Table 2 Needle EMG findings in patients with myelitis and atopic diathesis

	Fasciculation potential	Fibrillation potential	PSW	Giant MUP	Polyphasic MUP	Recruitment pattern
1	–	–	–	Bil. Triceps, FDI	Rt. triceps	reduced
2	–	Rt. FDI	Rt. FDI	Rt. vastus lateralis	–	reduced
3	–	–	–	–	Rt. ext. carpi ulnaris	reduced
4	–	–	–	Rt. abductor pollicis brevis	–	reduced
5	Rt. FDI, Rt. tibialis anterior	Rt. biceps	Rt. biceps	Rt. biceps	Rt. Biceps, Rt. tibialis anterior	reduced
	–	–	–	–	Bil. abductor pollicis brevis	
6	–	–	–	–	Rt. abductor pollicis brevis	reduced
7	–	Rt. FDI	Rt. FDI	Rt. FDI, Rt. gastrocnemius	Lt. Biceps, Lt. triceps	reduced
8	–	–	–	Lt. tibialis anterior, Lt. gastrocnemius	Rt. gastrocnemius	reduced
9	–	Rt. flex. pollicis brevis	–	Rt. flex. pollicis brevis	Lt. gastrocnemius	reduced
10	–	–	–	Rt. FDI, Lt. rectus femoris	Rt. flex. pollicis brevis	reduced
11	–	Lt. abductor digiti minimi	–	Bil. FDI, Lt. triceps	–	reduced
12	–	–	–	Rt. FDI	Lt. triceps	reduced
13	–	–	–	–	–	normal
14	–	–	–	–	–	normal
15	–	–	–	–	–	normal
16	–	–	–	–	–	normal
17	–	–	–	–	–	normal
18	–	–	–	–	–	normal
19	–	–	–	–	–	normal
20	–	–	–	–	–	normal

Bil.=bilateral, Rt.=right, Lt.=Left, PSW=Positive sharp wave, MUP=motor unit potential, FDI=First dorsal interossei, flex.=flexor, ext.=extensor.

right gastrocnemius in case 8 and left rectus femoris in case 11). In addition, these 4 patients had no compressive lesions on lumbar MRI.

Effects of immunotherapy

Muscle weakness as well as on-going denervation potentials were improved by immunotherapy such as PE or PE plus IVIG in both patients tried (cases 5 and 12). In case 5, muscle strength improved as follows: deltoid 4 (right) / 5 (left) to 5 / 5, finger flexor 3+/ 5 to 4 / 5, finger extensor 3+/ 5 to 4 / 5, iliopsoas 2+/ 3+ to 3+/ 4+, tibialis anterior 0 / 3 to 1-/ 4 and gastrocnemius 0 / 3 to 1-/ 4 on the Medical Research Council (MRC) scale. Fasciculation potentials in right first dorsal interossei and right tibialis anterior and fibrillation potentials and positive sharp waves in right biceps were disappeared on needle EMG performed 7 days after immunotherapy (three PEs followed by IVIG). MRI lesions were unchanged before and after the treatment in this case. In case 12, muscle strength improved as follows: deltoid 5 (right) / 4+ (left) to 5 / 5, triceps 4 / 4 to 5 / 5, wrist extensor 4+ / 4 to 5 / 5, wrist flexor 4+ / 4 to 5 / 5, iliopsoas 1 / 1 to 5 / 5, hamstrings 1 / 1 to 5 / 5, quadriceps 3 / 3 to 5 / 5, tibialis anterior 3 / 4 to 5 / 5, gastrocnemius 3 / 4 to 5 / 5, toe extensor 3 / 4 to 5 / 5, toe flexor 3 / 4 to 5 / 4+ on the MRC scale. Fibrillation potentials in left abductor digiti minimi muscle was disappeared on needle EMG performed on 9 days after three PEs. MRI was not studied after the treatment in case 12. Chronic neurogenic patterns were unchanged in both cases.

Discussion

The present study disclosed the frequent involvement of lower motor neurons in patients with AM. Among 13 patients showing the lower motor neuron involvement on needle EMG, only 1 had the definite muscle atrophy and none showed fascicula-

tion, suggesting that the lower motor neuron involvement is subclinical in most cases. Frequent lower motor neuron involvement in the relatively early course of the disease is consistent to the pathological observation that axons as well as myelin were lost in the biopsied spinal cord lesions[7)18)]. These findings are thus supposed to discriminate this condition from multiple sclerosis (MS).

It is important that one-third of the patients had subclinical involvement of the lower motor neurons in the spinal cord segments other than the overt spinal cord lesions shown by MRI. It thus suggests the scattered involvement of the spinal cord by the disease process, yet the preferential site of involvement is the cervical cord.

