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Analysis of cerebrospinal fluid cytokines and growth factors in multiple sclerosis patients with and without chronic headaches

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Abstract

Background: We previously reported that, in Japanese patients with multiple sclerosis (MS), the frequency of chronic headaches was significantly higher after administration of interferon beta (IFNβ). However, the mechanisms underlying IFNβ-related chronic headaches were unknown. *Objective*: To clarify the mechanisms underlying IFNβ-induced chronic headaches in MS patients by analyzing cytokine levels in cerebrospinal fluid (CSF). *Methods*: We measured the levels of 27 CSF cytokines and growth factors using a fluorescent bead-based immunoassay, during a headache-free period, in 34 MS patients enrolled in our previous survey on chronic headaches. *Results*: There were no significant differences in CSF cytokine levels between the 21 MS patients with chronic headaches and the 13 without chronic headaches. Among the 14 patients receiving IFNβ therapy, the 5 patients with chronic headaches showed significantly lower levels of interleukin (IL) 15, IL17 and chemokine (C-C motif) ligand 2 (CCL2) (also known as monocyte chemoattractant protein 1; MCP1) compared with the 9 patients without chronic headaches ($P < 0.05$).
Conclusions: The present survey revealed that in MS, chronic headache sufferers on IFNβ therapy had decreased levels of IL15, IL17 and CCL2 in CSF. This suggests that chronic headaches may tend to develop in good responders to IFNβ.

INTRODUCTION

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system, and is assumed to be mediated by myelin-autoreactive T cells.[1] In Western MS series, the prevalence of primary headaches in patients with MS ranges from 4 to 61.8%.[2] One reason for this high prevalence may be that MS plaques preferentially involve the periaqueductal gray matter, a migraine generator or modulator, thereby inducing migraine-like headaches.[3,4] Other studies indicate that administration of interferon beta (IFNβ) is related to the high prevalence and exacerbation of primary headaches in MS patients.[5,6] In Asians, we first reported that the frequency of chronic headaches, namely migraine, tension-type headache (TTH) and chronic secondary headaches, was much higher among 127 MS patients than that expected based on epidemiological surveys of the general Japanese population. Moreover, the presence of chronic headaches was independent of the MS clinical phenotype.[7]

In patients with chronic headaches, several studies have investigated cytokine levels in blood and cerebrospinal fluid (CSF), and reported that the levels of many pro-inflammatory cytokines were elevated, such as interleukin (IL) 1 beta (IL1β), tumor necrosis factor alpha (TNFα) and chemokine (C-C motif) ligand 2 (CCL2)/ monocyte attractant protein 1 (MCP1). Anti-inflammatory cytokines, such as IL1 receptor antagonist (IL1RN), IL4, IL10 and transforming growth factor beta (TGFβ), were also elevated; however, the role of these molecules remains uncertain.[8,9] IFNβ is well known to modulate cytokine balance, but the relationship between drug-induced cytokine alterations and the occurrence of headache is entirely unknown. To clarify the mechanism underlying IFNβ-related chronic headaches in MS patients, we measured and compared the levels of 27 cytokines and growth factors in the CSF of MS patients with and without IFNβ treatment.

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METHODS

Patients

Thirty-four patients who were diagnosed with clinically definite MS based on Poser's criteria[10] and were enrolled in our previous study[7], took part in this study after informed consent was obtained. Their donated CSF was cryopreserved at –70°C in our laboratory. The patients' disability was scored using the Expanded Disability Status Scale (EDSS) of Kurtzke.[11]Acute transverse myelopathy was defined according to Fukazawa *et al*.[12] Longitudinally extensive spinal cord lesions were defined as lesions extending over three or more vertebral segments on magnetic resonance imaging.[13] Anti-aquaporin 4 (AQP4) antibody levels were measured by immunofluorescence, as described previously.[14,15]

The demographic features of the MS patients are summarized in Table 1. Eight of the 31 patients examined were positive for anti-AQP4 antibody. IFNβ was administered to 14 of the 34 (41.2%) patients; 10 of the 23 (43.5%) patients without anti-AQP4 antibody, one of the 8 (12.5%) patients with anti-AQP4 antibody and 3 of the 3 (100%) patients whose anti-AQP4 antibody status was unknown.

