Table 3 *Demographic features and neuropathological findings of spinal cords in eight multiple sclerosis autopsy cases*

Cases	OS-multiple sclerosis						C-multiple sclerosis	
	1	2	3	4	5	6	7	8
Age/sex	31/M	35/F	37/F	54/F	28/F	44/F	39/M	47/F
Duration of disease (years)	6	7	10	3	4	1	19	7
No. of relapses	8	5	>10	5	6	8	>10	Progressive
CSF								
Cell count (/μl)	3	/	136	54	78	2	8	5
Neutrophilia	–	/	+(67%)	–	+(60%)	–	–	–
Total protein amount (mg/dl)	39	/	256	113	240	23	104	77
Spinal cord pathology								
Tissue destruction	+++	++	+++	+++	+++	++	+	+++
Myelin loss	+++	+++	++	+++	+++	++	+++	++
Axonal loss	+++	+++	++	+++	+++	++	+	++
Neutrophil infiltration	F (+++), D (+)	None	D (+)	None	None	F (++), D (+)	None	None
Macrophage infiltration	+++	+	++	+++	+	+++	++	+
T-cell infiltration	+++	+	+++	++	++	++	+	++
B-cell infiltration	+	+	+	+	+	+	+	+

OS-multiple sclerosis = opticospinal form of multiple sclerosis; C-multiple sclerosis = conventional form of multiple sclerosis; / = no data available. Tissue damage (evaluated by haematoxylin–eosin staining): + mild, ++ moderate, +++ severe. Myelin damage (evaluated by myelin basic protein immunostaining): + mild, ++ moderate, +++ severe. Axonal damage (evaluated by phosphorylated neurofilament immunostaining): + mild, ++ moderate, +++ severe. Neutrophil infiltration (evaluated by myeloperoxidase immunostaining): F = focal accumulation (none 0, + <20 per field, ++ 20–40 per field, +++ >40 per field), D = diffuse scattered (none 0, + <10 per field, ++ >10 per field). Each field had a magnification of ×200. Macrophage infiltration: + slight infiltration, ++ moderate infiltration, +++ severe infiltration. T-cell infiltration: none 0, + <10 per field, ++ 10–30 per field, +++ >30 per field in the lesion. B-cell infiltration: none 0, + <10 per field, ++ 10–30 per field, +++ >30 per field in the lesion.

and revealed that, even in the absence of inflammation, CSF cells showed a significant Th1 shift compared with PBLs, which is consistent with the observation that Th1 cells bearing CXCR3, a specific chemokine receptor for CXCL10 (IP-10), are enriched in CSF cells compared with PBLs (Trebst and Ransohoff, 2001). However, such a Th1 shift was far more marked in C-multiple sclerosis patients at relapse than in controls. In C-multiple sclerosis patients, the significant Th1 shift in PBLs was due mainly to a marked decrease in IFN-γ^-IL-4$^+$ CD4$^+$ T cells (Th2 cells), while that in CSF cells was mainly attributable to a large increase in IFN-γ^+IL-4$^-$ CD4$^+$ T cells (Th1 cells) and partly to a decrease in IFN-γ^-IL-4$^+$ CD4$^+$ T cells. In contrast, in OS-multiple sclerosis patients, a significant Th1 shift compared with controls was only seen in PBLs (Ochi *et al.*, 2001; present study), and not in CSF cells. The Th1 shift in PBLs was considered to be caused by a significant decrease in Th2 cells, while in the CSF cells, a significant increase in Th1 cells also occurred in OS-multiple sclerosis patients compared with controls, but at the same time Th2 cells were rather more increased in CSF than in PBLs. This latter increase partially cancelled out the increase in Th1 cells in CSF and caused a significant difference in the IFN-γ^-IL-4$^+$ CD4$^+$ T-cell percentages between the two multiple sclerosis subtypes. These findings suggest that the Th1/Th2 balance is differentially regulated in the peripheral blood and CSF compartments, and that OS-multiple sclerosis and C-multiple sclerosis have distinct systems of immune dysregulation.

A broad range of cytokine/chemokine concentrations was successfully measured in CSF supernatants in the present study. However, caution should be exercised with respect to the low ranges of sample concentrations (<1 pg/ml) until sufficient data for the various diseases have been obtained using multiplexed fluorescent bead-based immunoassays. Even when we set the cut-off level to 1 pg/ml and excluded patients on immunomodulatory therapies, increases of IL-17, IL-8 and MIP-1β in OS-multiple sclerosis and the increase of IL-8 and decrease of MCP-1 in C-multiple sclerosis in comparison with controls, and the difference of IL-17 between OS-multiple sclerosis and C-multiple sclerosis were all still significant. As we used SCD as controls, it is possible that SCD-related cytokine changes, if any, may have introduced a misleading element into our multiple sclerosis-related findings. However, as no inflammatory components have ever been reported in SCD CSF, we consider it appropriate for use as the control in this study, and the above-mentioned changes in multiple sclerosis to be relevant for each disease process.

In Western multiple sclerosis series, chemokines inducing Th1 cell mobilization, such as CXCL10 (IP-10) for CXCR3-bearing cells and RANTES for CCR5-bearing cells, have been shown to increase in the CSF at relapse, while chemokines for Th2 cells, such as MCP-1 for CCR2-bearing cells, decrease (Sørensen *et al.*, 1999; Franciotta *et al.*, 2001; Mahad *et al.*, 2002; Scarpini *et al.*, 2002). Furthermore, co-localization of CXCL10 and CXCR3 was noted in the

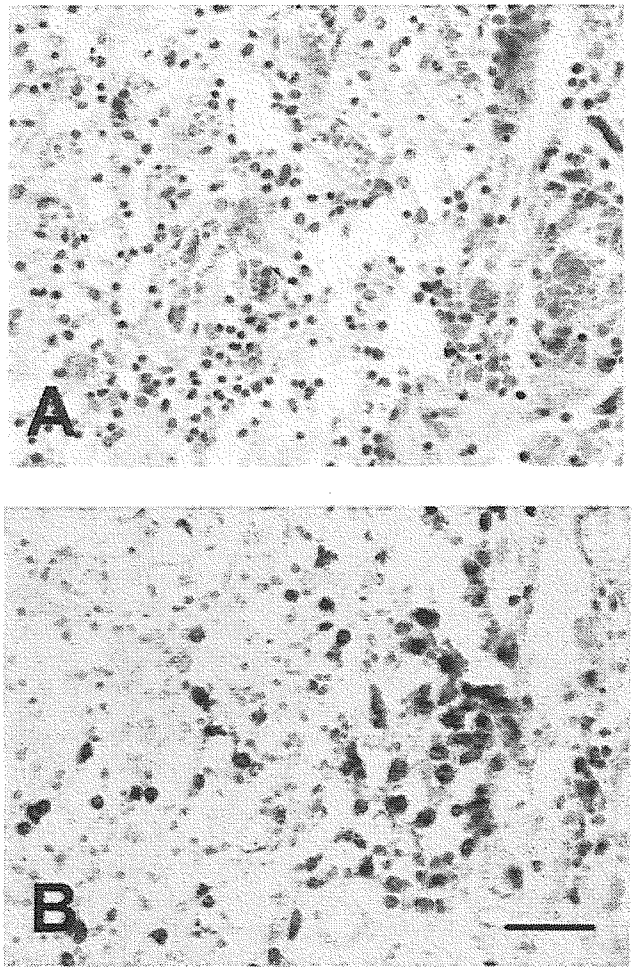

Fig. 5 Neuropathological findings of autopsied spinal cord specimens from OS-multiple sclerosis cases (case 1). The myelin and axon are severely damaged and vascular proliferation has occurred in the cornu laterale of the thoracic spinal cord. Numerous granulocytes containing myeloperoxidase (MPO)-positive neutrophils are accumulated and infiltrated in the lesion (**A**, haematoxylin–eosin stain; **B**, MPO immunostaining). The scale bar in **B** = 50 μm (also for **A**).

multiple sclerosis lesions (Sørensen *et al.*, 2002). These observations are consistent with the Th1 shift found during the intracellular cytokine analysis of CSF cells in C-multiple sclerosis in the current study.