Hopkins syndrome, JMADUE (Hirayama' s disease) and sporadic LMND have been shown to be more or less associated with airway allergy, such as bronchial asthma, allergic rhinitis and pollinosis[8)9)10)11)]. The results of the present study add AM to the list of lower motor neuron involvement associated with allergy, yet the degree of lower motor neuron involvement is mild and mostly subclinical. These observations support the notion that allergic tendency is one of the risk factors for lower motor neuron damage.

On the other hand, the neuropathology of the biopsied spinal cord lesions in atopic myelitis revealed it to be eosinophilic inflammation[7)18)]. Such neuropathological findings are in good agreement with the typical atopic disorders, such as bronchial asthma, allergic rhinitis and atopic dermatitis, where eosinophil infiltration is one of the cardinal features[19)20)]. Activated eosinophil products, such as eosinophil cationic proteins (ECP), have been shown to be deposited in the spinal cord tissues[7)]. ECP causes membrane damage through the formation of transmembrane channel pore[21)]. Such eosinophil products are well known to be neurotoxic[22)23)], yet the mechanism of neurotoxicity is ill-defined. Therefore, neurotoxic eosinophil products may in part contribute to the anterior horn cell damage in this condition.

We recently reported that PE is also beneficial for the lower motor neuron damage in JMADUE associated with airway allergy[24)]. Effectiveness of immunotherapy in lower motor neuron involvement of AM and JMADUE further supports the notion that immunological process contributes to the lower motor neuron damage in these conditions. PE may be beneficial for the motor neuron damage by removing mediators of allergic inflammation, anti-neuronal autoantibodies and Th2 cytokines enhancing their production.

Conclusion

In the present study, we found that subclinical lower motor neuron involvement is common in AM. Although the precise mechanism remains to be elucidated, it is likely to be immune-mediated and reversible by immunotherapy, and therefore such immunotherapies as PE and IVIG may thus be worth trying in the lower motor neuron involvement associated with atopic diathesis.

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Platelet-activating factor acetylhydrolase gene polymorphism and its activity in Japanese patients with multiple sclerosis

Manabu Osoegawa[a], Masaaki Niino[b], Hirofumi Ochi[a], Seiji Kikuchi[b], Hiroyuki Murai[a], Toshiyuki Fukazawa[c], Motozumi Minohara[a], Kunio Tashiro[b], Jun-ichi Kira[a,*]

[a] *Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan*
[b] *Department of Neurology, Hokkaido University Graduate School of Medicine, Sapporo, Japan*
[c] *Hokuyukai Neurology Hospital, Sapporo, Japan*

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Abstract

We evaluated the association of the plasma platelet-activating factor acetylhydrolase (PAF-AH) gene polymorphism ($G^{994} \rightarrow T$) and PAF-AH activity with susceptibility and severity of multiple sclerosis (MS) in Japanese. DNA was collected from 216 patients with clinically definite MS (65 opticospinal MS (OS-MS) and 151 conventional MS (C-MS)) and from 213 healthy controls. The missense mutation $G^{994} \rightarrow T$ that disrupts the PAF-AH activity was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). No statistically significant difference in the frequency of genotypes and alleles of the plasma PAF-AH polymorphism was observed among OS-MS patients, C-MS patients and healthy controls. However, the missense mutation tended to be associated with the severity of OS-MS, especially in females (GT/TT genotypes; 51.7% in female rapidly progressive OS-MS vs. 26.6% in female controls, $p=0.0870$). Moreover, PAF-AH activities were significantly lower in MS than in controls, irrespective of clinical subtypes, among those carrying the identical polymorphism in terms of nucleotide position 994 of the PAF-AH gene. These findings suggest that the PAF-AH gene missense mutation has no relation to either susceptibility or severity of C-MS, yet its activity is down-regulated, and that the mutation has no relation with susceptibility of OS-MS, yet it may confer the severity of female OS-MS.

