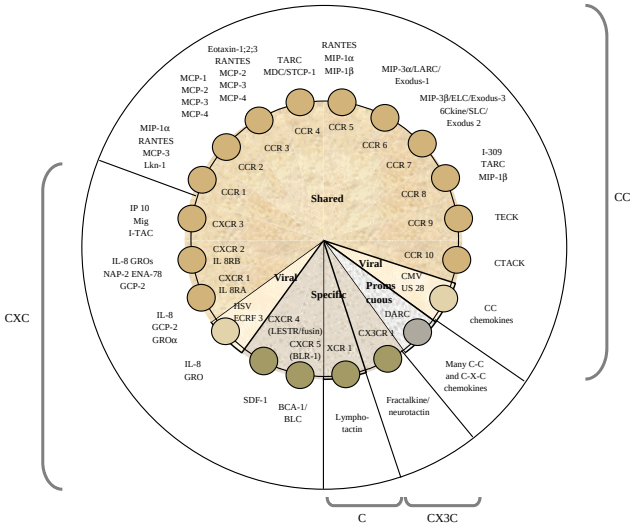

Fig. 1 The chemokine receptor overview
The catalogue of known chemokine receptors now includes 4 separate categories (adapted from the ref. 14).

CHEMOKINE RECEPTORS AS MARKERS OF T LYMPHOCYTE DIFFERENTIATION

Immune-mediated diseases can be classified as either Th1 (T helper 1) or Th2 (T helper 2) immunopathologies depending on the spectrum of cytokines produced by activated Th cells. Th1 cells produce mainly Interleukin (IL)-2, IFN-γ and IL-12 whereas Th2 cells secrete IL-4, IL-5, IL-10 and IL-13[15]. Th1 cells are the major players in the inflammation characterised by activated T cells and macrophages, and have been associated with diseases such as rheumatoid arthritis and delayed-type hypersensitivity reactions[9]. Th2 cells are involved in the responses leading to eosinophil and basophil recruitment and have therefore been implicated in the pathogenesis of allergic inflammatory diseases such as asthma and atopic dermatitis[16].

Recently, it has been demonstrated that chemokine receptors are differentially expressed on naive Th1 and Th2 subsets, and their expression is modulated by cytokines. Naive T cells primarily express CXCR4 and thus are responsive to SDF-1 (CXCL12, stromal cell-derived factor 1), a chemokine that has an important role in basal trafficking of naive T cells into lymphatic organs. Upon activation, T cells may express an array of chemokine receptors including CCR1, CCR2, CCR5, CXCR1 and CXCR4[17]. They thus become sensitive to inflammatory chemokines including MIP-1α (CCL3), MIP-1β (CCL4), MCP-3 (CCL7) and RANTES (CCL5), which are thought to mediate T-cell trafficking to secondary lymph node structures and sites of inflammation[6]. Chemokines have an important role in the induction of inflammatory responses and are also central in selective the type of immune response (Th1 vs Th2). During

bacterial or viral infections IP-10 (CXCL10), Mig (CXCL9), IL-8 (CXCL8) and I-TAC (CXCL11) production correlates with the presence of $CD4^+$ Th1 cells[17]. In contrast, during allergic inflammatory responses eotaxin (CCL11), RANTES (CCL5), MCP-2 (CCL8), MCP-3 (CCL7) and MCP-4 (CCL13) are induced and the majority of the $CD4^+$ T lymphocytes are of the Th2-type phenotype[17]. Characterisation of chemokine receptor expression on T lymphocytes suggests that this may be explained by the expression of CXCR3 and CCR5 predominantly on $CD4^+$ Th1 cells[18, 19, 20], whereas CCR3, CCR4, CCR8 (and perhaps CCR7) are restricted to $CD4^+$ Th2 cells[19, 20, 21]; the following molecules – CXCR4 and CCR2, and possibly also CCR1 – are expressed equally on both Th1 and Th2 cells, suggesting that these receptors as opposed to polarised (Th1 vs Th2) immune responses[18, 19, 23] (Figure 2).

