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CCR5 as a potential therapeutic target in multiple sclerosis

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CONTENTS

Abstract

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system. A number of chemokines and their receptors are implicated in the pathogenesis of MS. CCR5 is expressed on mononuclear inflammatory cells and its ligands are detectable in MS lesions and upregulated in the cerebrospinal fluid of MS patients. Furthermore, CCR5 appears to play an important role in the transmigration of leukocytes across the blood–brain barrier. We review the evidence implicating CCR5 in the pathogenesis of MS and discuss CCR5 antagonists as potential therapeutic agents in MS.

Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS) associated with neurodegeneration and astrogliosis (1). In North America and Europe, MS is the most common nontraumatic disabling neurological disease in young adults. Worldwide, 2.5 million individuals are estimated to be affected by MS. The majority of MS patients have a relapsing–remitting disease course with secondary progression (80%), while the remaining patients have a primary progressive or relatively 'benign' disease (2).

Inflammation is one of the neuropathological hallmarks of MS (3, 4). A detailed examination of acute MS lesions derived mainly from biopsy cases has implicated innate immunity (activated microglia and complement), as well as adaptive immune elements (T cells and antibodies), in the early demyelinating stages of MS (5). The destruction caused by inflammation in MS has been clearly shown in neuropathological studies (4). In MS tissue derived from patients with a typical disease course, there is a consistent correlation between the degree of inflammation and the extent of axonal damage (6, 7). Magnetic resonance imaging (MRI) studies with gadolinium indicating a breakdown in the blood–brain barrier have identified a correlation between inflammatory activity and clinical disease activity (8), and a variety of anti-inflammatory agents successfully reduce the relapse rate in MS (9). However, the therapeutic modulation of inflammation in MS is not without challenges (10). The interference with leukocyte transport across the blood–brain barrier by a monoclonal antibody that blocks the α_4 leukocyte integrins led to fatalities from progressive multifocal leukoencephalopathy (11, 12). It should also be noted that inflammation has a Janus-like face in MS, where it appears to facilitate spontaneous repair of myelin (13). Overall, there is little doubt that safe and efficacious modulation of inflammation will offer benefits to patients with MS.

Chemokines and chemokine receptors

Chemokines are small heparin-binding proteins that play an important role in the movement of inflammatory cells both from the circulation into the CNS and within the brain parenchyma (10, 14). Chemokines are classified into four families (CXC, CC, C and CX_3C) depending on the configuration of the positional conserved cysteine residues near the NH_2 terminus (15). This large number of molecules orchestrates a wide range of physiological and pathological functions, including leukocyte migration, angiogenesis and the proliferation of tumors (14). In terms of leukocyte and endothelial interactions, chemokines play a role in the firm arrest, locomotion to interendothelial junctions and diapedesis of leukocytes (15).

The response of inflammatory cells to chemokines depends on the pattern of chemokine receptor expression on their surface. Chemokine receptors are seven-transmembrane-domain G-protein coupled receptors that activate signaling pathways leading to actin polymerization and cell movement. The identity of a chemokine receptor depends on the class of chemokine that binds to it. Hence, there are four subgroups of chemokine receptors, termed CCR (CCR1-10), CXCR (CXCR1-7), XCR1 and CX_3CR1 (15).

CCR5 and CCR5 antagonists

The importance of CCR5 as a coreceptor for the cellular entry of human immunodeficiency virus type 1 (HIV-1) has led to an enormous interest in this chemokine receptor, which was further intensified by the finding of a common null allele in humans. The mutant nonfunctional form of *CCR5* is present in approximately 1% of the caucasian population, with the only functional adversity being increased susceptibility to West Nile encephalitis virus (16). The homozygotes for the Δ32 deletion (*CCR5-Δ32*) show resistance to infection by CCR5-tropic HIV-1 strains (17). As a result, a number of CCR5 antagonists have become available. The current leading CCR5 antagonists in development include maraviroc (UK-427857, Selzentry™, Celsentri®; Pfizer), which has demonstrated efficacy and tolerability in HIV-infected patients (18). Maraviroc has been approved in the U.S. and Europe for the treatment of HIV-infected adult patients who are infected with only CCR5-tropic HIV-1 strains resistant to multiple antiretroviral agents.

CCR5 is expressed on monocytes, macrophages, immature dendritic cells, T lymphocytes and B lymphocytes. The CCR5 ligands include CCL3, CCL4, CCL5, CCL7, CCL8, CCL11 and CCL13 (15). CCR5 antagonists may therefore offer benefits to patients with inflammatory disorders including MS. The use of CCR5 antagonists in patients with inflammatory disorders is unlikely to be totally without adverse effects, however, as highlighted by the increased risk of symptomatic West Nile encephalitis virus in patients with a *CCR5*-null allele (16). Furthermore, there was a slight increase in the number of malignancies (Hodgkin's disease, non-Hodgkin's lymphoma, gastric adenocarcinoma and human papillomavirus-related squamous cell carcinoma) in subjects receiving antiretroviral therapy and a CCR5 antagonist (vicriviroc) compared to the placebo group (19).

Genetic studies of CCR5 in MS

The remarkable heterogeneity of MS in terms of clinical, radiological and neuropathological features makes it an appealing disorder to explore the disease-modifying affects of *CCR5* genes, both the null (32-base-pair deletion in the coding region) and high-producer (*CCR5-303G,* which is a single nucleotide polymorphism [SNP] of allele *CCR5-303A*) alleles.