Keywords: Polymorphism; Platelet-activating factor acetylhydrolase; Multiple sclerosis; Opticospinal MS; Conventional MS

1. Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease of unknown etiology influenced by genetic background (Bell and Lathrop, 1996; Chataway et al., 1998). In Asian patients with MS, severe and selective involvement of the optic nerves and spinal cord is characteristic. We previously reported the existence of two subtypes of MS in Japanese; 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) showing disseminated lesions in the central nervous system (CNS), including the cerebrum, cerebellum or brainstem (Kira et al., 1996; Yamasaki et al., 1999). OS-MS has distinct features, such as a marked female preponderance, higher age at onset, higher Kurtzke's expanded disability status scale (EDSS) scores (Kurtzke, 1983) resulting from severe visual impairment and marked spinal cord dysfunction, and a lower number of brain lesions on MRI compared with C-MS. Severe inflammatory destruction is suggested in OS-MS because of the higher cell counts and amounts of protein in the cerebrospinal fluid (CSF), as well as long swollen lesions extending over several vertebral segments on spinal cord MRI (Kira, 2003). Pathological studies have also revealed severe inflammation and vascular changes in OS-MS lesions (Ikuta et al., 1982; Tabira and Tateishi, 1982). Gene polymorphism in HLA-DR (DRB1*1501) (Kira et al., 1996; Ma et al., 1998), Vitamin D receptor

Abbreviations: OS-MS, opticospinal multiple sclerosis; C-MS, conventional multiple sclerosis; EDSS, Kurtzke's expanded disability status scale; PAF-AH, platelet-activating factor acetylhydrolase.

* Corresponding author. Tel.: +81-92-642-5340; fax: +81-92-642-5352.
E-mail address: kira@neuro.med.kyushu-u.ac.jp (J. Kira).

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(Fukazawa et al., 1999; Niino et al., 2000) and osteopontin (Niino et al., 2003) were reported to be associated with susceptibility of C-MS while estrogen receptor polymorphism was associated with the severity of C-MS in Japanese (Kikuchi et al., 2002); however all of these results except for HLA-DRB1*1501 have not been independently confirmed by other groups and therefore still require further confirmatory studies. On the other hand, in OS-MS only the HLA-DPB1*0501 allele has been shown to be a disease-susceptibility gene (Ito et al., 1998; Yamasaki et al., 1999; Fukazawa et al., 2000), and no genetic background explaining the severe inflammation and vascular changes has ever been demonstrated.

There is a report showing an elevation in platelet-activating factor (PAF) in the CSF and plasma of relapsing–remitting MS patients, and the authors claimed that PAF is responsible for the early breakdown of the blood–brain barrier (BBB) in MS (Callea et al., 1999). The notion is supported by the observation that PAF receptors are upregulated in MS lesions (Lock et al., 2002). PAF is a biologically active phospholipid implicated as a mediator of physiological processes, such as the signaling and activation of proinflammatory cells and alteration of vascular permeability. In addition, PAF is also a very potent chemotactic stimulus for inflammatory cells such as eosinophils (Wardlaw et al., 1986) and polymorphonuclear neutrophils (O'Flaherty et al., 1981).

PAF is inactivated by plasma PAF acetylhydrolase (PAF-AH), which has its gene located in chromosome 6p12-21.1 and comprises 12 exons spanning at least 45 kilobase of DNA (Stafforini et al., 1996). In Japanese, a single point mutation (G → T transversion) at nucleotide position 994 in exon 9 results in a Val → Phe substitution at amino acid residue 279 of the mature protein, and is responsible for the loss of catalytic activity, has been shown to occur in 4% of the population, whereas no such mutation has ever been reported in Caucasians (Miwa et al., 1988). In previous reports, this mutation has been held up as a genetic risk factor for Japanese patients with vascular or autoimmune inflammatory diseases, such as stroke (Hiramoto et al., 1997), nonfamilial dilated cardiomyopathy (Ichihara et al., 1998), coronary artery disease (Yamada et al., 1998), and asthma (Stafforini et al., 1999). Moreover, the plasma PAF-AH gene mutation has been reported to influence the degree of proteinuria and the extent of mesangial proliferation in childhood IgA nephropathy (Tanaka et al., 1999).

Whether or not this missense mutation in the PAF-AH gene confers OS-MS susceptibility and severity in Japanese is of interest since the OS-MS is characterized by severe inflammation and vascular disruptions (Ikuta et al., 1982; Tabira and Tateishi, 1982; Kira, 2003). Moreover, OS-MS is preferentially seen in Japanese, who are reported to have higher frequency of the PAF-AH gene missense mutation, and is rare in Caucasians, in whom such mutation has not been reported. The present study was thus designed to ascertain the associations between the PAF-AH gene mutation and PAF-AH activities in Japanese MS patients by clinical subtypes.