Among the cytokines and chemokines measured in the CSF supernatant, both IL-17 and IL-8 had a significant correlation with the CSF/serum albumin ratio and the length of the spinal cord lesions on MRI in multiple sclerosis, suggesting an involvement of both cytokines in the destruction of the blood–brain barrier and the formation of inflammatory spinal cord lesions. In the present CSF study, the increases in IL-17 and IL-8 were significantly greater in OS-multiple sclerosis patients than in C-multiple sclerosis patients. A previous report on IL-17 in Western multiple sclerosis series found no change at the protein level in CSF (Saruhan-Direskeneli *et al.*, 2003), although all multiple sclerosis samples were below the detection limit, while IL-17 mRNA expression in CSF mononuclear cells was elevated in a fraction of multiple sclerosis patients, especially during clinical exacerbation (Matusevicius *et al.*, 1999), and gene microarray analysis of multiple sclerosis plaques revealed an increased level of IL-17 transcripts (Lock *et al.*, 2002). Our findings extend the latter observations at the mRNA level and suggest that the IL-17 response is much more prominent in OS-multiple sclerosis than in C-multiple sclerosis at the protein level. IL-17 is produced by activated memory Th1 cells (Aarvak *et al.*, 1999) and induces various downstream cytokines and chemokines, such as IL-8, IL-6, G-CSF and prostaglandin E_2 (Fossiez *et al.*, 1996; Dudler *et al.*, 2000; Hwang *et al.*, 2004). IL-17 causes neutrophil recruitment mainly through the release of IL-8, a CXC chemokine for neutrophils, and induces neutrophil activation, i.e. increases in myeloperoxidase and elastase activity (Laan *et al.*, 1999; Hoshino *et al.*, 2000; Linden and Adachi, 2002; Miyamoto *et al.*, 2003; Witowski *et al.*, 2004). Upregulation of IL-17 and IL-8 has been reported to cause severe destruction of tissues by neutrophilic inflammation in Th1 diseases, such as rheumatoid arthritis (Kotake *et al.*, 1999; Ziolkowska *et al.*, 2000; Miossec, 2003), as well as Th2 diseases, such as bronchial asthma (Linden, 2001; Molet *et al.*, 2001). CSF neutrophilia and the heavy infiltration of neutrophils seen in the necrotic spinal cord lesions of OS-multiple sclerosis may well be related to the increases in IL-17 and IL-8 in CSF. The observation that only IL-8 is significantly correlated with the EDSS score further underscores a critical role for IL-8-induced neutrophil recruitment in the destruction of the spinal cord tissues in Japanese patients with multiple sclerosis. Since longitudinally extensive spinal cord lesions are more frequently encountered in OS-multiple sclerosis than in C-multiple sclerosis, and represent one of the two main determining factors for irreversible disability in OS-multiple sclerosis, intrathecal activation of the IL-17/IL-8 axis is considered to be crucial in OS-multiple sclerosis. Interestingly, although the IL-8 levels have not been reported to differ in either unstimulated PBLs or CSF from multiple sclerosis patients and controls in a Western population series (Comabella *et al.*, 2002; Jalonen *et al.*, 2002; Kleine *et al.*, 2003), IL-8 expression in PBLs is markedly downregulated in IFN-β responders, but not in non-responders (Stürzebecher *et al.*, 2003), indicating a potentially important role for IL-8 in multiple sclerosis, even in Westerners. In our C-multiple sclerosis patients, IL-8 was significantly elevated in CSF, whereas IL-17 was not increased. Since IL-8 is also driven by TNF-α (Hoffmann *et al.*, 2002), the augmented TNF-α, but not IL-17, may potentiate IL-8 production in C-multiple sclerosis. Since surges of TNF-α are hardly detectable due to its extremely short half-life (30 min) (Li *et al.*, 2001), it is reasonable that IL-8 rather than TNF-α is found to be correlated with the EDSS score.

It is interesting to note that the downregulation of Th2 cells in CSF in C-multiple sclerosis at relapse was not

found in OS-multiple sclerosis, and Th2 cells were rather increased in CSF at relapse in comparison with PBLs. Th2 polarized cells directed against myelin proteins are encephalitogenic in immunocompromised animals (Lafaille *et al.*, 1997) and exacerbate experimental allergic encephalomyelitis (EAE) in non-human primates (Genain and Hauser, 1996). Moreover, in certain animal strains with Th2-prone genetic backgrounds, myelin oligodendrocyte glycoprotein (MOG)-induced EAE shows severe and selective involvement of the optic nerves and spinal cord (Storch *et al.*, 1998; Stefferl *et al.*, 1999). In these models, the accumulation of numerous neutrophils is a dominant feature (Lafaille *et al.*, 1997; Storch *et al.*, 1998; Stefferl *et al.*, 1999). We previously reported that MOG-autoreactive T cells were more frequently established than those reactive to myelin basic protein or proteolipid protein epitopes in OS-multiple sclerosis patients (Minohara *et al.*, 2001). Thus, in OS-multiple sclerosis, intrathecal activation of the IL-17/IL-8 axis by memory Th1 cells specific for myelin proteins such as MOG may contribute to the neutrophilic recruitment and destruction of the tissues under Th2-prone genetic backgrounds or even together with myelin protein-specific Th2 cells. Therefore, Th2-related genetic backgrounds as well as Th2 cell reactivity to myelin proteins could be future targets for studies on OS-multiple sclerosis.

IL-17 expression is induced by IL-23, a product of activated dendritic cells and macrophages/microglial cells (Becher *et al.*, 2003; Cua *et al.*, 2003), while IL-12 (p70), a disulphide-linked heterodimer of p35 and p40, has only marginal effects on IL-17 production (Aggarwal *et al.*, 2003), yet both IL-23 and IL-12 (p70) share a common p40 subunit. Furthermore, IL-23, but not IL-12 (p70), has been shown to be a critical cytokine for autoimmune inflammation of the brain in an EAE model using knockout mice for each of their subunits (Cua *et al.*, 2003). Previous reports have described that IL-12 (p40) was increased in multiple sclerosis patients with gadolinium-enhanced lesions on MRI (Fassbender *et al.*, 1998), whereas IL-12 (p70) was only detectable in CSF in ~10% of multiple sclerosis patients (Drulovic *et al.*, 1997; Fassbender *et al.*, 1998). Since we also found no increase in IL-12 (p70) in CSF from our multiple sclerosis patients, we consider that a further study on IL-23 in the CSF compartment is urgently required.

We found that IL-5 levels were significantly higher in OS-multiple sclerosis than C-multiple sclerosis patients. In the latter, the IL-5 level was possibly depressed in some cases, reflecting an intrathecal down-modulation of Th2 cells in the acute stage. Instead, some OS-multiple sclerosis patients showed an increase in IL-5 in the CSF, although the increase was not statistically significant in comparison with control patients as a whole. Although we could not find any eosinophil infiltration in OS-multiple sclerosis spinal cord lesions, degranulated eosinophils are hard to detect by haematoxylin–eosin staining. Thus, immunostaining of activated eosinophil products is required to determine eosinophil involvement in OS-multiple sclerosis.

Lucchinetti *et al.* (2002) reported that in nine autopsied cases of Devic's NMO, eight relapsing and one monophasic, 56% had prominent infiltration of neutrophils and eosinophils into the spinal cord lesions, and marked deposition of immunoglobulins and complements were seen in all cases. A distinction between and the identities of relapsing NMO and OS-multiple sclerosis have long been discussed (Cree *et al.*, 2002) and, in the recent study by Lennon *et al.* (2004), newly identified IgG autoantibody (NMO-IgG) was detected in both NMO and Japanese OS-multiple sclerosis patients. The considerable overlap between the two conditions suggests common pathomechanisms are operative. Our finding of marked increases of IL-17 and IL-8 in CSF may be relevant to neutrophil infiltration, while an IL-5 increase may relate to eosinophil infiltration. Moreover, a relative increase of Th2 cells in CSF compared with PBLs may correspond to the involvement of humoral immunity in relapsing NMO (Lucchinetti *et al.*, 2002; Lennon *et al.*, 2004). Further investigation into the deposition of immunoglobulins and complement proteins as well as activated eosinophil products in OS-multiple sclerosis may shed light on the contribution of the Th2 cell-mediated effector arm in this condition, and clarify the disease entities of relapsing NMO and OS-multiple sclerosis.

In summary, we successfully identified OS-multiple sclerosis-related CSF cytokine/chemokine changes, which may be useful both for monitoring disease activity and for developing future subtype-specific therapies, such as pharmacological blocking of neutrophil activation in OS-multiple sclerosis.

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Short communication

Platelet-activating factor receptor gene polymorphism in Japanese patients with multiple sclerosis

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Abstract

We evaluated the association of the platelet-activating factor receptor (PAFR) gene polymorphism (A224D) with the susceptibility and severity of multiple sclerosis (MS) in a Japanese population. DNA was collected from 162 Japanese patients with clinically definite 'conventional' MS (MS) and 245 healthy controls. The missense mutation A224D that impairs PAF-PAFR signaling was determined by polymerase chain reaction restriction fragment length polymorphism. The frequency of the AD/DD genotypes was significantly higher in MS patients (21.0%) than in healthy controls (13.5%) (p=0.045; odds ratio (OR), 1.71; 95% confidence interval (CI), 1.01–2.89). Moreover, the frequency of D allele in MS patients (11.7%) was also significantly higher than those in healthy controls (6.9%) (p=0.019; OR, 1.78; 95% CI, 1.10–2.89). These findings suggest that the PAFR gene missense mutation has a relation to the susceptibility for MS.

