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IL-6 and CCL2 levels in CSF are associated with the clinical course of MS: Implications for their possible immunopathogenic roles

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Abstract

Biological markers would provide valuable tools for tracking disease activity, immunopathological processes or therapeutic efficacy in MS. In this study we analysed a panel of Th_1/Th_2 cytokines and the chemokine CCL2 in serum and CSF from MS patients and healthy controls. Increased levels of IL-6 ($p<0.05$) and decreased levels of CCL2 ($p<0.001$), with the lowest levels during acute relapses, was found in CSF from patients with relapsing–remitting MS. CSF levels of CCL2 correlated with indices for intrathecal IgG production and the CSF level of the neurofilament light protein, a marker for axonal damage, indicating a immunopathogenic role for CCL2.

Keywords: MS; CCL2; MCP-1; IL-6; NFL; CSF markers

1. Introduction

Multiple sclerosis (MS) is considered to be an autoimmune disease of the central nervous system (CNS). The neuropathology is characterised by inflammatory cell infiltrates, demyelination, axonal degeneration and astrogliosis. Although most interest of the immunopathogenesis has focussed upon demyelination with myelin proteins as the most likely targets for the immune attack, the CNS inflammation probably plays a critical role in most of the pathological processes in MS (Hickey, 1999). Evidence for a predominantly destructive role of inflammation is suggested by the presence of inflammatory cell infiltrates at all stages of acute lesion evolution (Trebst et al., 2001), positive correlation of inflammation and the degree of axonal transection in MS lesions (Ferguson et al., 1997; Trapp et al., 1998), and the protective role of immunomodulating drugs on brain atrophy development (Hardmeier et al., 2005; Rudick et al., 1999).

The inflammation involves infiltration of monocytes and activated T-cells through the blood–brain barrier (BBB) into the CNS. The role of cytokines and chemokines in the inflammatory cascade of MS has been investigated in brain lesions (Cannella and Raine, 1995; Woodroofe and Cuzner, 1993; McManus et al., 1998) as well as in serum and CSF (Mahad et al., 2002b; Nicoletti et al., 1996; Scarpini et al., 2002; Sorensen et al., 2004, 1999). Although these studies are essentially descriptive, they suggest an important role for cytokines and chemokines in the immunopathogenesis of MS. The pattern of cytokines may reveal the inflammatory activity during different stages of MS as well as the possible association with different pathological processes in MS. Cytokines analysed in serum and CSF in the present study were chosen from their major characteristics along the Th_1/Th_2 axis: IFN-γ, TNF-α, predominantly Th_1 (Hakonarson et al., 1999); IL-6 considered to have multiple prosperities (Hirano, 1998); IL-4, mostly Th_2 (Kucharzik et al., 1997). We also determined the level of the chemokine CCL2, also designated MCP-1 (monocyte chemoattractant protein-1), which has previously shown to be associated with the clinical course of MS (Mahad et al., 2002b; McManus et al., 1998; Scarpini et al., 2002; Sorensen et al., 2004).

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In a previous study we found increased levels of the light subunit of the neurofilament protein (NFL), a biomarker for axonal damage, in all stages of MS with an almost 10-fold increase during acute relapse (Malmestrom et al., 2003). We also showed increased levels of the glial fibrillary acid protein (GFAP), a marker for astrogliosis, with the highest levels during secondary progressive MS (SPMS) (Malmestrom et al., 2003). The aim of the present study was to investigate a panel of Th_1/Th_2 cytokines and CCL2 as possible immunological markers for disease activity in MS and investigate if serum or CSF levels were correlated with those indicating axonal damage or astrogliosis.