2. Patients and methods

2.1. Patients

A total of 216 patients (56 men and 160 women) with clinically definite MS, according to the recommended diagnostic criteria (McDonald et al., 2001), were recruited from the Department of Neurology, Kyushu University Hospital, and the Department of Neurology, Hokkaido University Hospital, and Hokuyukai Neurological Hospital (Table 1). Hematological and biochemical studies and serologic tests for syphilis were performed in all patients. None of the patients were seropositive for human T-cell leukemia virus type 1. Age at examination was 41.5 ± 13.0 years (mean ± S.D.) and at disease onset 30.1 ± 12.0 years (mean ± S.D.). After at least a 1-year observation period, 169 were diagnosed as relapsing–remitting type MS and 47 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). EDSS scores were 3.8 ± 2.6 (mean ± S.D.) at the time of examination. In the present study, MS patients were classified into two subtypes before genotyping of the plasma PAF-AH according to the clinical criteria described previously (Kira et al., 1996; Yamasaki et al., 1999). The patients, whose clinically estimated lesions were confined to the optic nerve and

Table 1
Clinical profiles of MS patients

	MS (n=216)	OS-MS (n=65)	C-MS (n=151)
Male:Female*	56:160	9:56	47:104
Age at disease onset[a],**	30.1 ± 12.0	38.1 ± 13.9	26.7 ± 9.2
Age at examination[a],**	41.5 ± 13.0	49.3 ± 13.4	38.2 ± 11.3
Disease duration[a]	11.4 ± 8.7	11.2 ± 8.6	11.5 ± 8.8
EDSS score[b],**	3.8 ± 2.6	4.8 ± 2.3	3.5 ± 2.7
Rapidly progressive patients (%)**	29.3	52.3	29.8
Clinical course**			
R-R	169	62	107
S-P	47	3	44

OS-MS, opticospinal form multiple sclerosis; C-MS, conventional form multiple sclerosis; R-R, relapsing–remitting course; S-P, secondary progressive course; EDSS, Kurtzke's Expanded Disability Status Scale.

[a] Mean ± S.D. (years).

[b] Mean ± S.D.

*$p<0.05$ (OS-MS vs. C-MS).

**$p<0.001$ (OS-MS vs. C-MS).

spinal cord, were classified as having OS-MS and had no clinical evidence of disease in either the cerebrum or the cerebellum. The rest of the MS patients, who showed involvement of multiple sites in the CNS including the cerebrum, cerebellum, or brainstem, were classified as having C-MS. In each MS group, those who had a grave disability (EDSS score 5 and more than 5) within 10 years were classified as rapidly progressive type. Sixty-three of 216 (29.2%, 34 OS-MS and 29 C-MS) were considered to have a rapidly progressive disease. Moreover, in each MS group, those who had not a grave disability (EDSS score less than 5) beyond 10 years were classified as non-rapidly progressive type; 41 of 216 (19.0%, 12 OS-MS and 29 C-MS) were considered to have a non-rapidly progressive disease. The rests (112 patients; 19 OS-MS patients and 93 C-MS patients) were undetermined at the time of examination, since the observation period was less than 10 years and the EDSS scores were less than 5. The control group was composed of 89 unrelated healthy men and 124 unrelated healthy women (mean age $\pm$ S.D. $= 34.7 \pm 10.1$ years). Subjects' consent was obtained in accord with the declaration of Helsinki, and the Ethical Committee of the Institution in which the work was performed gave its approval.

2.2. Genotyping of the plasma PAF-AH

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 the plasma PAF-AH was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) according to the method of Stafforini et al. (Stafforini et al., 1996) without knowledge of the samples' clinical diagnosis. The sense primer (5′-CTATAAATTTATATCATGCTT-3′) and antisense primer (5′-TTTACTATTCTCTTGCTTTAC-3′) were used. Reactions were performed in a total volume of 50 μl containing 0.5 μg genomic DNA, 20 pmol of each primer, 0.2 mmol/l each of dATP, dGTP, dCTP, and dTTP, 1 U Taq DNA polymerase (Takara, Otsu, Japan), 50 pmol/l KCl, 1.5 mmol/l $MgCl_2$, and 10 nmol/l Tris hydrochloride (pH 8.3). The thermocycling procedure consisted of an initial denaturation at 94 °C for 5 min; five cycles of denaturation at 94 °C for 1 min, annealing at 56 °C for 1 min, and extension at 72 °C for 1 min; 30 cycles of 94 °C for 30 s, 52 °C for 30 s, and 72 °C for 30 s; and a final extension at 72 °C for 5 min. The genotype of the plasma PAF-AH was confirmed by digestion of the PCR products with the restriction endonuclease *Mae II* (Stafforini et al., 1996).

PCR products were analyzed by 2% agarose gel electrophoresis and visualized by ethidium bromide staining. Since the $G^{994} \rightarrow T$ transversion produces a new restriction site for *Mae II*, genotypes were classified as GG (normal), GT (heterozygous) and TT (homozygous deficient).