Keywords: Polymorphism; Platelet-activating factor receptor; Multiple sclerosis; Japanese; Missense mutation

1. Introduction

Increased levels of platelet-activating factor (PAF) receptor (PAFR) mRNA are reported in multiple sclerosis (MS) plaques together with several genes encoding molecules associated with allergic responses (Lock et al., 2002). In experimental allergic encephalomyelitis (EAE), an animal model of MS, transcripts for PAFR were reported as elevated in the CNS during the disease, and PAFR antagonist was shown to reduce the severity of EAE (Pedotti et al., 2003). Moreover, in cerebrospinal fluid and plasma of patients with the relapsing–remitting MS, elevation of PAF that correlated with the number of gadolinium-enhancing lesions was found on brain MRI (Callea et al., 1999). Taken together, the above findings are suggestive that PAF might have a proinflammatory role in MS.

PAF is a very potent chemotactic stimulant for inflammatory cells such as eosinophils (Wardlaw et al., 1986) and polymorphonuclear neutrophils (O'Flaherty et al., 1981). PAF not only promotes leukocyte adhesion and transmigration by the induction of intracellular adhesion molecule-1 (ICAM-1) on endothelial cells (Chihara et al., 1992), but also upregulates major histocompatibility complex (MHC) class I and II expressions in some brain cells that are critical in antigen presentation (Martin-Mondière et al., 1987). These proinflammatory and vasoactive actions of

Abbreviations: PAFR, platelet-activating factor receptor; MS, multiple sclerosis; OR, odds ratio; CI, confidence interval; EAE, experimental allergic encephalomyelitis; ICAM-1, intracellular adhesion molecule-1; MHC, major histocompatibility complex; EDSS, Kurtzke's Expanded Disability Status Scale; PI, progression index; TNF-α, tumor necrosis factor-alpha; IL, interleukin; TGF-β, transforming growth factor-beta; DTH, delayed-type hypersensitivity.

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PAF are mediated through a specific G-protein-coupled receptor, PAFR (Honda et al., 1991; Nakamura et al., 1991). A single amino acid substitution (A224D) in the third cytoplasmic loop of human PAFR that modifies its function has been reported, with the variant being relatively common in Japanese, with 13.8% heterozygous and 0.9% homozygous (Fukunaga et al., 2001). Fukunaga et al. also found that Chinese hamster ovary cells expressing A224D mutant PAFR displayed a partial but significant reduction of PAF-induced intracellular signaling, and that the variant exhibited impaired coupling to G-proteins.

The present study aimed to elucidate the effect of the PAFR polymorphism on the development of MS in Japanese; thus, we investigated the PAFR gene polymorphism (A224D) in MS patients, and correlated the findings with clinical parameters.

2. Patients and methods

2.1. Patients

A total of 162 patients (59 men and 103 women) with MS, according to the recommended diagnostic criteria (McDonald et al., 2001), were recruited from the Department of Neurology, Kyushu University Hospital, the Department of Neurology, Hokkaido University Hospital, and the Hokuyukai Neurological Hospital. Hematological and biochemical studies and serologic tests for syphilis were performed in all patients and the results were not contributory. None of the patients was seropositive for human T-cell leukemia virus type 1. Age at examination was 38.2±11.3 years (mean±S.D.) and at disease onset 27.1± 9.9 years (mean±S.D.). Subjects were "conventional" MS patients as described previously (Fukazawa et al., 1992) (i.e., clinical features were similar to those of MS patients in Western countries). Patients with opticospinal MS (OS-MS) whose lesions were clinically confined to the optic nerve and spinal cord were excluded from this study because this group of patients seems to constitute a distinct subgroup (Kira et al., 1996; Yamasaki et al., 1999; Kira, 2003). After at least a 1-year observation period, 120 were diagnosed as relapsing–remitting type MS and 42 as secondary progressive type MS, in which the onset of progressive disease was defined as continual worsening of symptoms and signs for a period at least 6 months, with or without superimposed relapses (Lublin and Reingold, 1996; Confavreux et al., 2000). Primary progressive MS was not included in the present study. MS severity was evaluated by Kurtzke's Expanded Disability Status Scale (EDSS) scores (Kurtzke, 1983) and progression index (PI) (Miyagishi et al., 2003). PI was calculated as a measure of accumulated disability over time (PI=EDSS/disease duration in years). EDSS score was 3.3±2.6 (mean±S.D.) and PI was 0.40±0.42 (mean±S.D.) at the time of examination. The control group was composed of 107 unrelated healthy men and 138 unrelated healthy women (mean age±S.D.= 34.0±10.1 years). Subjects' informed consent was obtained in accord with the declaration of Helsinki, and the ethical committees of the institutions in which the work was performed gave their approval.

2.2. Genotyping of PAFR

Total blood genomic DNA was extracted from leukocytes with a QIAamp DNA Blood Midi Kit (QIAGEN, Tokyo, Japan) following the manufacturer's instructions. The genotype of human PAFR was determined by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) according to the method of Fukunaga et al. (2001) without knowledge of the samples' clinical diagnosis. The sense primer used was (5′-CCACAGCGCCC-GGCGCTTGACTGCA-3′) and the antisense primer was (5′-ATCGTGTTCAGCTTCTTCCTGGTCT-3′). Reactions were performed in a total volume of 50 μl containing 0.5 ìg of genomic DNA, 20 pmol of each primer, 0.4 mmol/l each of dATP, dGTP, dCTP, and dTTP, 1 U Taq DNA polymerase (Takara, Otsu, Japan), 100 pmol/l KCl , and 20 nmol/l Tris hydrochloride (pH 8.0). The thermocycling procedure consisted of an initial denaturation at 94 °C for 10 min, 30 cycles of 94 °C for 1 min, 58 °C for 1 min, and 72 °C for 1 min. PCR-amplified DNA was digested with *Pst*I (Fukunaga et al., 2001) at 37 °C overnight. PCR products were analyzed by agarose gel (0.7% agarose+2.5% NuSieve) electrophoresis and visualized by ethidium bromide staining. This genetic variant results in the loss of a *Pst*I restriction site; thus, the wild-type allele yielded 105-bp and 24-bp fragments, while the mutant allele remained undigested (129-bp).

2.3. Statistical analysis

Allele and genotype frequencies of the PAFR were compared between MS patients and controls, using chi-square and Fisher's exact tests. Statistical analysis between the genotype PAFR polymorphism (AA vs. AD/DD) and clinical parameters were tested in MS patients, using Mann–Whitney U and chi-square tests. Values of $p<0.05$ were considered statistically significant. Statistical analyses were performed with StatView/Mac software.

3. Results

3.1. PAFR genotype and allele frequencies in MS

The proportions of PAFR genotypes (AA, AD, and DD) and alleles (A allele, D allele) in MS patients and healthy controls are shown in Table 1. The frequency of the AD/DD genotypes was significantly higher in MS patients (21.0%) than in healthy controls (13.5%) (p=0.045; odds ratio (OR), 1.71; 95% confidence interval (CI), 1.01–2.89). In control

Table 1
Genotype and allele frequency of the PAFR polymorphism in patients with MS and healthy controls

	MS (n=162)	Healthy controls (n=245)
Genotype frequencies		
AA	128 (79.0)[a]	212 (86.5)[a]
AD	30 (18.5)	32 (13.1)
DD	4 (2.5)	1 (0.4)
AD/DD	34 (21.0)[a]	33 (13.5)[a]
Allele frequencies		
A allele	286 (88.3)[b]	456 (93.1)[b]
D allele	38 (11.7)[b]	34 (6.9)[b]

MS=multiple sclerosis. Percentages are in parentheses.
[a] p=0.045; OR, 1.71; 95% CI, 1.01–2.89.
[b] p=0.019; OR, 1.78; 95% CI, 1.10–2.89.

subjects, the genotype frequencies are similar to those found in other Japanese studies (Fukunaga et al., 2001). Moreover, the frequency of D allele in MS patients (11.7%) was also significantly higher than those in healthy controls (6.9%) (p=0.019; OR, 1.78; 95% CI, 1.10–2.89).

3.2. The relation between PAFR polymorphism and clinical parameters

There was no association between the PAFR polymorphism and clinical parameters such as age at onset, sex, clinical phenotype, EDSS, and PI (data not shown).