These observations suggest that chemokine receptors expression on T cells together with tissue-specific chemokine expression are important factors in controlling the composition of lymphocyte infiltrates in different types of inflammatory pathologies.

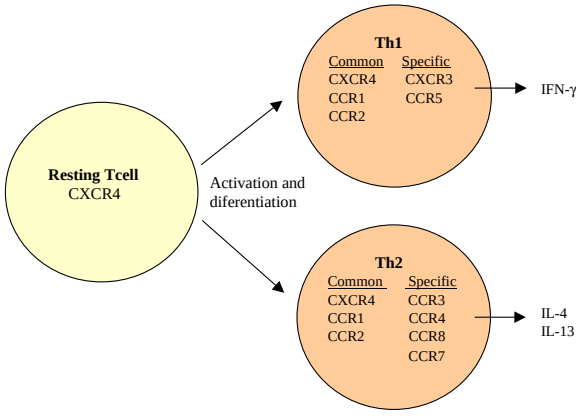

Fig. 2 The chemokine receptors as markers for T lymphocyte differentiation (adopted from the ref. 6).

CHEMOKINE RECEPTORS AND HIV INFECTION

Human Immodefficiency virus (HIV-1) is the aetiologic agent of AIDS which results from the destruction of $CD4^+$ lymphocytes in infected individuals. The entry of HIV-1 into its targets cells is mediated by the viral envelope glycoproteins such as gp120, which binds the cellular receptor CD4, resulting in conformational change that exposes the V3 loop in gp120 and permits subsequent interaction with a chemokine receptor. The primary cellular receptor for all strains of HIV-1 is CD4 molecule[24], but strain-specific chemokine receptors are required as coreceptors for fusion and entry[25]. CXCR4 is coreceptor for strains of HIV-1 that infect T-cell lines (T-tropic strains)[25, 26] and CCR5 is coreceptor for HIV-1 isolates that infect macrophages and activated T cells (M-topic strains)[27, 28].

The importance of chemokine receptors in the pathophysiology of HIV infection became apparent when it was discovered that in an individual who was homozygous for a 32 bp deletion in the human CCR5 gene (CCR5δ32 allele), a functional CCR5 protein cannot be synthesised, and such individuals are generally not found in HIV-1 positive cohorts[29, 30, 31]. Furthermore, in persons who are heterozygous for the mutation, the rate of progression of HIV-1 infection is slower than in those without the mutation[29]. CCR5δ32 appears to have originated in northeaster Europe, and it occurs with an average allelic frequency of 10 % in North American Caucasians; it is not found in native Asians and Africans. The frequency of the deletion mutation in the Czech population is no different from that in other European Caucasian populations[32].

Many other polymorphisms, relevant to HIV infection, have been described in chemokine receptor genes (Table 2). Another much rarer mutation, CCR-m303 (which contains a premature stop codon at position 303) has also been reported to confer resistance to HIV-1 infection, and is presumed to act like δ32[33]. The other two affect the rate of disease progression but may not affect susceptibility to initial infection, and their mechanism us unclear. One of these polymorphisms caused a conservative amino acid change in CCR2 (V64I, substitution of valine for isoleucine in the first transmembrane domain)[34] and the other is single-nucleotide polymorphism in the CCR5 promoter (59029A/G), which has a particularly strong affect and is very common in all racial groups tested[35, 36]. Easterbrook[37]et al. studied effects on HIV disease progression of other polymorphisms in the promoter region of CCR5 (positions 59353 and 59402), and found that 59353 C allele was associated with a delayed progression.