Classification of chronic headaches

Headache types were determined according to the International Classification of Headache Disorders criteria, second edition.[16] Chronic headaches identified included migraine with aura, migraine without aura, episodic TTH, chronic TTH and other types of headache including cluster headache and other trigeminal autonomic cephalgias, other primary headaches and chronic secondary headaches, as in our previous study.[7]

Measurement of cytokines

CSF samples were taken from 15 patients within one month of a relapse, and from 19 patients during a convalescence period. The liquid phase of the CSF was simultaneously analyzed for 27 cytokines/chemokines and growth factors, namely, IL1β, IL2, IL4, IL5, IL6, IL7, IL9, IL10, IL12/p70, IL13, IL15, IL17, IFNγ, TNFα, IL8/C-X-C motif ligand 8 (CXCL8), CXCL10/inducible protein 10 (IP-10), CCL2, CCL3/macrophage inflammatory protein (MIP)-1α, CCL4/MIP-1β, CCL5/RANTES, CCL11/eotaxin, granulocyte colony stimulating factor (CSF3), granulocyte-macrophage colony stimulating factor (CSF2), platelet-derived growth factor beta (PDGFβ), basic fibroblast growth factor (FGF2), vascular

Table 1: Demographic features of patients with multiple sclerosis

	Total (*n* = 34)
Sex (F/M)	27/7
Age at onset (years)	32.8±10.5
Disease duration (years)	11.5±10.1
Relapse/Remission	15/19
EDSS	3.4±2.2
Barkhof criteria	19/34 (55.9%)
Paty Criteria	28/34 (82.4%)
Severe ON (≥FS 5)	6/33 (18.2%)
ATM	9/34 (26.5%)
LESCL	12/34 (35.3%)
anti-AQP4 antibody	8/31 (25.8%)
Administration of IFNβ	14/34 (41.2%)
Chronic headaches	21/34 (61.8%)

AQP4 = aquaporin 4; ATM = acute transverse myelopathy; EDSS = Expanded Disability Status Scale of Kurtzke[11]; IFNβ = Interferon beta; LESCL = longitudinally extensive spinal cord lesions; ON = optic neuritis.

endothelial growth factor (VEGF) and IL1 receptor antagonist (IL1RN). The Bio-Plex Cytokine Assay System (Bio-Rad Laboratories, Hercules, CA) was used according to the manufacturer's instructions. The detailed methodology was described in our previous report.[17]

Statistical analysis

Statistical analyses were performed using the Wilcoxon test. Significance was set at $P < 0.05$.

RESULTS

Twenty-one out of 34 (61.8%) patients had chronic headaches; one patient had migraine with aura, 9 had migraine without aura, 10 had TTH and one had headache not classified elsewhere.

There were no significant differences in cytokine and growth factor levels between the 21 patients with chronic headaches and the 13 without chronic headaches (Table 2). Among the 23 patients without anti-AQP4 antibody,

Table 2: Levels of cytokines and growth factors in CSF from multiple sclerosis patients with and without chronic headaches

