Review

The Role of CCR5 in HCV Infection

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Abstract

Efficient recruitment and activation of immuno-competent cells is crucial for an effective immune response to hepatitis C virus (HCV) infection. Chemokines and chemokine receptors have been shown to be critically involved in these processes.

The CCR5 chemokine receptor is expressed on several cells of the immune system and has been suggested to influence the susceptibility to HCV infection as well as natural course and progression of hepatitis C. However, these reports are still controversial.

This review will summarize and discuss the available data regarding the potential role of CCR5 and its ligands in hepatitis C.

Key words: chemokine, chemokine receptor, CCR5, HCV, immune response

Chemokines and Chemokine Receptors

Chemokines, a group of chemotactic cytokines exert chemotactic and immunoregulatory actions [1]. Furthermore, these molecules are involved in modulation of adhesion processes at the endothelium and thus promote the transendothelial migration of leukocytes (haptotaxis). In addition, some chemokines stimulate angiogenesis or angiostasis and thus may play a further role in the suppression of tumor growth or the establishment of an inflammatory response.

Currently, about 50 human chemokines and 20 chemokine receptors have been described. Traditionally, chemokines and chemokine receptors have been classified into four families (CXC, CC, C and CX3C).

Several years ago, a new nomenclature was introduced in which each ligand and each receptor is identified as a member of a sub-family and carries a special number [2]. For example, a chemokine designated CCL5 is a member of the CC sub-family with the number 5. This special chemokine was formerly known as RANTES.

Despite their essential importance for the immune system chemokines and chemokine receptors have been associated with an extraordinary number of diseases [3]. These include autoimmune diseases [4, 5], cancer [5], lung [3], and vascular diseases [6] as well as transplant rejection [7-10].

In addition, the human immunodeficiency virus (HIV) uses different chemokine receptors as co-receptors for entry into the cell [11].

CC chemokines are an important subset of chemokines. They activate specific inflammatory cells such as monocytes or T lymphocytes and recruit them to the site of inflammation. These CC chemokines bind to specific G protein-coupled receptors, thereby initiating activation and migration of cells.

The CC chemokines CCL3 (MIP-1α), CCL4 (MIP-1β), CCL5 (RANTES), and CCL3L1 are the natural ligands of the CC-chemokine receptor CCR5 (CC-CKR5, ChemR13 or CMKBR5). The coding sequence for this receptor is located at position 21 on the short arm of chromosome 3 and has a coding sequence of 1056 base pairs, which is translated into a protein of 352 amino acid length.

Expression of Chemokine Receptors

Based on their cytokine profile CD4-positive T-cells can be divided into different subclasses: TH1 cells (T helper cells type 1) secrete IL-2, IFN-γ and lymphotoxin, whereas TH2 cells produce IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13. TH1 cells primarily control the cellular immune response and appear to be involved in chronic inflammatory processes. Th2 cells support the formation of specific antibodies by increasing the B-cell proliferation and their differentiation into plasma cells and play an important role in the development of allergic reactions due to the promotion of IgE production. Interestingly, the expression of specific chemokine receptors is closely associated with the differentiation and activation of specific inflammatory cells. Thus, the CCR5 receptor is expressed on TH1 lymphocytes [12] but not on TH2 lymphocytes, which primarily express CCR3, and CCR8 and CCR4.

Beyond TH1 cells, CCR5 has also been detected on monocytes, memory T cells (CD45RO), stem cells, dendritic cells, microglia, and on a main group of cytotoxic CD8 (+) T lymphocytes.

HCV and CCR5

Infiltration of mononuclear inflammatory cells is a central feature of hepatitis C virus (HCV) infection. Here, many of the liver-infiltrating cells express high levels of the CCR5 receptor. Moreover, high levels of the chemokines CCL3, CCL4 and CCL5 can be found in the HCV-infected liver [13].

In vitro data suggest that the hepatitis C virus can directly influence the secretion of these chemokines. For instance, it could be shown that binding of the HCV E2 envelope protein to the tetraspanin CD81, which serves as a co-receptor for HCV, induces release

of the CCR5 ligand CCL5 by CD8 (+) T cells [14]. In addition, the HCV core and NS5A proteins have been demonstrated to affect CCL5 secretion by modulating CCL5 promoter activity [15].

Of note, CCR5 has been shown to promote hepatic fibrosis in mice and marked up-regulation of CCL5 and CCR5 has been found in patients with hepatic cirrhosis, confirming activation of the CC chemokine system in human fibrogenesis [16, 17].