2.3. Measurement of PAF-AH activity

PAF-AH activity was measured using a spectrophotometric method with an H-7170 automatic analyzer (HITACHI) as described previously (Kosaka et al., 2000, 2001). Briefly, a 3-μl volume of sample and 240 μl of Reagent 1 (200 mmol/l NaCl, 5 mmol/l EDTA, 10 mmol/l sodium 1-non-anesulfonate, 10 mmol/l CHAPS, and 200 mmol/l HEPES, pH 7.6) were mixed, and preincubated at 37 °C for 5 min. The reaction was then started by adding 80 μl of Reagent 2 (19 mmol/l citric acid monohydrate, 9.5 mmol/l sodium 1-non-anesulfonate, and 4.8 mmol/l L-myristoyl-2-(4-nitrophenylsuccinyl) phosphatidylcholine, pH 4.5). Absorption was measured at 2 and 5 min after the addition of the substrate solution (Reagent 2). The activities were calculated using the differences between the absorbances of the above measuring points and the extinction coefficient of 4-nitrophenol. The sera from 24 OS-MS patients, 28 C-MS patients and 82 healthy controls and the plasma from eight OS-MS patients, five C-MS patients and 43 healthy controls were measured for their PAF-AH activities. Samples from OS-MS and C-MS patients were analyzed at a clinical remission phase in all patients and, in addition, in 13 patients with OS-MS and in nine patients with C-MS PAF-AH activities were analyzed both at the time of clinical relapse (within 2 weeks after the onset of acute exacerbation) and the time of clinical remission. Here we defined MS patients in remission as those who had been clinically stable for at least 1 month without any immunosuppressive medication, such as corticosteroids, interferon beta and azathioprine; and MS patients in relapse as those who developed new symptoms or a significant aggravation of pre-existing symptoms lasting for more than 2 days.

2.4. Statistical analysis

Plasma PAF-AH genotypes and allele frequencies were compared between OS-MS, C-MS and healthy controls, using Chi-square tests. As the calculated p values were not corrected for multiple comparisons, p values were multiplied by a factor of 10 (the number of comparisons) to calculate the corrected p values. The association between plasma PAF-AH activities and serum PAF-AH activities was examined using Pearson's correlation coefficient. Serum PAF-AH activities in the three genotypes were compared using the Kruskal–Wallis H test followed by multiple comparisons. Once differences among clinical groups were identified, we compared the results using one-way analysis of variance followed by Bonferroni's correction to determine statistical differences after multiple comparisons. In addition, for serum PAF activities, other comparisons were conducted using the Kruskal–Wallis H test followed by multiple comparisons. Values of $p < 0.05$ were considered statistically signifi-

Table 2
Genotype and allele frequencies for the PAF-AH gene mutation ($G^{994} \rightarrow T$) in MS patients and healthy controls

PAF-AH	MS			OS-MS			C-MS			Healthy controls		
	Total ($n=216$)	Male ($n=56$)	Female ($n=160$)	Total ($n=65$)	Male ($n=9$)	Female ($n=56$)	Total ($n=151$)	Male ($n=47$)	Female ($n=104$)	Total ($n=213$)	Male ($n=89$)	Female ($n=124$)
Genotype frequency												
GG	159 (73.6)	41 (73.2)	118 (73.7)	44 (67.7)	8 (88.9)	36 (64.3)	115 (76.2)	33 (70.2)	82 (78.8)	155 (72.8)	64 (71.9)	91 (73.4)
GT	54 (25.0)	14 (25.0)	40 (25.0)	20 (30.8)	1 (11.1)	19 (33.9)	34 (22.5)	13 (27.7)	21 (20.2)	53 (24.9)	21 (23.6)	32 (25.8)
TT	3 (1.4)	1 (1.8)	2 (1.3)	1 (1.5)	0 (0.0)	1 (1.8)	2 (1.3)	1 (2.1)	1 (1.0)	5 (2.3)	4 (4.5)	1 (0.8)
Allele frequency												
Allele G	372 (86.1)	96 (85.7)	276 (86.2)	108 (83.1)	17 (94.4)	91 (81.2)	264 (87.4)	79 (84.0)	185 (88.9)	363 (85.2)	149 (83.7)	214 (86.3)
Allele T	60 (13.9)	16 (14.3)	44 (13.8)	22 (16.9)	1 (5.6)	21 (18.8)	38 (12.6)	15 (16.0)	23 (11.1)	63 (14.8)	29 (16.3)	34 (13.7)

OS-MS, opticospinal form multiple sclerosis; C-MS, conventional form multiple sclerosis. The table indicates frequency of genotypes and alleles (percentage).

cant. Statistical analyses were performed with StatView/Mac software.