Cytokine	Headache (–) (n = 13)	Headache (+) (n = 21)	Wilcoxon test
IL1RN	31.29±47.71	40.76±81.86	NS
IL1β	0.37±0.51	0.47±1.09	NS
IL2	0.48±0.00	0.48±0.00	NS
IL4	0.17±0.20	0.17±0.19	NS
IL5	0.53±0.21	0.52±0.27	NS
IL6	151.5±515.7	164.2±420.7	NS
IL7	1.44±0.85	1.49±0.58	NS
IL8/CXCL8	58.01±31.30	64.22±74.05	NS
IL9	11.11±3.83	9.95±3.67	NS
IL10	1.08±1.20	1.46±2.76	NS
IL12/p70	4.78±5.38	4.37±5.41	NS
IL13	0.09±0.03	0.10±0.45	NS
IL15	8.23±2.20	7.02±1.33	NS
IL17	1.33±1.65	1.08±1.41	NS
CSF3/G-CSF	11.84±21.56	12.07±21.33	NS
CSF2/GM-CSF	6.42±4.67	4.76±4.84	NS
IFNγ	4.24±9.82	4.27±8.21	NS
CCL2/MCP-1	193.14±52.50	195.25±117.31	NS
CCL4/MIP-1β	12.28±7.59	13.32±9.64	NS
TNFα	1.33±2.48	1.34±2.40	NS
VEGF	4.09±2.37	3.39±1.62	NS
CCL5/RANTES	2.80±3.58	2.10±3.74	NS
PDGFB	0.63±0.49	0.49±0.46	NS
CCL3/MIP-1α	0.60±0.42	0.54±0.30	NS
FGF2/bFGF	3.78±2.97	3.05±1.49	NS
CXCL10/IP-10	2639±2638	2073±1265	NS
CCL11/eotaxin	2.44±0.77	2.06±0.44	NS

Mean ± SD is shown.
NS, not significant.

Table 3: Levels of cytokine and growth factors in CSF from multiple sclerosis patients on IFNβ with and without chronic headaches

Cytokine	Headache (–) (n = 5)	Headache (+) (n = 9)	Wilcoxon test
IL1RN	15.76±5.40	15.64±2.25	NS
IL1β	0.23±0.00	0.23±0.00	NS
IL2	0.48±0.00	0.48±0.00	NS
IL4	0.11±0.00	0.11±0.00	NS
IL5	0.46±0.19	0.35±0.07	NS
IL6	6.75±5.46	5.62±3.27	NS
IL7	1.20±0.00	1.20±0.00	NS
IL8/CXCL8	36.86±4.34	31.62±9.94	NS
IL9	9.98±2.85	10.50±2.52	NS
IL10	0.55±0.39	0.57±0.29	NS
IL12/p70	5.64±5.81	4.27±4.58	NS
IL13	0.08±0.00	0.08±0.00	NS
IL15	7.09±0.76	6.01±0.38	P = 0.0077*
IL17	1.97±1.87	0.50±0.42	P = 0.0343*
CSF3/G-CSF	6.19±3.07	5.20±3.70	NS
CSF2/GM-CSF	4.99±5.20	4.69±3.72	NS
IFNγ	1.04±0.76	1.20±0.84	NS
CCL2/MCP-1	202.46±31.71	159.14±30.98	P = 0.0329*
CCL4/MIP-1β	9.18±1.92	12.52±5.88	NS
TNFα	0.66±0.25	0.53±0.01	NS
VEGF	3.31±2.11	3.37±0.91	NS
CCL5/RANTES	1.48±1.37	1.21±0.53	NS
PDGFB	0.33±0.16	0.32±0.15	NS
CCL3/MIP-1α	0.35±0.31	0.46±0.19	NS
FGF2/bFGF	2.42±1.48	3.40±0.68	NS
CXCL10/IP-10	1466±517	2044±771	NS
CCL11/eotaxin	2.13±0.45	1.87±0.30	NS

Mean ± SD is shown.
NS, not significant; *P < 0.05.

there were no significant differences in cytokine and growth factor levels between the 15 patients with chronic headaches and the 8 without chronic headaches (data not shown). Among the 8 patients with anti-AQP4 antibody, there were no significant differences in cytokine and growth factor levels between the 5 with chronic headaches and the 3 without chronic headaches (data not shown). On the other hand, among the 14 patients receiving IFNβ, the 9 with chronic headaches showed significantly lower levels of IL15 (P = 0.0077), IL17 (P = 0.0343) and CCL2 (P = 0.0329) in the CSF, compared with the 5 patients without chronic headaches (Table 3 and Figure 1). There were no significant differences among the 20 patients without IFNβ therapy in the presence (n = 12) or absence (n = 8) of chronic headaches (data not shown).