In contrast to the infected liver significantly reduced numbers of CCR5-expressing lymphocytes can be found in the peripheral blood of HCV-infected patients. This may be due to HCV-induced intrahepatic chemokine secretion mediating the hepatic recruitment of CCR5-mediated hepatic (+) T-cell and thus reflect altered compartmentalization of CCR5(+) lymphocytes.

A strong anti-viral type 1 immune response has been shown to be associated with a successful elimination of the virus, both during the acute phase of infection and an antiviral therapy [18-20]. This would suggest that accumulation of CCR5 (+) TH1 cells in the HCV-infected liver contributes to an effective antiviral immune response. However, these relationships appear to be more complex. We recently showed that CCR5 (+) lymphocytes are characterized by a high expression of the inhibitory NK cell receptor NKG2A [21]. Therefore, these cells are very sensitive to NKG2A-mediated inhibition of cytotoxic function. As hepatitis C is associated with a high intrahepatic expression of the NKG2A ligand HLA-E, intra-hepatic accumulation of CCR5(+)NKG2A(+) lymphocytes could interfere with an effective immune response. In addition, CCR5 (+) T cells may have a role in immune-mediated liver cell damage during chronic hepatitis C [22].

THE CCR5Δ32 POLYMORPHISM

The CCR5 gene is subject to various mutations, including a deletion of 32 base pairs. This mutation, called CCR5Δ32, leads to a shift in the reading frame, resulting in the formation of a truncated, non-functional protein, which is not expressed on the cell surface [23, 24]. In a Caucasian population the CCR5Δ32 allele frequency is about 10-20% [23].

The frequency of the CCR5Δ32-polymorphism decreases from Northern to Southern Europe. Moreover, the Δ32 allele is absent in African and Asian cohorts. Thus, it is believed that this mutation first occurred in Northern Europe not so long ago.

Individuals who are homozygous for the mutation and thus "genetically negative" for CCR5, develop normal and are healthy. The same was observed in CCR5 knockout mice. Therefore, it was originally presumed that the CCR5 receptor has no relevant importance to the immune system. In further studies, however, it could be shown that lack of CCR5 is associated with a significantly altered immune response in mice. For example, infection with Cryptpcoccus neoformans is often fatal in CCR5-deficient mice, whereas wild-type mice survive. Furthermore, experiments using CCR5 knockout demonstrated that lack of CCR5 is associated with an increased T-cell immune response in various infectious diseases [25-27].

Even in non-infectious inflammatory reactions CCR5 seems to play an important role in the regulation of T-cell functions. Recently, it has been reported that CCR5-deficient mice, in which a T cell-mediated autoimmune hepatitis had been induced, displayed a significantly greater liver damage in comparison to CCR5-positive mice [28].

Studies on the clinical relevance of CCR5Δ32 mutation in humans indicated that absence of the CCR5 receptor may, under certain circumstances, have either a positive or a negative effect. Patients carrying a homozygous CCR5Δ32-genotype have a higher risk of death during infection with West Nile virus [29]. On the other hand, it is presumed that the absence of CCR5 confers some protection against infection with smallpox (or the plague), which is reflected by the relatively high prevalence of this mutation in Central and Northern Europe.

Moreover, CCR5 has been suggested to play a role in transplant rejection [7-10, 30]. For instance, a prospective, biopsy-controlled study suggested that the local expression of the CCR5-ligand RANTES (CCL5) leads to the directional movement of activated CCR5 bearing T cells into the renal allograft thereby mediating acute rejection [10]. Fischrieder et al. reported patients homozygous for CCR5Δ32 to show longer survival of renal transplants than those with other genotypes [9]. In contrast, Bickerstaff and co-workers found rapid rejection of renal allografts in CCR5–/– mice with many histopathologic features observed during AHR of human renal allografts [8]. Thus, the exact role of CCR5/CCR5 polymorhpisms in renal transplantation remains controversial [30].

HCV AND CCR5Δ32

With respect to HCV infection data regarding the importance of CCR5Δ32 mutation are conflicting. Woitas et al. reported that patients with chronic HCV infection were significantly more likely to exhibit the homozygous CCR5-Δ32/CCR5-Δ32 genotype compared to a healthy control group. Moreover, CCR5-Δ32 homozygosity in this study was associated with significantly increased HCV viral loads and CD8+ T lymphocyte counts in the peripheral blood [31]. Thus, the results of this study would suggest that individuals with a CCR5Δ32/CCR5Δ32 genotype display an increased susceptibility to HCV infection.