Table 2. Polymorphisms in genes for CC chemokine receptors/HIV-1 coreceptors that alter susceptibility to HIV-1 infection and progression

Molecule	Polymorphism	Type	Phenotypes		Mechanism
			-/-	+/-	
CCR5	CCR5δ32	Del	R	DP	Truncation
	m303	SNP	R	ND	Truncation
	59029 G/A	SNP	DP	-	ND
	59353 T/C	SNP	DP	-	ND
	59402 G/A	SNP	-	-	ND
CCR2	V64I	SNP	ND	DP	ND

Abbreviations: -/-, homozygous for the given allele; +/-, heterozygous for the given allele; SNP, single nucleotide polymorphism; Del, deletion; R, HIV-1 resistance; DP, delayed progression to AIDS relative to other genotypes; ND, not determined
adapted from the references no. 1 and 37

CHEMOKINE RECEPTOR (CCR2, CCR5, CXCR1 AND CXCR2) GENE POLYMORPHISMS IN OTHER DISEASE

Several studies have recently suggested association of certain chemokine receptor gene polymorphisms with other immunological diseases than HIV infection (Table 3).

Hall[38] et al. reported that individuals carrying the CCR5δ32 mutation are at reduced risk of asthma. This has not, however been confirmed in the Hungarian population, where CCR5δ32 mutation in non-asthmatic but atopic children did not indicate a reduced risk[39].

Petřek[40] et al. have shown an association between CC chemokine polymorphisms with pulmonary sarcoidosis in the Czech population. Their study has shown a decrease of the CCR2-64I allele, and a significant increase in the frequency of CCR5δ32 allele in patients with sarcoidosis, CCR5δ32 allele was associated with advanced disease. CC chemokine receptor polymorphisms should therefore be considered to be protective (CCR2-64I) or susceptibility (CCR5δ32) factors for the development of sarcoidosis. In agreement with the above findings, Hizawa[41] et al., have reported that CCR2 polymorphism (CCR2-64I) is associated with decreased susceptibility to sarcoidosis in Japanese population.

A role of CCR2 and CCR5 gene polymorphisms in other immune-mediated diseases, such as multiple sclerosis (MS) and insulin-dependent diabetes mellitus (IDDM) was not confirmed[42, 43], however, a mutation in the CCR2 gene was postulated to possibly contribute to the susceptibility to the IDDM[43].

The studies of association CCR5δ32 with susceptibility to rheumatoid arthritis have yielded with quite conflicting results. Gómez-Reino[44] et al. suggested that a functional CCR5 receptor was required for the development of rheumatoid arthritis (RA) and this conclusion was based on the absence of the homozygous CCR5δ32 deletion in patients with RA. The findings of Cooke[45] et al. were opposite: they have found two patients with homozygous CCR5δ32 who had typical disease, and they concluded that blockade of CCR5 was unlikely to have therapeutic benefit. Another report on this topic by Mack[46] et al., showed that the number of CCR5-positive cells in the synovial fluid did not differ between the CCR5/CCR5 wild-type and the CCR5δ32/CCR5 heterozygous patients with RA. This finding suggests that a complete deficiency in the CCR5 receptor expression does not prevent the development of RA and other chemokine receptors (e.g., CCR2, CXCR3) may substitute for the CCR5 deficiency.

Renzoni[47] et al. described 4 novel polymorphisms in the CXCR1 and CXCR2 genes. In the CXCR1 gene a novel polymorphism at nucleotide +2607 was found, resulting in a conservative amino acid substitution from serine to threonine at the 276 amino acid residue of the CXCR1 protein. In the CXCR2 gene 3 novel polymorphisms at nucleotide +785 (C or T), +1208 (T or C), and +1440 (G or A) were identified, the first of which resulted in a silent codon change and the others were in the 3 untranslated area of exon. The authors[47] studied an association between these polymorphisms and systemic sclerosis (SSc) and cryptogenic fibrosing alveolitis (CFA) and have reported an association between two polymorphisms in the CXCR2 gene (CXCR2 +785 and CXCR2 +1208) and systemic sclerosis, independent of the presence of fibrosing alveolitis. This finding suggest a role for CXCR2 receptor in the pathogenesis of systemic sclerosis disease. No association was found between novel CXCR1 and CXCR2 polymorphisms and cryptogenic fibrosing alveolitis.