DISCUSSION

The present study has revealed, for the first time, that in MS patients on IFNβ therapy, those

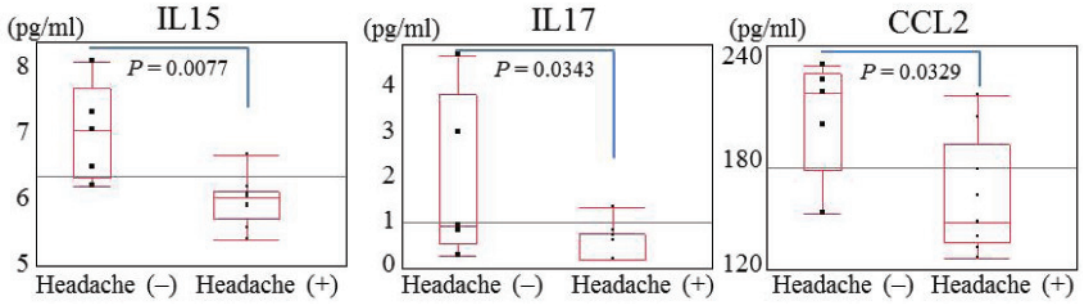

Figure 1. Levels of IL15, IL17 and CCL2 in CSF from MS patients receiving IFNβ, with and without chronic headaches.

suffering from chronic headaches had significantly lower levels of IL15, IL17 and CCL2 in the CSF compared with those without chronic headaches. In this study, we did not separately analyze patients with migraine and those with TTH, partly because the number of patients was small; this may impose some caution on interpreting the data. However, according to previous studies, IFNβ administration caused an increase in both migraine and TTH.[5,6] We believe that the alterations in CSF cytokine levels observed in our MS patients with chronic headaches during IFNβ therapy may have some relevance to the occurrence of the headaches.

Patients with migraine have been reported to show higher levels than healthy controls of IL4, IL5[18] and TGFβ1[19], and lower levels of IL10[18] in sera during headache-free periods. In contrast, during headache attacks, migraine patients demonstrated decreases in IL4[20,21] and IL5[20], and increases in IL6[22,23], IL10[20,23,24], CCL5[23], IL1β[24], intercellular adhesion molecule 1 (ICAM1) and TNFα.[22] On the other hand, the only study that has investigated CSF cytokine levels in patients with migraine during headache attacks revealed an elevation of IL1RN, CCL2 and TGFβ1.[9] These cytokines are thought to contribute to generation of neurogenic inflammation in migraine. In contrast, in the present study, we did not find any increases in either pro-inflammatory or anti-inflammatory cytokines in the CSF of MS patients with chronic headaches compared with those without headaches. This may be partly explained by the fact that cytokine changes related to headache were masked by cytokine alterations associated with relapse, and that CSF was drawn during a headache-free period.

Nonetheless, we did find a headache-associated decrease in IL15, IL17 and CCL2 in MS patients on IFNβ treatment. IL15, produced by blood monocytes, macrophages and glial cells, has similar biological activities to IL2, and elevation of IL15 in sera and CSF has been reported in MS patients.[25,26] CCL2 induces migration and infiltration of monocytes/macrophages, and intrathecal synthesis of CCL2 has been observed in patients with MS.[27,28] IL17 is produced by T helper (Th) 17 cells, which are now regarded as initiators of autoimmune inflammation, including in MS.[29] Thus, all these cytokines have crucial roles in the pathogenesis of MS. Increasing evidence suggests that Th1 and Th17 cells are responsible for the autoimmune inflammation in MS[1,30,31]; IFNβ corrects such Th1 and Th17 shifts[28,32,33], thereby exhibiting significant therapeutic effects. Therefore, it is conceivable that the headache-associated decrease in IL15, IL17 and CCL2 in MS patients on IFNβ may reflect the therapeutic effects of the drug, and that chronic headache may tend to develop in MS patients who show excellent responses to IFNβ. In other words, good responders to IFNβ may be prone to develop chronic headaches during treatment. This preliminary finding supports a positive correlation between IFNβ treatment and the development of chronic headaches in MS patients; however, further studies of CSF cytokines during headache attacks are required to elucidate the exact mechanisms underlying chronic headaches in MS patients receiving IFNβ.

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