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Clinical study on CXCL13, CCL17, CCL20 and IL-17 as immune cell migration navigators in relapsing—remitting multiple sclerosis patients

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ABSTRACT

Background: There has been a growing evidence for the role of chemokines in the pathology of multiple sclerosis. Recently, there has been great emphasis placed on humoral immunity and the T(H)-17 response, which has not yet been thoroughly described in MS. The aim of this study was to investigate the role of specific chemokines involved in B-cell migration (CXCL13) and in the T(H)-17 immune response (IL-17, CCL17, CCL20).
Methods: Using ELISA, the chosen chemokine concentrations were measured in the serum and cerebrospinal fluid of relapsing—remitting MS patients with both active and stable disease, and the relapse prediction rate was calculated.
Results: We found that the CSF concentrations of CXCL13 in patients with RRMS both, during relapse and remission, were significantly higher than in controls. CCL17 and CCL20 were not detected in CSF in either of the groups, whereas serum CCL20 level was significantly higher in remission than during relapse. Intravenous methylprednisolone treatment of patients with relapse did not influence serum CXCL13 and CCL20 levels. However, it did lower CCL17 and IL-17 concentrations.
Conclusions: CXCL13 is an important mediator in MS that is strongly linked to the neuroinflammatory activity of the disease. However, more studies are needed for elucidating the roles of CCL17, CCL20 and IL-17 in MS pathology.

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1. Introduction

Multiple sclerosis (MS) is a chronic inflammatory demyelinating and neurodegenerative disease of the central nervous system (CNS) [1]. MS is characterized by multiple areas of CNS white matter inflammation, demyelination, glial scarring (sclerosis), perivascular leukocyte infiltration [2], axonal damage and neuronal loss. While for many years demyelination was considered to be the major aspect of MS pathology, it is now an indisputable fact that axonal loss determines brain and spinal cord atrophy leading to disease progression in MS patients [3].

Immunohistopathologically, MS is characterised by recruitement of activated T cells and macrophages into the CNS, and by persistent intrathecal synthesis of immunoglobulins, such as oligoclonal IgG of unknown specificity [4]. The putative autoimmune origin of MS, with myelin-reactive T cells as the key mediators in demyelinative damage has recently been questioned raising the importance of the humoral aspects of this disease. Undoubtedly, neuroinflammation is a fundamental process in MS pathology with the critical step being immune cell migration into the CNS. Trafficking of immune cells is guided mainly by cytokines, chemokines and their receptors, and other immune system molecules, such as adhesive agents.

Chemokines, also known as chemotactic cytokines, are a family of small peptide mediators which attract leukocytes to sites of inflammation. Thus far, about 50 human chemokines, categorized into four families, have been described [5]. A number of chemokines have already been investigated in body fluids and brain samples from MS patients, and there is growing evidence for their pathogenic role in this disease pathology [6]. Specifically, CXCL13 is a chemokine which is critical for secondary lymphoid tissue development and for B-cell migration [7]. Its primary G-protein coupled receptor, namely CXCR5, is expressed mainly on B cells, monocytes and dendritic cells. However, CXCL13 has also been shown to activate *in vitro* CXCR3, which is a known receptor for CXCL9, CXCL10 and CXCL11 chemokines, and thus to recruit activated TH1 cells to sites of inflammation [8]. Krumbholz et al. detected CXCL13 in perivascular infiltrates within actively demyelinating lesions, but not in chronic inactive ones or in normal CNS. In addition, Krumbholz detected a significant correlation between CSF CXCL13 levels, which were elevated in relapsing-remitting MS (RRMS) patients and the number of B cells, plasma blasts and T cells in the cerebrospinal fluid [9]. This clearly suggests that CXCL13 may be

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one of the key mediators responsible for the neuroinflammation which underlies MS pathology.

So far there have been only a few reports on the role of CCL20 chemokine in MS [10–12]. CCL20 is produced by activated cells, including monocytes and T cells, and has selective chemotactic activity for lymphocytes and dendritic cells. Also, it has the ability to bind with the G protein-coupled chemokine receptor CCR6 [13], which is expressed on memory T cells, B cells and dendritic cells [14]. In 2008 Michalowska-Wender et al. described a group of 30 MS patients, whose serum CCL20 levels did not differ significantly from those in the healthy controls group, and were not influenced by intravenous methylprednisolone treatment [10]. Furlan et al. showed that peripheral blood mononuclear cells (PBMCs) mRNA levels of CCL20 were increased in MS patients [12]. CCL20 was also found to be elevated at disease onset in an animal model of MS, namely in experimental autoimmune encephalitis (EAE) [15,16]. Moreover, CCL20 has been implicated in priming pathogenic T cells in EAE [15]. Interestingly, it has recently been found that mice lacking CCR6 were highly resistant to the induction of EAE. The authors of this report suggested that the CCR6-CCL20 axis in the choroid plexus controls immune surveillance of the CNS [17]. Therefore, one might suspect it could also be crucial for human pathology.