Table 3. Chemokine receptor CCR2, CCR5, CXCR1 and CXCR2 polymorphisms associated with different disease

Disease	Polymorphism						References
	CCR5δ32	CCR2-64I	CXCR1 (+2607)	CXCR2 (+785)	CXCR2 (+1208)	CXCR2 (+1440)	
Asthma	+, –	–	–	–	–	–	38, 39
Sarcoidosis	+	+	–	–	–	–	40, 41
Multiple sclerosis	–	–	–	–	–	–	42
IDDM	–	+	–	–	–	–	43
Rheumatoid arthritis	+,–	–	–	–	–	–	44, 45, 46
Systemic sclerosis – FASSc	–	–	–	+	+	–	47
– NFASSc	–	–	–	+	+	–	47
CFA	–	–	–	–	–	–	47
HIV	+	+	–	–	–	–	29, 31, 33, 34, 35, 36, 37

Abbreviations: +, polymorphism is associated with disease; –, polymorphism is not associated with disease; CFA, cryptogenic fibrosing alveolitis; CCR5δ32, a 32-bp deletion in the CCR5 gene; CCR2-64I, a substitution mutation in the CCR2 gene; CXCR1 (+2607), polymorphism at nucleotide +2607 (G/C) in the CXCR1 gene; CXCR2 (+785), polymorphism at nucleotide +785 (C/T) in the CXCR2 gene; CXCR2 (+1208), polymorphism at nucleotide +1208 (T/C) in the CXCR2 gene; CXCR2 (+1440), polymorphism at nucleotide +1440 (G/A) in the CCR2 gene; IDDM, insulin-dependent diabetes mellitus; FASSc, systemic sclerosis with fibrosing alveolitis; NFASSc, systemic sclerosis without fibrosing alveolitis

KNOCKOUT MICE AND EXPRESSION LEVELS AS TOOLS FOR TARGET VALIDATION

Several chemokine and their receptors have been deleted in mice to address whether they a play a specific role in normal maintenance of the immune system or in diseased states where the immune balance is disturbed. There have already been some interesting results (Table 4).

Deletion of the CXCR2 homologue in mice caused impaired neutrophil recruitment upon infection, suggesting that CXCR2 antagonists may be useful in managing tissue damage during acute lung inflammation or sepsis[48].

Deletion of either CXCR4 or its ligand SDF-1 (CXCL12) impaired bone marrow myelopoiesis, B cell lymphopoiesis, cerebellar development and gastric vascularization, and was lethal[50, 51, 52]. Gutierrez-Ramos[65] studied of the *in vivo* role of CXCR4 and its ligand, SDF-1 (CXCL12), during allergic airway disease (AAD). This study has been limited by the fact that transgenic mice that have been made deficient in either molecule die early in life. The author[65] presents data supporting that lungs from asthmatic patients as well as inflamed lung of mice subjected to a model of AAD show modulation of CXCR4 mRNA and protein expression during diseases. The blockade of CXCR4 *in vitro* lead to reduce the migration of both eosinophils and lymphocytes to the lung (BAL fluid and lung interstitium) by 50 and 60%, respectively, indicating that CXCR4-mediated signals contribute to lung inflammation during allergic processes. CXCR4 and SDF-1 (CXCL12) have a critical role during AAD and the potential relevance of this receptor and its ligand in other inflammatory processes[65].

BLR-1/CXCR5 knockout do not develop inguinal lymph nodes or B cell areas in secondary lymphoid tissues[53]. The chemokine receptor BLR-1/CXCR5 is the first G protein-coupled receptor involved in the regulation of B cell migration and localisation of these cells within specific anatomic compartments[53].