4. Discussion

In the present study, we disclosed a significant association between susceptibility for MS and the PAFR polymorphism that has a partial but significant reduction of PAF-induced intracellular signaling.

PAF is a proinflammatory mediator produced early in response to several immunological stimuli, including immune complexes and proinflammatory cytokines (Camussi et al., 1981; Valone and Epstein, 1988). Moreover, PAF itself mediates some of the biological effects exerted by cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1, and IL-8 (Dubois et al., 1989; Poubelle et al., 1991; Denault et al., 1997). Considering the proinflammatory nature of PAF, it is rather unexpected that a missense mutation partially disrupting PAFR signaling is a susceptibility factor for MS. There are two possible explanations for this. First, not all the biological effects of PAF are proinflammatory. It has been shown that PAF is involved in the inhibition of proinflammatory cytokine production during macrophage phagocytosis of apoptotic cells, possibly through the synthesis of transforming growth factor-beta (TGF-β) (Fadok et al., 1998). PAF also suppresses the induction of delayed-type hypersensitivity (DTH) and enhances the transcription of COX-2 and IL-10—two important mediators of systemic immune suppression (Walterscheid et al., 2002). Thus, the missense mutation of PAFR may downmodulate immunosuppressive actions, which then enhance susceptibility for MS in some patients.

Second, PAF preferentially enhances Th2-mediated immune responses (Harada et al., 1996; Kusuhara et al., 2000). Huang et al. (1996) reported that PAF activates Th2 cells to produce IL-4, which is completely inhibited by PAF receptor antagonist. Since activation of Th2 cells inhibit Th1-related cellular immunity, the missense mutation of PAFR may enhance susceptibility for MS, in which Th1 cells are supposed to play a major role, through down-regulation of Th2 cells.

In summary, we analyzed the PAFR polymorphism in Japanese patients, and found that the PAFR AD/DD genotype seems to confer a risk for the development of MS. Further studies on PAF/PAFR signal transduction in MS patients will be necessary to determine whether the PAFR polymorphism is involved in the pathogenesis of MS.

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Comparison of the Clinical Courses of the Opticospinal and Conventional Forms of Multiple Sclerosis in Japan

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Abstract

We evaluated the clinical courses of 216 patients with multiple sclerosis (MS) diagnosed according to the recommended diagnostic criteria of McDonald et al (10). Sixty-five patients clinically displaying selective involvement of the optic nerves and spinal cord were classified as opticospinal MS (OS-MS), while the other 151 showing disseminated involvement of the central nervous system were classified as conventional MS (C-MS). The disease duration did not differ significantly between the two subtypes (11.2 years vs. 11.5 years). In addition to a higher age of onset, female preponderance and higher Kurtzke's expanded disability status scale (EDSS) scores, the OS-MS patients showed a markedly lower frequency of secondary progressive MS than the C-MS patients (4.6% vs. 29.1%, p=0.0001). The EDSS scores of the C-MS patients were significantly correlated with the disease duration, while those of the OS-MS patients were not. Among the C-MS patients, the frequency of secondary progressive MS was significantly more common in patients with a disease duration of more than 10 years than in those with a shorter duration. These results suggest that the irreversible disability in OS-MS is determined by relapses, rather than by chronic progression, whereas C-MS has a similar clinical course to MS in Westerners.
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Key words: multiple sclerosis, opticospinal form, conventional form, progression

Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease of unknown etiology, and an increasing amount of evidence suggests that it is heterogeneous. According to its clinical course, MS can be described as relapsing-remitting type (RR-MS), secondary progressive type (SP-MS), in which a progressive phase follows an initial relapsing-remitting phase, or primary progressive type (PP-MS), in which the disease shows a progressive course from its onset. On the other hand, concerning the sites of involvement, there have been several reports that Oriental MS patients more commonly show clinical evidence of major involvement of the optic nerves and spinal cord than Caucasian MS patients (1). In addition, we previously reported the clinical and immunogenetic characteristics of two further MS subtypes in Japanese populations, namely opticospinal MS (OS-MS), in which the clinically estimated main lesions are confined to the optic nerves and spinal cord, and conventional MS (C-MS), which shows disseminated lesions in the central nervous system (CNS), including the cerebrum, cerebellum or brainstem (2–4). OS-MS has distinct features, such as marked female preponderance, higher age at onset, higher Kurtzke's expanded disability status scale (EDSS) scores (5) resulting from severe visual impairment and marked spinal cord dysfunction, and a lower number of brain lesions on magnetic resonance imaging (MRI) compared with C-MS (6). Severe inflammatory destruction is indicated in OS-MS by the higher cell counts and increased amounts of protein in the cerebrospinal fluid (CSF), and the long swollen lesions extending over several vertebral segments on spinal cord MRIs (6). Pathological studies have also revealed severe inflammation and vascular changes in OS-MS lesions (7, 8).

OS-MS is relatively common among Asians compared

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with Westerners, while early studies indicated that the progressive type is rare among Japanese MS patients (9). However, in these studies, the follow-up periods and disease durations were not sufficient and the number of MS patients was not large enough to determine the clinical course of Asian MS. Moreover, the clinical courses have not been compared between each clinical subtype in Asian MS patients. In the present study, we therefore studied the clinical courses of large numbers of MS patients with an average disease duration of more than 10 years according to each clinical subtype, and found that OS-MS and C-MS showed distinct clinical courses.

Patients and Methods

A total of 216 patients (56 males and 160 females) with MS diagnosed according to the recommended diagnostic criteria of McDonald et al (10) were recruited from the Department of Neurology, Kyushu University Hospital, the Department of Neurology, Hokkaido University Hospital, and Hokuyukai Neurological Hospital between August 1989 and November 2001. They represented consecutive patients who gave consent in accordance with the declaration of Helsinki. Hematological and biochemical studies and serologic tests for syphilis were performed in all patients. No patients were seropositive for human T-cell leukemia virus type 1. The mean ages at examination and disease onset were 41.5±13.0 years (mean±SD) and 30.1±12.0 years, respectively, and the mean disease duration was 11.4±8.7 years. After at least 1 year of observation, 169 of the patients were diagnosed as RR-MS, and 47 were diagnosed as SP-MS, in which the onset of a progressive disease was defined as continual worsening of the symptoms and signs over a period of at least 6 months, with or without superimposed relapses (10, 11). PP-MS patients were not included in the present study because their number was too small for statistical analysis. The MS severity was evaluated by Kurtzke's EDSS scores (5). The medical records of all the patients were reviewed retrospectively. Overall, the frequency of SP-MS was 21.8% (47/216), and the mean EDSS score at the time of examination was 3.8±2.6.

In the present study, the MS patients were classified into the two clinical subtypes. The patients whose clinically estimated lesions were confined to the optic nerves and spinal cord were classified as OS-MS. These patients showed no clinical evidence of disease in either the cerebrum or cerebellum, and those with minor brainstem signs, such as transient double vision and nystagmus, in addition to the opticospinal involvement were included. In Japan, Devic disease is diagnosed for cases with a monophasic course and, since such monophasic cases potentially contain acute disseminated encephalomyelitis cases, patients with Devic disease were excluded. Recurrent optic neuritis and recurrent transverse myelitis involving the identical level were also excluded from OS-MS. The remaining MS patients, who showed the involvement of multiple sites in the CNS, including the cerebrum, cerebellum or brainstem, were classified as C-MS. This study was approved by the Ethical Committee of the Institution in which it was performed.

Statistical analyses of the clinical parameters between OS-MS and C-MS were performed, using the Mann-Whitney U-test and chi-square test. Spearman's correlation was used for statistical analyses of the disease duration and EDSS scores. Values of $p<0.05$ were considered statistically significant. Statistical analyses were performed with StatView/Windows software.

Results

Comparisons of the clinical findings of OS-MS and C-MS

The proportion of females was significantly higher in OS-MS than in C-MS (1 : 6.2 vs. 1 : 2.2, p=0.0079) (Fig. 1). The age at disease onset was significantly higher in OS-MS than in C-MS (38.1±13.9 years vs. 26.7±9.2 years, $p<0.0001$), while the disease duration did not differ significantly (11.2 ±8.6 years vs. 11.5±8.8 years). The EDSS scores were significantly greater in OS-MS than in C-MS (4.8±2.3 vs. 3.5± 2.7, p=0.0004).