What seems to be of high importance is that CCR6 is a chemokine receptor characteristic of T(H)-17 cells, that produce interleukin 17 (IL-17), known to induce proinflammatory cytokines, such as TNF-α, IL-1β, IL-6, and chemokines, including CCL20 [18]. IL-17 seems to play a crucial role in MS pathogenesis. A significant increase in the number of T(H)-17 cells has been demonstrated in active rather than inactive areas of MS lesions [19]. Also, it was found that patients with MS had an increased number of IL-17 mRNA expressing PBMCs during clinical relapses compared to remissions. In addition, MS patients have a higher number of IL-17 mRNA expressing mononuclear cells and are found in CSF rather than in peripheral blood [20]. Moreover, T(H)-17 cells have recently been shown to transmigrate efficiently across blood−brain barrier endothelial cells, promoting CNS inflammation through CD4+ lymphocytes recruitment [21]. In one study, a significant increase in IL-17 CSF levels was described in a population of Asian patients with opticospinal MS, in comparison with the control group [22]. This was contrary to the results from a study conducted on European MS population, in which no difference between IL-17 CSF levels was found between patients and controls [23].

IL-17-producing human CD4+ T cells have been found to express not only CCR6, but also CCR4 receptor, which binds to CCL17 chemokine [24]. In another study, CCL17 levels were found to be elevated in CSF of MS patients [25]. CCL17 binds to CCR4 receptor, which is expressed on PBMCs and human T-cell lines, but not on B cells, NK cells, or granulocytes, and to the lymphoid tissue CCR8 receptor. Both CCR4 and CCR8 are transiently upregulated on activated T cells, preferentially on the T(H)-2 subset [26–28]. The role of CCL17 has not yet been fully investigated in the pathogenesis of MS.

The aim of the present study was to estimate the levels of CCL20, CCL17, CXCL13 chemokines and IL-17 cytokine in the sera and CSF of RRMS patients during relapse and in remission, and to compare the results with those obtained from patients with other, noninflammatory neurological disease. Moreover, we tried to establish which measurement gives the best insight into defining the clinical relapse in RRMS patients by performing a ROC curve analysis. Lastly, we wanted to evaluate the influence of methylprednisolone (MP) treatment on CCL20, CCL17, CXCL13 and IL-17 serum concentrations in patients with RRMS.

2. Materials and methods

2.1. Patients

We studied two groups of patients with clinically established multiple sclerosis according to revised McDonald criteria [29]: one group consisted of 17 patients with RRMS during relapse, which was defined as a sudden appearance of new symptoms and signs lasting at least 24 h with an increase of at least 1.0 point in the EDSS score; and the other group included 15 RRMS patients in a stable phase of the disease (remission). The control group consisted of 20 patients with noninflammatory neurological diseases (tension type headaches), matched with the RRMS group according to age. Additionally, in 15 patients from the relapse group we examined the influence of methylprednisolone therapy (a 5-day treatment with MP at a dose of 1 g i.v. once daily; Solu-Medrol, Pharmacia NV/SA, Puurs, Belgium) on the chosen chemokine serum concentrations. The interval between the attack onset and the beginning of steroid therapy was of no more than 3 weeks.

2.2. Cytokine assays

The levels of CXCL13, CCL17, CCL20 and IL-17 were measured in sera and in CSF by ELISA according to the manufacturer's instructions (Quantikine Human CXCL13/BLC/BCA-1 Immunoassay; Quantikine Human CCL20/MIP-3α Immunoassay, Quantkine Human IL-17 Immunoassay, Quantikine Human CCL17/TARC Immunoassay, R&D Systems, USA). The minimum detectable dose for CXCL13, CCL20, CCL17 and IL-17 was 1.64, 0.47, less than 7 pg/ml and less than 15 pg/ml, respectively. CSF derived of cells and serum samples were frozen and stored at −80 °C until the analysis was performed. CXCL13, CCL17 and CCL20 were measured in the CSF and in the serum of MS patients during relapse, remission, and in controls. In addition, these markers were also measured before relapse treatment and 24 h after the final dose of intravenous methylprednisolone. IL-17 has been measured in serum of MS patients during relapse, both before and after steroid therapy, in patients during remission and in a control group. IL-17 CSF levels have been estimated for remission and control groups only.

2.3. Statistical analysis

Statistical analysis, using Statistica 8.0 and R CRAN environment (www.r-project.org), was utilized. It included the nonparametric Mann−Whitney *U* test for comparing two different groups, the one-way ANOVA test for multiple group comparison, the Wilcoxon test for comparison of paired data, and Friedman test for time-dependent observations. The p value<0.05 was considered statistically significant. The receiver operating characteristic (ROC) curve analysis was performed using ROCR package in R CRAN environment [30] to discriminate between MS remission and relapse states by using the chosen chemokines' concentrations. We next calculated the sensitivity and specificity pairs for each concentration threshold and constructed the ROC curve. Area under the curve (AUC) was calculated for serum CCL20 (AUC = 0.83), serum CXCL13 (AUC = 0.52) and CSF CXCL13 (AUC = 0.58) curves. Further analysis was done only when relevant, namely for serum CCL20. If serum CCL20 concentration was below threshold in a relapse patient, it was considered a true positive (TP) finding. If a patient with relapse had the value above threshold, it was considered a false negative (FN) finding. If a patient with remission had serum CCL20 below threshold, it was considered a false positive (FP) finding, and if a patient with remission had the value above threshold, it was considered a true negative (TN) finding. We also identified serum CCL20 concentration, providing the maximum accuracy (ACC), defined as fraction of correct (true) classifications among all classifications as follows: $ACC = (TP + TN)/n$, where n is the number of all patients. Sensitivity, specificity and positive and negative predictive values were calculated for maximum accuracy threshold.

3. Results

3.1. Patients

The relapse group consisted of 6 males and 11 females with a mean age of 34.8 years (range 21–53 years) whose mean EDSS score on

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