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Original article

Concentration gradient of CXCL10 and CXCL11 between the cerebrospinal fluid and plasma in children with enteroviral aseptic meningitis

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ABSTRACT

Background: Lymphocyte migration from the blood into the CNS is mediated by chemokines and chemokine receptors. Chemokines CXCL10 and CXCL11 are important for the recruitment of CXCR3-expressing Th1 lymphocytes to the site of inflammation.
Aims: To determine the concentrations of CXCL10 and CXCL11 in the CSF and plasma of children with enteroviral aseptic meningitis (EV AM) and controls and the contribution of these chemokines to the chemokine concentration gradient between the periphery and the CNS.
Methods: The study included 26 pediatric patients with EV AM and 16 controls in whom CNS infection is excluded by negative CSF examination. Chemokines were quantified by using enzyme immunoassay. Etiological diagnosis of EV AM was based on the detection of enteroviral RNA in the CSF using real-time PCR.
Results: CXCL10 (median 12 725 pg/ml) and CXCL11 (median 187 pg/ml) concentrations in CSF of patients with meningitis were significantly higher compared to plasma (median 173 pg/ml and median 110 pg/ml; $p < 0.001$, $p = 0.026$ respectively). CXCL10 concentrations in the CSF (median 198 pg/ml) and plasma of controls (median 124 pg/ml) were not significantly different ($p = 0.642$). CXCL11 concentrations in the CSF of controls (median 89 pg/ml) were significantly lower compared with plasma (median 139 pg/ml, $p = 0.004$). Chemokine concentration gradient was not influenced by pleocytosis, nor dependent on cytologic CSF formula or the presence of proteinorrachia.
Conclusion: CXCL10 and CXCL11 concentration gradient between the CSF and plasma in children with EV AM suggests an important role of these chemokines in the T-cells recruitment into the CNS and local immunoreaction.

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1. Introduction

Enteroviruses (EV), RNA viruses belonging to *Picornaviridae* family, cause a wide spectrum of illnesses, from benign and self-limiting diseases manifested by fever alone to severe life threatening infections, including neonatal sepsis, myocarditis, encephalitis and myelitis.[1–3] However, the most common clinical presentation of EV disease that deserves the attention of clinicians especially during the summer and fall season is aseptic meningitis (AM).[1,4] The importance of EVs in AM etiology clearly underlines the fact that more than 80% of all AM cases in which the causative agent was defined are caused by one of those viruses.[1] The widespread use of standardized real-time polymerase chain reaction (PCR) assays for etiological diagnostics of neurotropic viruses in the cerebrospinal fluid (CSF) of patients with AM has further increased the number of diagnosed cases and thus confirmed the leading position of EVs in the etiology of this syndrome.[1] Although the clinical characteristics and laboratory findings in EV AM have been well studied, the knowledge of the local immune response is still scarce.[1,4–6] The majority of studies dealing with this topic have focused on patients with more severe forms of CNS inflammation caused by EVs, especially those with enterovirus 71 (EV71) brainstem encephalitis.[7,8]

The leading, although nonspecific laboratory hallmark of EV AM is pleocytosis, usually predominantly lymphocytic.[4] Some earlier studies demonstrated a preferential recruitment of memory CD4+ T-cells from the blood to the CSF.[5,9,10] These results support the hypothesis that activated memory CSF CD4+ T-cells are the principal cellular subpopulation responsible for the local immune response in patients with inflammatory CNS diseases, including those with EV AM.[5]

After crossing the blood brain barrier (BBB) memory CD4+ T cells can encounter and recognize their specific antigen and trigger the local inflammatory response. Alternatively, in case that memory CD4+ T cells do not encounter an antigen, they will recirculate back into the periphery.[11]

The lymphocyte migration through the BBB into the CSF in response to infection with neurotropic viruses is mediated by chemokines and their corresponding receptors.[12] Chemokines are small, soluble, basic proteins that can be classified into four subfamilies: CXC, CC, CX3C and C, depending on the number and distribution of cystein residues within the amino terminus of the molecule.[13] The current hypothesis holds that upon recognition of viral DNA or RNA, Toll-like receptors activate intracellular signaling cascade resulting in enhanced expression of genes coding for various cytokines, particularly interferons and chemokines.[14] *In vitro* and *in vivo* studies showed that astrocytes and microglia cells are the principal sources of chemokines following viral infections of the CNS. Studies in both animal and human models showed complex and variable patterns of chemokine synthesis in various CNS viral infections.[14]

Chemokines CXCL10 (interferon-inducible protein-10, IP-10) and CXCL11 (interferon-inducible T-cell alpha chemoattractant) are CXC chemokines that are synthesized upon stimulation with IFN-γ during infection or inflammation.[11] Both chemokines bind to the chemokine receptor CXCR3 and are important in pathologies mediated by activated Th1 CD4+ T-cells. The biological role of CXCL10 appears to be complex. CXCL10 recruits antigen-specific activated CXCR3-positive T-cells into the CNS enabling the efficient control of viral replication.[13] However, persistent overexpression of CXCL10 can lead to continuous infiltration of inflammatory cells that can subsequently lead to neurotoxicity, cell death or an immune-mediated demyelinating disease.[14–16]

Several studies investigated the expression of CXC10 and CXCL11 as well as possible contribution to the immunopathogenesis of CNS infections caused by tick-borne encephalitis virus, West Nile virus, lymphocytic choriomeningitis virus, human immunodeficiency type 1 virus, herpes simplex virus and neuroborreliosis in adult patients.[17–23] However, literature data on the expression of these chemokines in pediatric viral CNS infections are scarce. The Taiwanese investigators demonstrated the presence of CXCL10 in the CSF of children with brainstem encephalitis developing as a consequence of infection with enterovirus 71.[8] To our knowledge, the expression of CXCL10 and CXCL11 in the CSF of Caucasian children with non-complicated clinical course of EV AM has not been previously investigated.

The aim of this study was to investigate the presence of CXCL10 and CXCL11 in the CSF and plasma of children with EV AM and compare the observed pattern of expression with controls in whom the diagnosis of CNS inflammatory disease was excluded. The results of this study are expected to determine whether overexpression of CXCL10 and/or CXCL11 in the CSF contributes to the induction of local cellular immune response in patients with EV AM.

Additionally, extensive research on the role of chemokines in the pathogenesis of various inflammatory diseases consequently lead to the development of a large number of molecules targeting chemokines and chemokine receptors as candidates for human therapeutics. So far, two drugs targeting chemokines and chemokine receptors are currently in use; a CCR5 inhibitor for the treatment of HIV-infection and a CXCR4 antagonist that stimulated hematopoietic stem cell mobilization that is essential for the treatment of hematological malignancies.[24] Therefore, the results of this study can provide an important insight into possible relevance of chemokine/chemokine receptor inhibitors/antagonists as potential therapeutics in the treatment of inflammatory CNS diseases.

2. Patients and methods

2.1. Study design and patients

This prospective, cross-sectional study was carried out at the University Hospital for Infectious Diseases "Dr. Fran Mihaljevic" (UHID), Zagreb, Croatia between January 2009 and November 2010. The study included 42 patients aged less than 14 years of whom 26 with EV AM, and 16 controls. The diagnosis of AM was based on clinical signs and symptoms, accompanied with CSF cytological and biochemical findings, negative Gram stain and bacteriological culture.[25] The EV