Comparisons of the clinical courses of OS-MS and C-MS

In OS-MS, 62 patients were RR-MS and 3 were SP-MS, while in C-MS, 107 patients were RR-MS and 44 were SP-MS. The frequency of SP-MS was significantly lower in OS-MS than in C-MS (4.6% vs. 29.1%, p=0.0001) (Fig. 1). When the frequency of SP-MS was compared among C-MS patients with long (≥10 years) and short (<10 years) disease durations, the frequency was significantly higher in the former (45.7 % vs. 14.8%, $p<0.0001$) (Fig. 2). Among the C-MS patients, SP-MS was also significantly more common in those with moderate to severe disabilities (EDSS ≥4) than in those with mild disabilities (EDSS <4) (73.6% vs. 3.1%, $p<0.0001$). The EDSS scores of the C-MS patients were significantly correlated with the disease duration ($r=0.415$, $p<0.0001$), while those of the OS-MS patients were not ($r=0.185$, $p>0.1$) (Fig. 3). When the proportions of patients with moderate to severe disabilities, that is, those with an EDSS score of 4 (limited walking ability) or more, were compared in each tertile of disease duration (<10 years, 10–20 years and ≥20 years), the OS-MS patients showed a significantly higher frequency of moderate to severe disabilities in the lower tertile than the C-MS patients (55.9 vs. 17.3%, $p<0.0001$), while the other tertiles did not differ (Fig. 4).

Comparisons of the clinical findings between Kyushu and Hokkaido cases

The proportion of OS-MS was significantly higher in Kyushu cases than in Hokkaido cases (OS-MS : C-MS, 1.0 : 1.0 vs. 1.0 : 4.0, $p<0.0001$). Reflecting the higher population of OS-MS in the Kyushu cases, the frequency of SP-MS was significantly lower in Kyushu cases than in

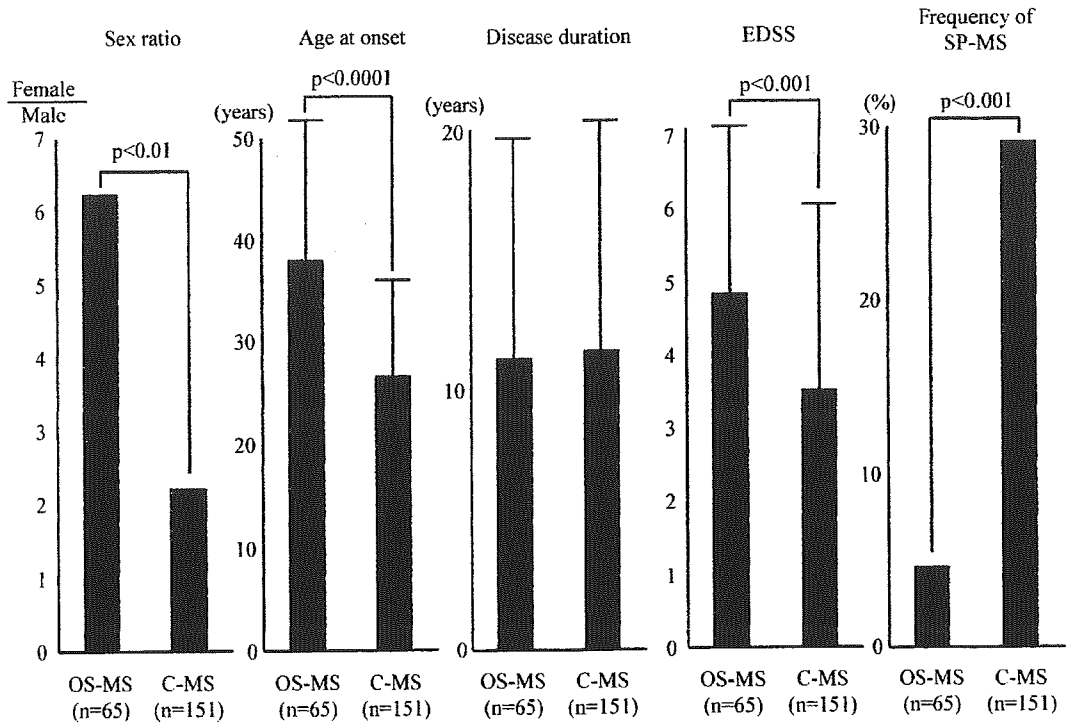

Figure 1. Comparisons of the clinical findings of OS-MS and C-MS.

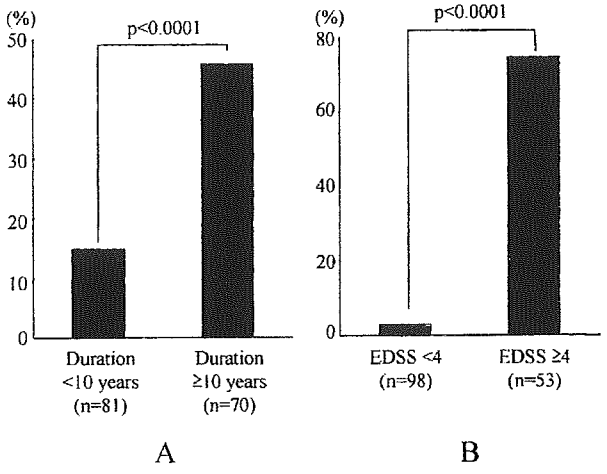

Figure 2. Comparisons of the frequency of SP-MS among C-MS patients according to the disease duration (A) and disability (B).

Hokkaido cases (11.3% vs. 26.9%, p=0.0089). The age at disease onset was also significantly higher in Kyushu cases than in Hokkaido cases (33.3±12.4 years vs. 28.6±11.5 years, p=0.0093). However, other than these points, there were no statistically significant differences in other clinical parameters between the Kyushu and Hokkaido cases.

Discussion

In the present study, we focused on comparing the clinical courses of OS-MS and C-MS by retrospectively analyzing large numbers of MS patients. The results revealed that SP-MS is significantly more uncommon in OS-MS than in C-MS, even at more than 10 years after the disease onset, and that the disability in OS-MS patients is primarily determined by relapses, rather than by chronic progression of the disease.

The retrospective nature of the present study partly limits the validity of the results. In addition, although experienced clinical neuroimmunologists from both institutions used the same evaluation protocols after a consensus was reached, the data collection from two different institutions may have imposed some inter-institutional variation. However, an EDSS score of 4 can easily be determined retrospectively and is frequently adopted in other studies to check disease progression (12). The proportion of OS-MS patients with an EDSS score of 4 was significantly higher than that of C-MS patients within 10 years after the disease onset. It is therefore considered that in the early course of the disease, OS-MS patients experience moderate to severe disabilities following relapses. A similar trend was reported in African Americans (13). In that report, more African Americans had early pyramidal system involvement than Caucasians, leading to greater EDSS scores, while the rates of disease progression at later stages were nearly identical. Since Africans and their

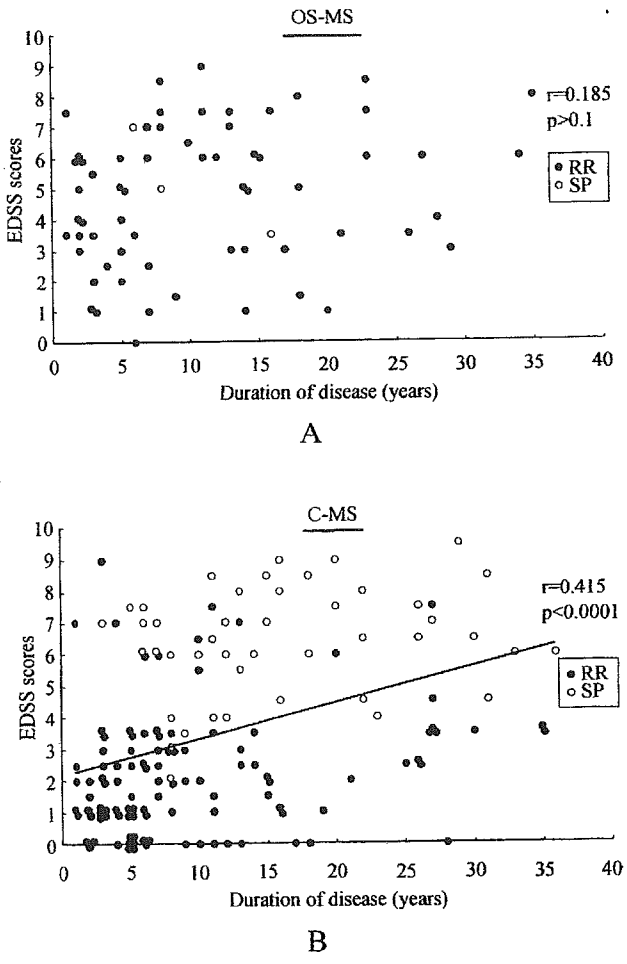

Figure 3. Relationships between the disease duration and EDSS scores in OS-MS (A) and C-MS (B) patients. The EDSS scores of the C-MS patients were significantly correlated with the disease duration (r=0.415, p<0.0001), while those of the OS-MS patients were not (r=0.185, p>0.1).