Table 4. Function of chemokine receptors *in vivo*: phenotypes associated with targeted gene disruptions in mice and naturally-occurring inactivating mutations in humans

Disrupted gene	Viable	Major phenotypes	References
Mouse CXCR2	Yes	– Neutrophil and B cell expansion in blood, lymph nodes, spleen, and bone marrow	48
		– Impaired neutrophil recruitment to intraperitoneal thioglycollate	
Mouse CXCR3	Yes	– Absence of acute or chronic cardiac allograft rejection	49
Mouse CXCR4	No	– Ventricular septal defect	50, 51, 52
		– Impaired B cell lymphopoiesis	
		– Impaired bone marrow myelopoesis	
		– Impaired cerebellar development	
		– Impaired gastric vascularization	
Mouse CXCR5	Yes	– Absent inguinal lymph nodes	53
		– Absent or abnormal Peyer's patches	
		– Defective B cell trafficking and localization	
		– Disturbed lymphoid architecture and impaired immune response	
Mouse CCR1	Yes	– Impaired lung granuloma formation to *S.mansoni* eggs	49, 54, 55, 56,
		– Abnormal Th1/Th2 cytokine balance in *S.mansoni* egg challenge	57
		– Reduced pancreatitis-induced pulmonary inflammation	
		– Increased susceptibility to *A.fumigatus*	
		– Abnormal steady state and induced trafficking and proliferation of myeloid progenitor cells	
		– Pathogenetically important role in modulating the effector phase of the immune response during nephrotoxic nephritis	
		– Absence of acute or chronic cardiac allograft rejection	
Mouse CCR2	Yes	– Reduced monocyte recruitment after intraperitoneal thioglycollate	58, 59, 60
		– Reduced size lung granuloma of elicited by PPD challenge	
		– Increased susceptibility to *Listeria*	
		– Decreased atherogenesis	
		– Abnormal Th1/Th2 cytokine balance in PPD challenge	
Mouse CCR5	Yes	– Increased susceptibility to *Listeria*	61
		– Increased susceptibility to lipopolysaccharide endotoxemia	
		– Increased humoral response to T cell-dependent antigenic challenge	
		– Enhanced DTH reaction	
Mouse CCR7	Yes	– Delayed kinetics regarding the antibody response and lack contact sensitivity	62
		– Delayed type hypersensitivity reactions	
		– Impaired migration of lymphocytes	
		– Profound morphological alterations in all secondary lymphoid organs	
Human CCR5	Yes	– Resistance to HIV-1 and AIDS	29, 30
Human Duffy	Yes	– Resistance to *P. vivax* form of malaria	63, 64

Adapted from the ref. 1

CCR1 deletion impaired the granulomatous response to *Schistosoma mansoni* eggs[54], and decreased the severity of symptoms in models of multiple sclerosis. CCR1 knockout also have reduced pancreatitis-associated pulmonary inflammation[55].

Deletion of CCR2 resulted in reduced monocyte and macrophage recruitment in response to inflammatory stimuli, and a decreased pathology in an atherosclerotic mouse model, suggesting a role for CCR2 antagonists in this disease[60].

Macrophage and T cell accumulation in autoimmune and degenerative disorders is associated mainly, but not exclusive, with CC chemokines. There are also appear to be important in allergic inflammation in diseases such as asthma, based on the local presence of eotaxin (CCL11), MCP-3 (CCL7) and RANTES (CCL5); and expression of their shared receptor, CCR3, on eosinophils, basophils and Th2 cells, which accumulate at sites of allergic inflammation[21, 66, 67].

In spite of the high level expression of CCR4 seen *in vitro* on Th2 polarised cells, *in vivo*, in an ovalbumin (OVA) model of inflammation (OVA induced lung inflammation, which is a predominantly Th2 driven disease), CCR4 does not appear to be required for development of a Th2 response[68]. Yet surprisingly, blockade of a CCR4 ligand, mouse MDC (CCL22), in the OVA model significantly reduces both bronchial hyperreactivity and leukocyte infiltration into the lung interstitium[68]. This suggests that there may be some compensatory mechanism for the loss of CCR4 *in vivo* or that a second receptor for MDC (CCL22) exists in mice[68].