3. Results

3.1. Plasma PAF-AH genotype and allele frequencies in MS

The proportions of plasma PAF-AH genotypes (GG, GT, and TT) and alleles (G allele, T allele) in OS-MS patients, C-MS patients and healthy controls are shown in Table 2. In control subjects, the genotype frequencies are similar to those found in other Japanese studies (Hiramoto et al., 1997; Ichihara et al., 1998; Yamada et al., 1998; Stafforini et al., 1999; Tanaka et al., 1999). No statistically significant difference in the frequency of genotypes and alleles of the plasma PAF-AH polymorphism was observed among OS-MS patients, C-MS patients and healthy controls.

3.2. Plasma PAF-AH genotype and allele frequency changes by severity in each MS subgroup

In the rapidly progressive OS-MS subgroup, the frequency of the GT/TT genotypes was higher than in healthy

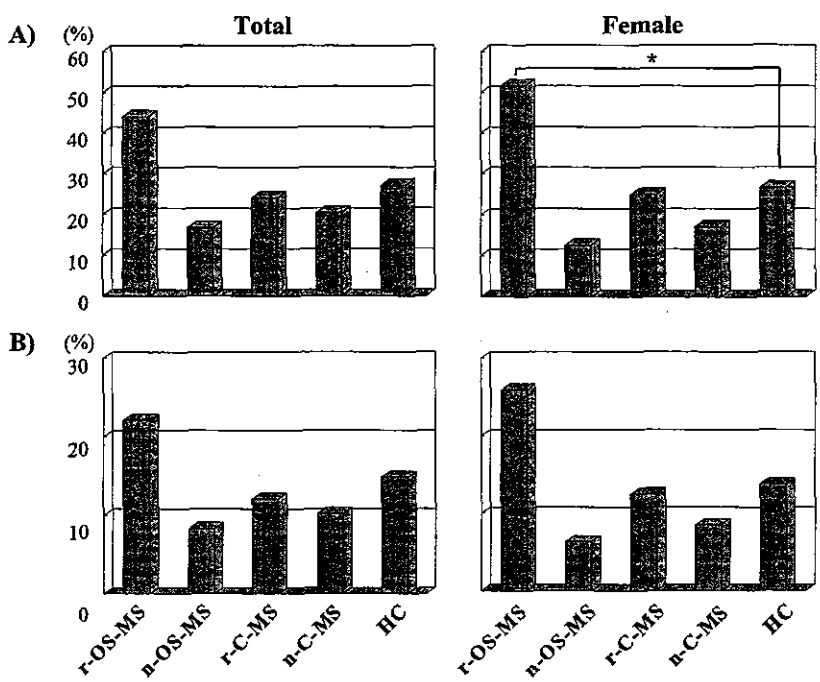

Fig. 1. Comparison of frequencies of GT/TT genotypes (A) and T allele (B) among rapidly progressive OS-MS, non-rapidly progressive OS-MS, rapidly progressive C-MS, non-rapidly progressive C-MS and healthy controls in total (left) and female (right) subjects. The asterisks (*) indicates that the frequency of GT/TT genotypes in female rapidly progressive OS-MS tended to be higher than in female healthy controls (15 of 29 female rapidly progressive OS-MS (51.7%) vs. 33 of 124 female healthy control (26.6%), $p=0.087$). No statistically significant difference in the frequency of genotypes and alleles of the plasma PAF-AH polymorphism was observed in other comparisons. r-OS-MS; rapidly progressive opticospinal multiple sclerosis ($n=34$); n-OS-MS, non-rapidly progressive opticospinal multiple sclerosis ($n=12$); r-C-MS, rapidly progressive conventional multiple sclerosis ($n=29$); n-C-MS, non-rapidly progressive conventional multiple sclerosis ($n=54$); HC, healthy controls ($n=213$).

controls (44.1% vs. 27.2%), but did not reach statistical significance. The frequency of GT/TT genotypes in female patients with rapidly progressive OS-MS tended to be higher than those in female healthy controls (51.7% vs. 26.6%, $p=0.087$) (Fig. 1). In female OS-MS patients, although the frequency of GT/TT genotype was much higher in the rapidly progressive type than in the non-rapidly progressive type (51.7% vs. 12.5%), it did not reach a statistical significance due to the small sample size. As for C-MS, no statistically significant difference in the frequency of either genotypes or alleles of the plasma PAF-AH polymorphism was observed.