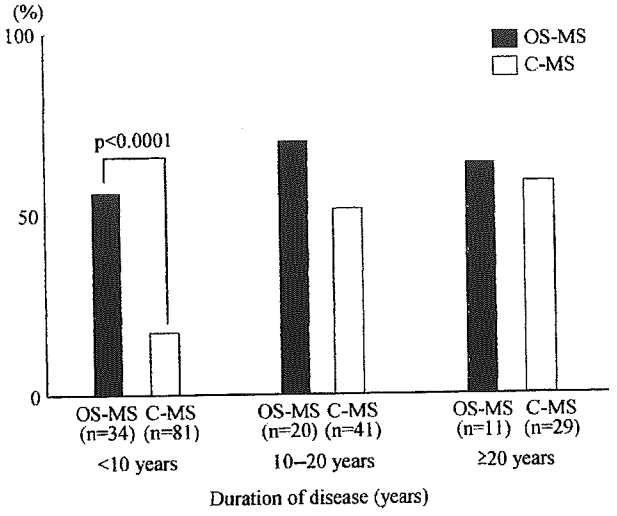

Figure 4. Comparison of the frequency of patients with moderate to severe disabilities (EDSS scores ≥4) between OS-MS and C-MS according to the disease duration.

descendants are known to preferentially show opticospinal involvement (14, 15), it is possible that early deterioration is universally characteristic of OS-MS, regardless of race.

Early studies on Asian MS reported that the progressive form was infrequent. For example, Shibasaki et al (9) retrospectively reviewed 204 British and 60 Japanese MS cases and revealed that the progressive phase was observed in 36% of the former but in only 12% of the latter with or without superimposed relapses. However, in that study, the clinical courses were not analyzed according to the MS subtype and the disease duration of the Japanese patients was 8 years on average (9). In the present study, classification into the two subtypes revealed that C-MS frequently showed a secondary progressive disease in the late stage of illness, especially in patients with grave disabilities, and that there was a significant correlation between disease severity and disease duration. On the contrary, secondary progression was significantly less common in OS-MS than in C-MS, yet the disease durations were similar between the two. Moreover, OS-MS showed no relationship between disease severity and disease duration. These observations suggest that the infrequent occurrence of SP-MS in Asians is attributable to the rarity of the secondary progressive phase in OS-MS, and that the disability in OS-MS patients is determined mainly by the severity of relapses, while that in C-MS is determined largely by chronic progression.

A similar retrospective study recently performed on 1,844 MS patients in Western countries with a mean disease duration of 11±10 years revealed that relapses had no significant influence on the progression of irreversible disability (12). This finding therefore indicates that irreversible disabilities are determined by chronic progression in Western MS patients. Based on the results of the present study, the same appears to be true for Japanese C-MS patients. Although earlier studies reported that the progressive phase was rare in Asians(9), the results of the present study demonstrate that, after longer disease durations, a considerable proportion of C-MS patients enter a similar progressive phase, suggesting that identical pathomechanisms are operative in C-MS even in Asians. Therefore, the process of C-MS is considered to be neurodegeneration plus superimposed inflammation similar to Western MS (16), while that of OS-MS is thought to be purely inflammatory.

In summary, the results of the present study indicate that OS-MS is distinct from C-MS in terms of their clinical courses, further suggesting the possibility that distinct mechanisms might be operative. However, once OS-MS patients contract grave disabilities as a result of a severe

relapse, it is difficult to evaluate the chronic progression retrospectively. This may be explained in part by the rarity of SP-MS in OS-MS. Considering the rarity of MS in Japan, it is desirable to create a nationwide prospective study system using the same evaluation scale, such as the EDSS score, in the future.

Acknowledgements: This work was supported in part by grants from the Ministry of Education, Culture, Sports, Science and Technology, Japan, and the Neuroimmunological Disease Research Committee, and by a Research on Brain Science grant from the Ministry of Health, Labor and Welfare, Japan.

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□ CASE REPORT □

Exacerbation of Chronic Inflammatory Demyelinating Polyradiculoneuropathy during Interferonβ-1b Therapy in a Patient with Childhood-onset Multiple Sclerosis

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Abstract

Interferonβ-1b (IFNβ-1b) is commonly used for relapsing-remitting multiple sclerosis (MS). We report a 23-year-old woman with childhood onset relapsing-remitting MS treated with IFNβ-1b who developed overt chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) immediately after therapy. A baseline conduction study before IFNβ-1b therapy revealed decreased motor conduction velocities and prolonged F wave latencies in several nerves, but there was no neurological sign indicating neuropathy. The existence of subclinical demyelinating neuropathy before IFNβ-1b treatment was suggested, although the clinical criteria for CIDP were unfulfilled. Following two months of IFNβ-1b therapy, numbness of her right upper and lower limbs progressively worsened and all tendon reflexes were depressed. Electrophysiologically, F waves were not evoked in any limbs except for the left ulnar and tibial nerves, which showed marked prolongation of F wave latencies. Moreover, subclinical hyperthyroidism developed in association with high titers of anti-thyroglobulin and anti-thyroid peroxydase antibodies, which were negative before IFNβ-1b therapy. These findings indicated that peripheral demyelination worsened at the nerve roots after IFNβ-1b therapy. In addition to the development of autoimmune thyroid disease, the patient now fulfilled the criteria for probable CIDP. Along with the results of a previous report demonstrating IFNβ-induced CIDP development in patients with childhood MS, this case underscores IFNβ as a potential risk factor for CIDP in patients with childhood onset MS.

(Internal Medicine 44: 68–72, 2005)

Key words: interferonβ-1b; chronic inflammatory demyelinating polyradiculoneuropathy; multiple sclerosis

Introduction

The occurrence of multiple sclerosis (MS) and chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) in the same subject is uncommon (1–4), yet both are thought to be autoimmune diseases that target myelin. Peripheral nervous system involvement in MS patients has been reported by some authors (5–7). For example, Zee et al (6) conducted a retrospective study on 150 MS patients and found clinical and electrophysiological evidence of radiculopathy in 13 (8%) and peripheral neuropathy in 4 (3%) patients. However, exacerbation of CIDP following IFN-β has only been reported by Pirko et al (4). They described three children with MS who responded to interferon-β (IFNβ), but developed CIDP that responded to intravenous immunoglobulin (IVIG) administration. It was considered that IFNβ treatment somehow contributed to the development of CIDP. This hypothesis is supported by recent reports documenting the onset of CIDP in patients receiving type I IFN (8–10). Here, we report a female patient who developed MS in childhood and underwent interferon β-1b treatment in early adult life, which caused CIDP that was successfully treated by oral corticosteroids.

Case Report

A 23-year-old woman developed left optic neuritis that responded well to IV corticosteroids in 1989 at the age of 10 years. In 1996 she noticed numbness of her right lower limb

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Table 1. Peripheral Never Conduction Study of the Patient before and after Interferon β-1b Therapy

	Motor nerve															Sensory nerve					
	MCV (m/s)			DL (ms)			CMAP (mV)			F-No (%)			F-Lat (ms)			SCV (m/s)			SNAP (μV)		
	Feb	May	June	Feb	May	June	Feb	May	June	Feb	May	June	Feb	May	June	Feb	May	June	Feb	May	June
R. Median	38 (45~65)	37	36	3.4 (<4.5)	3.8	3.5	13 (4~25)	13	12	NE (>70%)	NE	56	NE (24~29)	NE	44	55 (45~68)	60	61	5 (10~60)	18	23
Ulnar	49 (45~65)	47	45	3 (<4.0)	2.9	2.8	11 (3~25)	8	6	63	NE	NE	32 (24~29)	NE	NE	48 (45~65)	50	53	8 (10~60)	7	8
Tibial	30 (40~60)	46	32	5.1 (<7.5)	6	4.8	34	20	25	100	NE	100	53 (43~52)	NE	68	–	–	–	–	–	–
Peroneal	47 (40~60)	–	–	6.2 (<7.0)	–	–	13 (7~40)	–	–	56	–	–	58 (43~52)	–	–	–	–	–	–	–	–
Sural	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	36	46	40	13 (15~40)	7	12
L. Median	38 (45~65)	34	37	3.6 (<4.5)	3.5	3.3	17 (4~25)	18	17	63 (>70%)	NE	88	32 (24~29)	NE	37	52 (45~68)	61	65	8 (10~60)	20	33
Ulnar	45 (45~65)	38	41	3.3 (<4.0)	3.5	3	9 (3~25)	10	9	44	100	75	34 (24~29)	37	35	50 (45~65)	52	53	10 (10~60)	3	8
Tibial	34 (40~60)	36	35	5.3 (<7.5)	4.8	4.1	38	32	27	100	88	100	59 (43~52)	63	61	–	–	–	–	–	–
Peroneal	48 (40~60)	40	–	6.1 (<7.0)	4.9	–	2.1 (7~40)	4.4	–	44	NE	–	60 (43~52)	NE	–	–	–	–	–	–	–
Sural	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	35 (40~60)	42	44	13 (15~40)	12	9