However, in subsequent studies this association could not be confirmed. In this regard, it has been speculated that increased CCR5Δ32-frequency may be a specific characteristic of haemophilic patients with chronic hepatitis C but without HIV infection - and rather reflects resistance to HIV-1 than increased susceptibility to HCV. However, a detailed statistical analysis showed that the high frequency of CCR5Δ32 in chronic hepatitis C reported by Woitas et al. could not be explained by the HIV-mediated selection pressure alone [32].

Alternatively, the strategy of patient selection in the Woitas study (haemophilia versus chronic liver disease) or the analysis of heterogeneous cohorts (stage and duration of infection, infecting HCV genotype, demographic factors) might explain these discrepant results.

This was examined in two independent largely homogeneous cohorts of HCV-infected women with a known source of infection. In the late 70s these women were infected by anti-D immunoglobulin contaminated with hepatitis C virus (HCV) genotype 1b from a single erythrocyte donor administered for prophylaxis of rhesus isoimmunization throughout East Germany and Ireland, respectively. In contrast to the data published by Woitas and co-workers the CCR5Δ32 mutation was associated with spontaneous elimination of the virus in the Irish anti-D cohort [33]. However, in the Eastern German "Anti-D cohort," we found carriers of the CCR5Δ32 polymorphism to be significantly more likely to develop chronic infection than women with a homogeneous wild-type genotype (Nattermann et al., AASLD 2009).

Similar discrepant results have been published regarding a possible association between the CCR5Δ32 polymorphism and the degree of the HCV-associated hepatitis. Data obtained in the Irish "Anti-D cohort" [33] and in two other studies [34, 35] showed a less pronounced hepatic inflammation in carriers of the CCR5Δ32 mutation as compared to patients with a homozygous wild type. However, these results could not be confirmed in other publications [36-38].

In contrast to these controversial data, it is widely accepted that the CCR5Δ32 mutation does not affect response to standard combination therapy with pegylated interferon and ribavirin. Although the CCR5Δ32 polymorphism was associated with a significantly poorer response to monotherapy with standard interferon [39], no study could confirm a similar effect for patients treated with pegylated interferon in combination with ribavirin [32, 39, 40].

Recently, Dolan et al. published a very elegant and comprehensive study which might provide some explanations regarding the controversial data on the role of CCR5 in hepatitis C as this work shed light on the complexity of such associations [41]. In this study different cohorts of HIV-positive and HIV–negative persons were analyzed with regard to polymorphisms in the CCR5 gene and the gene of the CCR5 ligand CCL3L1. CCR5 is an important co-receptor for HIV and earlier studies demonstrated that the CCR5D32 mutation blocks infection of CD4 lymphocytes. Dolan et al. identified another mechanism by which CCR5 in association with its ligands affect the course of HIV infection.

In this work the study subjects were classified into three 'genetic risk groups' (GRGs) based on variation in CCR5-CCL3L1 genotypes that are associated with risk of acquiring HIV infection and on HIV replication.

Interestingly the authors could show a direct association of these GRKs with the cell-mediated immune response. As a marker they determined the delayed-type immune response (type IV), which is a common 'readout' of cellular immunity. This immune reaction is based on activation of TH1 lymphocytes induced by a soluble antigen which in turn results in activation of macrophages. A typical example is the tuberculine test which induces an immune reaction of pre-sensitized T-lymphocytes after inoculation of tuberculine into the skin. Dolan and co-workers showed an increased type-IV immune response in those GRGs associated with a delayed disease progression.

Thus, the authors concluded that CCR5 and its ligands not only interfere with viral entry of HIV into the cell but also affect anti-viral responses via modulating cellular immunity.

The complexity of these associations was further emphasized by the observation that the CCR5 haplotype (HHG*2) comprising the Δ32-mutation (both homozygous or in combination with another specific haplotype (HHE)) is a predictor of a weak cell-mediated immune response and a rapid progression of HIV infection. However, the same CCR5 HHG*2 haplotype in combination with the CCR5 HHC haplotype was associated with a slower progression of infection and possibly with a stronger immune response.

Similar complex relationships could also play a role in HCV infection. For instance, variations of the CCL3L1 gene have recently been shown to affect HCV infection [42]. Moreover, Ahlenstiehl et al. could show a significantly decreased HCV-specific IFN-γ response of CD8(+) T-cells in carriers of the CCR5Δ32 allele as compared to cells from patients with a homozygeous wildtype genotype. This finding suggests the CCR5Δ32 polymorphisms to possibly affect cellular immune responses in HCV infection [43].

In summary there is clear evidence for a critical role of the CCR5 receptor and its´ ligands for the regulation of immune response which might also play a role in HCV infection. However, further studies with larger number of patients and a more detailed genetic analysis are necessary to precisely define the role of CCR5 in HCV infection.

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