3.3. Plasma and serum PAF-AH activities in each MS subgroup and healthy controls

As the number of sera stored in −80 °C and available for the present study was much larger than that of deep-frozen plasma, we first measured PAF-AH activities in both plasma and serum and evaluated the correlation between the two. Plasma PAF-AH activities significantly correlated with serum PAF-AH activities in MS patients and healthy controls ($r=0.9949$, $p<0.0001$), and therefore the serum PAF-AH activities were used for the statistical comparisons that followed. When we assorted serum PAF-AH activities in the MS subgroups and healthy controls into three groups according to genotype, we found that in each group, enzyme activities were significantly higher in subjects with the GG genotype (523.2 ± 133.7 IU/l) than those with GT or TT genotype (GT; 233.0 ± 52.8 IU/l, TT; 40.8 ± 4.2 IU/l, respectively), and also significantly higher in individuals with the GT genotype than in those with the TT genotype ($p<0.0001$) (Fig. 2). In addition, when we assayed PAF-AH activities at the time of a clinical relapse and at the time of a clinical remission in 22 MS patients, no statistically significant difference was observed ($p>0.1$). In subjects with the GG genotype and those with the GT genotype, PAF-AH activities in MS patients were significantly lower than those in healthy controls (GG genotype; 493.7 ± 146.0 vs. 546.9 ± 131.0 IU/l, $p=0.0223$, GT genotype; 204.7 ± 47.1 vs. 258.0 ± 45.2 IU/l, $p=0.0044$). In OS-MS subjects with the GG genotype, PAF-AH activities in rapidly progressive patients were lower than those in other patients (473.4 ± 81.1 IU/l in 5 vs. 505.9 ± 131.3 IU/l in 10). Moreover, in OS-MS subjects with the GT genotype, PAF-AH activities in rapidly progressive patients were lower than those in other patients (213.0 ± 45.7 IU/l in 6 vs. 241.0 ± 23.3 IU/l in 2). However, these comparisons did not reach a statistical significance due to the small sample size.

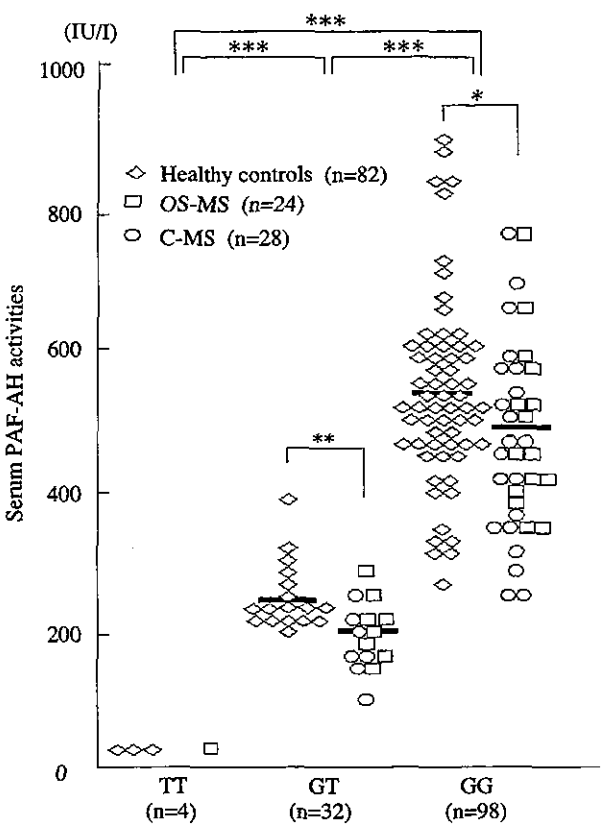

Fig. 2. Serum PAF-AH activities in healthy controls, and OS-MS and C-MS patients. Serum PAF-AH activities are significantly higher in subjects with the GG genotype (523.2 ± 133.7 IU/l) than those with GT or TT genotypes (233.0 ± 52.8 and 40.8 ± 4.2 IU/l, respectively), and also in individuals with GT genotype compared to those with TT ($p<0.0001$). In subjects with GG and GT genotypes, PAF-AH activities in MS patients are significantly lower than in healthy controls (GG genotype, 493.7 ± 46.0 vs. 546.9 ± 131.0 IU/l, $p=0.0223$; GT genotype, 204.7 ± 47.1 vs. 258.0 ± 45.2 IU/l, $p=0.0044$). The asterisks, *, ** and ***, indicate $p<0.05$, $p<0.01$ and $p<0.0001$, respectively. Each bar indicates the mean value of each group.

4. Discussion

In the present study, no significant association of the inactivating mutation of the PAF-AH gene with MS or with either subtype of MS was found, although the PAF-AH activities in MS were significantly lower than in healthy controls, even among populations carrying identical polymorphisms in terms of nucleotide position 994 of the PAF-AH gene. It is therefore suggested that the PAF-AH gene inactivating polymorphism plays no role in MS susceptibility.