MCV: motor conduction velocity, DL: distal latency, CAMP: compound muscle action potential, F-No: F wave evoked frequency, F-Lat: F wave latency, SCV: sensory conduction velocity, SNAP: sensory nerve action potential, NE: not evoked, –: not examined. Normal values are indicated in parentheses.

with mild weakness, which was resolved within 2 months. In May 1999 she developed right facial palsy followed by mild weakness of the right upper and lower limbs. At that time, tendon reflexes in all four limbs were normal. A brain MRI revealed multiple areas of increased signal intensity on T2-weighted images in the periventricular white matter of the bilateral cerebral hemispheres and some showed gadolinium-enhancement. She was thus diagnosed as having MS and she took oral prednisolone (1 mg/kg) with gradual tapering, after which she almost completely recovered except for minimal numbness of her right hand. In March 2002 she once again developed right facial palsy, and again responded well to oral prednisolone, which brought about complete remittance of her symptoms after three weeks of treatment. Eight months later, she developed left followed by right leg numbness. Neurological examination showed mild weakness in the left lower limb with a mild decrease in superficial sensations in both legs. Tendon reflexes were normal in the upper limbs but mildly hyperactive in the lower limbs. The Babinski sign was elicited in her left lower limb. She was once again treated with oral prednisolone, with complete recovery from leg weakness. However, minimal numbness in the distal parts of her left lower limb persisted. Although her illness clinically responded well to prednisolone, a follow-up MRI obtained one month after initiation of corticosteroids showed new multiple gadolinium-enhancing white matter lesions in the bilateral cerebral hemispheres. From February 2003, she was therefore started on 8×10^6 units of IFNβ-1b given subcutaneously every other day. Two months later, she showed a progressive worsening of the numbness in her right upper and lower limbs (Table 1 and Fig. 1).

Neurological examinations revealed temporal pallor of the left optic disc and mild weakness and superficial sensory disturbance in the distal parts of the right upper and lower limbs. Right triceps reflex and both sides ankle jerks were absent, and others were hypoactive. The Babinski sign was elicited bilaterally. Laboratory examinations disclosed an increase of liver enzymes [aspartate transaminase: 46 U/*l* (normal <33 U/*l*) and alanine transaminase: 101 U/*l* (normal <30 U/*l*)] indicating IFNβ-1b-induced liver damage. In February 2003 her baseline thyroid function had been normal [TSH: 2.35 μIU/ml (0.27<normal<4.20) and free-T4: 1.04 ng/dl (1.00<normal<1.80)] with an upper normal limit of antithyroid autoantibody titers. However, at this time, after IFNβ-1b therapy, she had subclinical compensated hyperthyroidism (TSH: 0.03 μIU/ml, free-T4 1.43 ng/dl) and elevated serum autoantibodies against human thyroglobulin (TG) and thyroid peroxidase (TPO) [2,094.5 IU/ml (45 in February 2003) and 167.6 IU/ml (7 in February 2003)]. Anti-ganglioside antibodies and other common autoantibodies

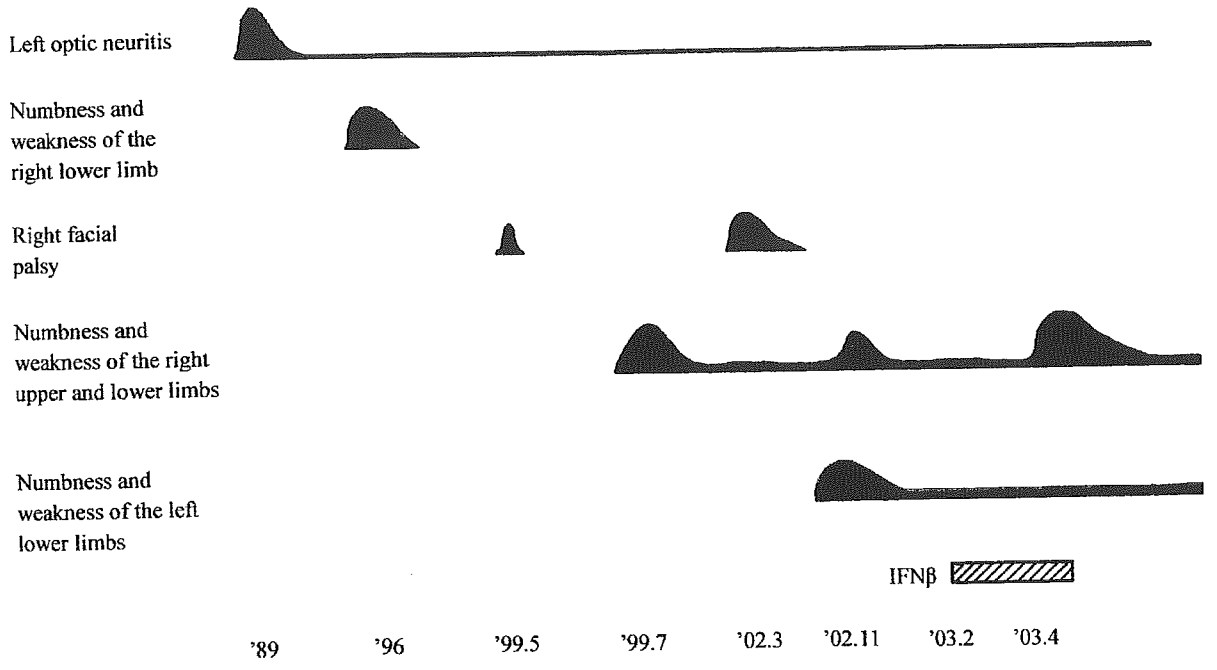

Figure 1. Clinical course of the presented patient.

such as anti-nuclear antibodies, anti-double strand DNA antibodies, anti-RNP antibody, anti-SSA antibody, anti-SSB antibody, anti-Sm antibody and perinuclear and cytoplasmic anti-neutrophil cytoplasmic antibodies were all negative. CSF examination showed 5 nucleated cells/μl (all lymphocytes) and an elevated protein level of 81 mg/dl. A baseline conduction study conducted in February 2003 revealed decreased motor conduction velocities and prolonged F wave latencies in several nerves (Table 2), indicating the existence of subclinical demyelinating neuropathy, yet the criteria for CIDP (11) were still not fulfilled. After two months of treatment, however, most of the F waves were not evoked and in the left ulnar and tibial nerves the F wave latencies were markedly prolonged (Table 2). These findings indicated that the demyelinating process had worsened at the nerve roots after IFNβ-1b therapy; at this point the patient fulfilled the criteria for probable CIDP (11). As for MS, a brain MRI disclosed multiple small periventricular white matter lesions without gadolinium enhancement (Fig. 2). The number of lesions on the T2-weighted images did not increase compared with those seen in January 2003. A spinal MRI also revealed high signal intensity lesions at the C7 level on the right side and at the T6-7 levels on T2-weighted images (Fig. 2), which were unchanged since February 2003 and not enhanced by gadolinium. Spinal roots including cauda equina were not enhanced by gadolinium. We then discontinued IFNβ-1b therapy and treated the patient with oral prednisolone at a dose of 1 mg/kg with a gradual taper. Her weakness was resolved, tendon reflexes were normalized, and sensory impairment was recovered to her baseline level (Table 1). After corticosteroid therapy, both the F wave evoked frequencies and F wave latencies were markedly improved (Table 2).

Discussion

Although baseline peripheral nerve conduction abnormalities might have partly contributed to the patient's neurologic symptoms prior to IFNβ therapy, the preserved tendon reflexes and presence of pathological reflexes together with spinal cord lesions on the MRI suggested that central demyelination was responsible for the earlier neurological symptoms. After IFNβ therapy, however, the decreased tendon reflexes and further worsening of peripheral nerve conduction abnormalities suggested that these worsening neurologic symptoms after IFNβ therapy were attributable to peripheral demyelination.