CCR5 expression is enhanced in the course of in vitro *Mycobacterium tuberculosis* infection and during active pulmonary tuberculosis[69]. CCR5 deletion have enhanced delayed hypersensitivity reactions and increased humoral responses to cell-dependent antigenic challenge[61]. Consistent with the notion that the chemokine system supports host defense, CCR1 and CCR5 knockout mice have increased susceptibility to inoculation with *Aspergillus fumigatus* and *Listeria monocytogenes*, respectively[61]. However, neither these nor any of the other chemokine/receptor knockout have increased susceptibility to spontaneous infection, indicating that sufficient redundancy exists within the system for baseline host defense[1]. Thus therapeutic "anti-chemokines" that are eventually developed (none has reached clinical trials yet) may profit from the specifity that certain chemokine appear to demonstrate in the amplification of pathologic inflammation versus the redundancy that appears to characterise chemokine regulation of immune function[1].

CCR6 in interaction with MIP-3α (CCL20) plays an important role in the trafficking of immature dendritic cells (DC) to chemokine production sites such as injured or inflamed peripheral tissues, where dendritic cells undergo maturation on contact with antigens[70].

The deletion of the receptor CCR7 is embryonally not lethal. The phenotypical analysis will show whether CCR7 mutant mice represent a second animal model showing an *in vivo* function of chemokine receptors as regulators of homeostatic trafficking of lymphocyte subsets and functional compartmentalisation of secondary lymphoid organs[71].

These results indicate complex and not yet understood roles for each of these molecules that extend the chemokine paradigm beyond simple chemotaxis and suggest a role for chemokines in modulating cytokine regulation of the inflammatory response. Analysis of gene expression patterns in knockout animals will lead to new insights into immune control *in vivo*.

Summarising the data from the different target validation approaches discussed above (monitoring expression levels, targeted gene disruption, use of blocking antibodies, modified chemokines) and tentatively infer pathogenic correlations relating various inflammatory diseases to particular chemokine receptors (Table 5).

Table 5. Involvement of chemokine receptors expressed on specific cell types in some inflammatory and infectious disease infered from different target validation.

Receptor	Cell type	Validation basis	Disease	References
CXCR1	Neutrophil	Knockout mouse	Acute inflammation	72, 73, 74, 75
CXCR2	Neutrophil	Knockout mouse	Acute lung inflammation or Sepsis	48
CXCR3	Th1 T cell	Up-regulation of expression	Multiple sclerosis, Nephrotoxic nephritis	56, 76
CXCR4	T cells	Bicylam-type antagonists	HIV	65, 77, 78, 79
	Eosinophil	The blockade of receptor	Allergic airway disease	
CCR1	T cells	Knockout mouse	Rheumatoid arthritis, Multiple sclerosis,	56, 75, 80
	Macrophage	Met-RANTES	Nephrotoxic nephritis	
		Up-regulation of expression		
CCR2	Monocyte	Knockout mouse	Atherosclerosis, Nephrotoxic nephritis,	60, 81, 82
	Macrophage	MCP-1 knockout mouse	Granulomatous inflammation	
	T cells			
CCR3	Eosinophil	Up-regulation of expression	Allergic disease (Asthma, Atopic dermatitis)	83, 84, 85, 86
	Th2 T cell	Eotaxin knockout mouse		
		Antibodies		
CCR4	Th2 T cell	Antibodies	Allergic disease (Asthma, Atopic dermatitis),	86
CCR5	Macrophage	Knockout mouse	HIV, Rheumatoid arthritis, Multiple sclerosis,	29, 30, 31, 56, 61, 80
	Th1 T cell	hCCR5δ32 knockout	Nephrotoxic nephritis, Granulomatous	
		Up-regulation of expression	inflammation	

Adapted from the ref. 90

CONCLUSIONS

The spectrum of chemokine and their receptors roles has expanded since the first comprehensive review on this topic[87]. Nowadays, detailed understanding of chemokine receptor structure and signalling suggests new treatment modes for immune-mediated disease. Therapeutic targeting of chemokines and their receptors represents extension of their traditional understanding as proinflammatory mediators[88] and has been already envisaged[89]. Moreover, recent suggestion that chemokine receptor gene polymorphism may play a role in susceptibility to immune disease, is a novel concept and brings new aspect to the complex field of chemokines and chemokine receptors.

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