There is consistent evidence that CCR5 does not play a primary role in susceptibility to MS (20-26). The *CCR5*-null allele, which leads to retention of truncated CCR5 in the endoplasmic reticulum, is equally frequent in MS patients as in controls, as shown in a study by van Veen *et al.* and their meta-analysis of 1,925 MS patients and 1,390 controls (20). Furthermore, CCR5 does not appear to influence the pattern of demyelination in terms of T cell- and antibody-mediated *versus* primary oligodendrocyte dysfunction (27).

In contrast, evidence for a disease-modifying effect for CCR5 is less consistent. In a multifaceted study, van Veen *et al.* reported an increase in clinical disease severity and earlier disease onset in high producers of CCL5 and CCR5, as well as a decrease in axonal degeneration and T2 and T1 lesion volumes on MRI in lower producers of CCL5 and in those with a *CCR5*-null allele (20). A number of previous studies support the disease-modifying affects of CCR5 (23, 24, 28), but others have found no effect or the opposite effect (25, 26).

Expression of CCR5 and its ligands in MS lesions

Increased expression of CCL3, CCL4 and CCL5 has been described in MS lesions (29, 30). CCR5 may be involved in the early stages of leukocyte infiltration, as CCL5 immunoreactivity is seen in endothelial cells in normal-appearing white matter (29). CCR5 expression has been detected on perivascular monocytes and T cells, as well as microglia and macrophages, in MS (31-33). Up to 90% of perivascular T cells express CCR5 in MS lesions (34, 35), and almost all monocytes and foamy macrophages in MS lesions express CCR5, which tends to be upregulated during lesion maturation in a subset of acute MS lesions (pattern II) (36). Activated microglia also express CCR5 in MS lesions (32).

Expression of CCR5 and its ligands in blood and CSF of patients with MS

Compared with blood T cells, CSF T cells are significantly enriched in CCR5 expression (32, 35, 37). Interestingly, the enrichment of CCR5-positive T cells in the CSF appears to be independent of CNS inflammation and can be largely explained by the higher frequency of

$CD4^+/CD45RO^+$ T cells in the CSF (38). Less than 20% of circulating monocytes express CCR5, compared with 70% of CSF monocytes ($CD14^+$), independent of CNS pathology (33).

CCL5 expression is increased in the CSF of patients with MS (32). However, the low sensitivity of ELISA assays and the presence of proteins in low pg/ml quantities have made the detection of CCL3, CCL4 and CCL5 in the CSF difficult. The relative enrichment of $CCR5^+$ T cells and monocytes in the CSF suggests that CCR5 might be necessary for transmigration of these cells across the blood–brain barrier or that CCR5 is an indirect marker of mononuclear cells capable of migration. The therapeutic modulation of CCR5 will depend on whether CCR5 is necessary for T cell and monocyte transmigration across the blood–brain barrier.

CCR5 antagonists in models of MS

$CCR5^{-/-}$ mice showed the same susceptibility to myelin oligodendrocyte glycoprotein (MOG)-induced experimental autoimmune encephalomyelitis (EAE) as wild-type mice. Met-RANTES (*N*-terminal-modified human CCL5 molecule) (39), which antagonizes CCR5 and CCR1, did not alter the susceptibility to EAE but reduced the long-term neurological disability and axonal loss during the plateau phase of EAE (40). The *in vivo* expression of an immunotoxin (DT390-RANTES-SRα), which is highly toxic to activated mouse T cells expressing CCR5, in muscle reduced the severity of EAE (41).

Modulation of CCR5 expression upon transmigration across the blood–brain barrier

The modulation of CCR5 on T cells ($CD4^+/CD45RO^+$) and monocytes ($CD14^+$) during transmigration across the blood–brain barrier has been investigated using an *in vitro* model (42). Monocytes appeared to be selectively activated through contact with endothelial cells, augmenting CCR5 expression on their surface. This was not the case for $CD3^+$ cells. Furthermore, the upregulation of CCR5 on monocytes was not a generic feature of transmigration in this model. CCR5 expression was enriched on monocytes that spontaneously migrated across the blood–brain barrier, whereas T cells required a chemotactic signal (addition of CCL5 to the bottom chamber) for migration across the blood–brain barrier. After migration, CCR5 was downregulated on both T cells and monocytes in the presence of CCL5. A functional neutralizing monoclonal antibody against CCR5 significantly reduced CCL5-driven transmigration of T cells and monocytes *in vitro* (43). These observations suggest that systemically administered CCR5 antagonists may modulate the infiltration of inflammatory cells into the CNS in MS.

A number of processes regulate the cell-surface expression and function of CCR5, including ligand-induced internalization with subsequent recycling or degradation. The fate of the internalized CCR5 partly depends on the *C*-terminal receptor phosphorylation by G-protein coupled receptor kinases (44, 45). The phosphorylated receptor, bound by β-arrestins, is linked to clathrin in order to mediate receptor internalization by endocytosis. Ligand-activated CCR5 may undergo caveolae-dependant endocytosis, independent of receptor phosphorylation. Once internalized, CCR5 accumulates in the perinuclear recycling endosomes and may return to the plasma membrane in a dephosphorylated form (46). Furthermore, the cell-surface expression of CCR5 depends on the removal of ligands through sequestration, dissociation from the receptor and endosomal degradation.

Conclusions

CCR5 is a potential therapeutic target in MS. Nonlipophilic CCR5 antagonists might reduce transmigration of receptor-bearing leukocytes into the CNS, without a requirement for agents to enter the CNS. The overall impact of CCR5 antagonists on the “compartmentalized” CNS inflammatory response, including microglial activation and chronic effects of activated monocytes and macrophages in MS lesions, will depend on the ability of such agents to penetrate the CNS.

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