The present case is similar to those of Pirko et al (4) in that MS onset occurred in childhood, and that IFNβ did not prevent the development of clinically overt CIDP. In their cases, the time lags between the initiation of IFNβ and CIDP development were 4 months, 1 year and 4 years, while our patient's CIDP worsened after just 2 months of initiating IFNβ therapy, suggesting that in our case IFNβ played a role as an exacerbating factor for CIDP. In the present patient, autoimmune thyroiditis also developed after IFNβ therapy. IFNβ thus appeared to trigger organ-specific autoimmune diseases in our patient. Exacerbation of autoimmune

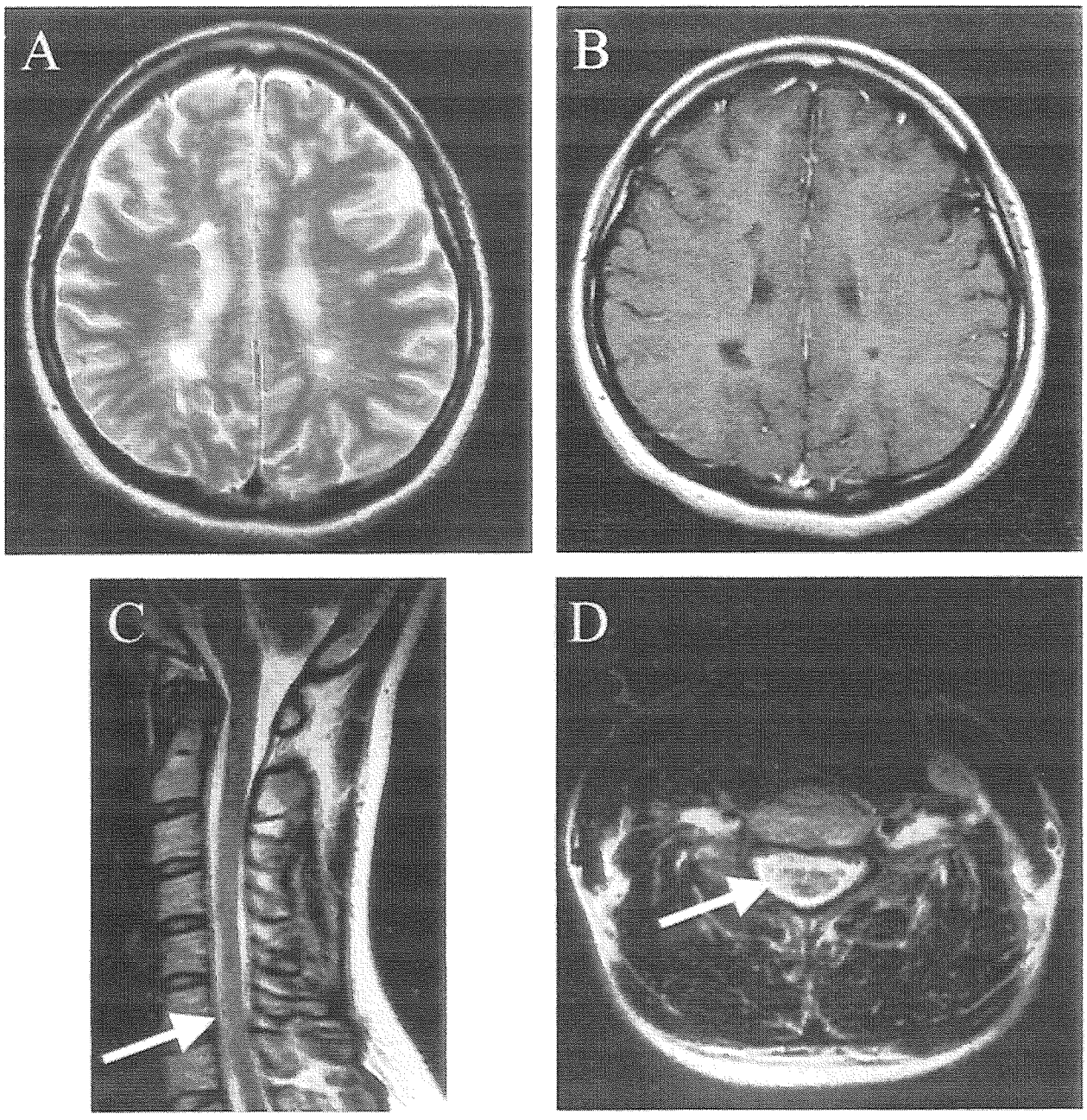

Figure 2. T2-weighted (A) and gadolinium-enhanced T1-weighted (B) brain MRI and T2-weighted spinal cord MRI (C and D) of the patient at the time of CIDP exacerbation. Note the multiple periventricular lesions without gadolinium enhancement. Arrows show the high signal intensity lesions at the C7 level and at the T6-7 levels. The spinal cord MRI also shows a discrete lesion at the C7 spine level that is not enhanced by gadolinium (not shown).

phenomena has been frequently reported with IFNα (12–15) but not for IFNβ (16), but both share a common receptor. Dayal et al (17) reported that IFN-γ secreting cells increased in the early course of IFNβ therapy. Moreover, it has been demonstrated by micro-array analysis that IFNβ therapy upregulates many Th1 genes (18). In CIDP, CXCL-10 (IP-10), a chemokine that attracts mainly Th1 cells, has been demonstrated to be elevated in CIDP CSF (19, 20). Moreover, T cells bearing CXCR3, which is a chemokine receptor for CXCL10 and specific for Th1 cells, are shown to mainly infiltrate into the biopsied sural nerves (19). Therefore, in CIDP, Th1 cells are thought to play a crucial

role. Therefore, IFNβ might also induce autoimmune diseases targeting peripheral nerve myelin through activation of type 1 cytokines.

The present case together with Pirko's cases (4) suggests that patients with childhood onset MS carry a higher risk of developing CIDP from IFNβ administration. Childhood MS reportedly has some distinct clinical and immunological features compared with adult-onset MS, such as a lower frequency of oligoclonal IgG bands in CSF (21), higher CSF cell counts (22), a higher frequency of EEG abnormalities (21), and a higher frequency of relapsing-remitting type (21, 23). Moreover, fever, asthenia, and anorexia are frequently present during the first episode in childhood MS in association with symptoms related to involvement of the spinal cord or cerebellum, suggesting an acute postinfectious autoimmune disorder (22). Such immunological characteristics might also be related to the predisposition for CIDP after IFNβ therapy in childhood-onset MS.

The present case further supports the notion that central and peripheral demyelination might have a distinct pathogenic mechanism (4). Although the presence of subclinical peripheral conduction abnormality was uncertain in Pirko's cases (4), initiation of IFNβ should be cautiously undertaken in the presence of subclinical demyelinating neuropathy in MS patients, especially in those with childhood onset.

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Autoimmunity against neurofilament protein and its possible association with HLA-DRB1*1502 allele in glaucoma

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Abstract

Glaucoma is understood as a neurodegenerative disease and intraocular pressure has been regarded as the major risk factors for the optic nerve damages. However, recent studies suggested that several risk factors including autoimmunity are also shown to play important roles in glaucoma. To identify the retinal antigen in glaucoma, we used the serological analysis of recombinant cDNA expression libraries (SEREX) approach and quantified IgG antibodies directed against the identified antigens in an ELISA. We identified neurofilament protein and the prevalence of anti-bovine neurofilament light subunit (NF-L) autoantibodies in glaucomatous patients was significantly higher than in healthy controls and patients with other uveitic and optic nerve diseases ($P<0.05$). In addition, our immunogenetic analysis showed a possible association between HLA-DRB1*1502 allele and the patients positive for anti-NF-L autoantibodies. It suggests that the HLA class II-linked gene may be involved in development of autoimmunity in patients with glaucoma.

Keywords: Glaucoma; Serological analysis of recombinant cDNA expression libraries (SEREX); Neurofilament protein; Autoantibodies; HLA-DRB1*1502 allele

1. Introduction

Glaucoma is classified as a neurodegenerative disease, in which the onset and progression of optic nerve damages results in visual field defects. Intraocular pressure (IOP) has been regarded as the major risk factor for the progression of optic nerve damage. However, recent clinical and experimental studies suggest that several risk factors such as ischemia, glutamate and genetic background also play important roles in the onset and progression of glaucoma [1,2]. These risk factors are likely associated with the occurrence of apoptotic cell death in retinal ganglion cells in glaucomatous eyes [3,4].

Recently, several investigators have suggested that autoimmunity directed against retinal proteins may be related to the development of glaucomatous optic neuropathy in glaucomatous patients. For example, Wax and his colleagues revealed that various autoantibodies reactive to retinal antigens such as rhodopsin, 27 kDa heat shock protein (hsp27), α–β crystalline, glycosaminoglycans, glutathione *S*-transferase (GST) were found in the sera of glaucomatous patients [5–8]. Also, a recent study using sera obtained from Japanese glaucomatous patients demonstrated the presence of anti-γ-enolase autoantibodies. Interestingly, vitreous

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