2. Materials and methods

2.1. Patients and controls, serum and CSF sampling and routine analysis

The study was approved by the Ethical Committee of the University of Gothenburg, Sweden and informed consent was obtained from all patients and healthy control subjects. Sixty-three patients with MS according to the McDonald criteria (McDonald et al., 2001) were consecutively recruited at the Department of Neurology, Sahlgrenska University Hospital, Gothenburg. To minimize interpretation difficulties due to variation in levels of the chosen biomarkers caused by other neurological diseases, healthy controls were chosen. Forty-four persons were recruited from the blood-donor registry. None of them had a history of neurological symptoms indicating an acute or chronic neurological disease and all had a normal neurological examination. Twenty-one patients were recruited by the time of an acute relapse and designated the relapsing–remitting MS-relapse group (RRMS-rel). Seventeen patients with clinically stable RRMS (i.e. no relapse for the last 3 months) were included in the RRMS-remission group (RRMS-rem). Twenty-five patients formed the SPMS group and none of them had had a relapse for the last 3 months. Three patients had experienced a relapse within the last year. One RRMS-rel and one RRMS-rem patient had ongoing interferon-β treatment at the time of their inclusion.

Patients were examined and neurological deficits were scored according to EDSS (Kurtzke, 1983). CSF samples were obtained by non-traumatic lumbar puncture (Lp). The first 12 ml of CSF were mixed and centrifuged, then frozen in 0.5 ml aliquots and stored at −80 °C. In the group of patients who had an ongoing acute relapse, 13 were followed with re-examination and repeated Lp. The delay between relapse onset and the first Lp was 16 days (median, range 5–34 days), and they had a second Lp at 5 weeks (median 34 days, range 26–53 days). CSF from the first Lp was missing in one patient. Three patients were lost to follow-up after the second Lp and the remaining 10 patients had a third Lp at 15 weeks after relapse onset (median 106 days, range 88–142 days). Thus, three consecutive CSF samples were obtained from 9 out of 13 patients. Six of the prospectively followed patients received treatment of their relapses with i.v. methylprednisolone 1000 mg daily for 3 days. Treatment was initiated shortly after the first Lp and hence approximately 2–3 weeks prior to the second Lp. Three of them started on interferon-β treatment during their follow-up. Albumin was measured by rate nephelometry. To evaluate the integrity of the BBB and the intrathecal IgG production we calculated the albumin ratio and the IgG-index (Tibbling et al., 1977). Demographic, clinical and CSF data are presented in Table 1. Two healthy controls had oligoclonal IgG bands in CSF. This finding is in accordance with the frequency reported from similar control populations (Tourtellotte et al., 1985; Kostulas et al., 1987).

2.2. Cytokine and chemokine analysis

Serum and CSF samples were analysed for IFN-γ, TNF-α, IL-4, IL-6, and CCL2 with Bio-Plex human cytokine assays (Bio-Rad Laboratories AB, Sundbyberg, Sweden). The system can separate up to 100 different colour tagged sets of beads and is suitable for multiple analyses on small sample volumes. Beads coated with capture antibodies (5000 beads per cytokine) were

Table 1
Characteristics of patients and healthy control subjects

Group	RRMS-relapse	RRMS-remission	SPMS	Healthy controls
N	21	17	25	44
Female/Male (*n*)	11/10	12/5	16/9	13/31
Age [mean (range); y]	33.1 (17–52)	38.3 (21–58)	47.7 (32–57)	36.0 (21–59)
MS duration [mean (range); y]	8.1 (0.5–25)	13.4 (5–27)	20.4 (4–37)	N/A
EDSS score [median (range)]	2.5 (1.5–6.5)	3 (1–4)	6.5 (2–8)	N/A
Subject with OB (*n*)	19	16	24	2
Albumin ration [mean (S.D.)]	5.12 (3.38)	5.33 (2.20)	6.1 (2.27)	5.16 (2.28)
IgG index [mean (S.D.)]	1.03 (0.53)	1.12 (0.68)	0.95 (0.60)	0.46 (0.04)

RRMS=relapsing–remitting multiple sclerosis; SPMS=secondary progressive multiple sclerosis; y=years; EDSS=Expanded Disability Status Scale of Kurtzke; OB=oligoclonal bands; S.D.=standard deviation; N/A=not applicable.