In C-MS, the frequency of the PAF-AH gene missense mutation did not differ either by gender or by disease severity; while in OS-MS it tended to be higher in female patients with the rapidly progressive type than in female controls and female patients with the non-rapidly progressive type; however, the difference did not reach statistical significance due to the small sample size. It might suggest that the missense mutation is a possible genetic severity factor for OS-MS in female. Such a difference in the association of the missense mutation with diseases by gender has also been reported in cardiac infarction; in which

male showed a higher frequency of the mutation (Yamada et al., 1998).

It has been reported that PAF activity is significantly higher in relapsing–remitting MS than in secondary progressive MS (Callea et al., 1999), suggesting that PAF may be more operative in the relapse than in the progressive phase. Secondary progression was rare in OS-MS in the present as well as in earlier studies (Shibasaki et al., 1981), and the disability was determined largely by the severity of relapses in this condition. Therefore, in OS-MS, a decrease in PAF inactivation due to the missense mutation might strengthen inflammatory destruction of the CNS during relapses through enhanced PAF activity. As PAF not only promotes leukocyte adhesion and transmigration by the induction of intracellular adhesion molecule-1 (ICAM-1) expression on endothelial cells (Chihara et al., 1992), but also upregulates MHC class I and II expressions in some brain cells that are critical in antigen presentation (Martin-Mondiere et al., 1987), PAF may contribute to the disease process of OS-MS by reinforcing inflammatory cell response in the CNS. Moreover, since PAF is well known as a strong vascular permeability factor, enhancement of PAF activity might contribute to an alteration of vascular permeability in OS-MS lesions, as shown pathologically (Ikuta et al., 1982; Tabira and Tateishi, 1982).

Similar decrease of PAF-AH activity, as seen in the present MS patients, compared to controls has been reported in other autoimmune inflammatory conditions, such as systemic lupus erythematosus (Tetta et al., 1990); yet the mechanism is unknown. Such a decrease of PAF-AH activity may in part be responsible for the reported increase of PAF in MS plasma and CSF (Callea et al., 1999), and therefore could contribute to the inflammation and vascular permeability changes in the CNS of MS.

Although the $G^{994} \rightarrow T$ mutation in the plasma PAF-AH gene has not been found in Caucasians, three different polymorphisms that change the amino acid sequence of the protein have been reported (Bell et al., 1997; Kruse et al., 2000). Kruse (Kruse et al., 2000) reported that both the I198T and A379V variants were significantly associated with atopy and asthma in patients from Germany and the UK, and also that Thr198 and Val379 lowered the substrate affinity of PAF-AH, therefore prolonging PAF activity. It is intriguing to note that relapsing neuromyelitis optica (NMO), which shares considerable similarities with OS-MS in Asians (Kira, 2003), also shows vascular permeability changes and eosinophilic infiltration (Lucchinetti et al., 2002). Since PAF is an eosinophil chemoattractant as well as a vascular permeability factor, it might be rewarding to investigate the frequency of such inactivating mutations of the PAF-AH gene in relapsing NMO in non-Asian populations.

In conclusion, the gene mutation, $G^{994} \rightarrow T$, of the PAF-AH gene is not considered to confer MS susceptibility. However, in female OS-MS patients, it may be a possible severity factor. In that case, PAF inhibitors might be useful for such patients carrying the PAF-AH gene missense mutation. As the rarity of OS-MS even in Japanese populations renders a large-scale study difficult to perform for any single institution, a nation-wide genetic study may be called for.

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Presence of IgE Antibodies to Bacterial Superantigens and Increased IL-13-Producing T Cells in Myelitic Patients with Atopic Diathesis

Hirofumi Ochi Manabu Osoegawa Hiroyuki Murai Motozumi Minohara
Takayuki Taniwaki Jun-ichi Kira

Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

Key Words
Enterotoxin · Superantigen · IgE · IL-13 · Myelitis · Atopy

Abstract
Background: Superantigens are considered to exacerbate autoimmune inflammation through the expansion of autoreactive T cells; however, the immune response to bacterial superantigens has not been extensively studied in any type of myelitis. We recently reported the occurrence of a distinct type of myelitis in patients with atopic diathesis, which in a recent nationwide survey was reported to be widespread in Japan. The aim of this study was to investigate the presence of IgE antibodies to bacterial superantigens and the proportion of IL-13- or IL-5-producing CD4+ or CD8+ T cells in patients with myelitis and atopic diathesis. ***Methods:*** Twenty-four myelitic patients with and 12 myelitic patients without hyperIgEemia, 28 patients with multiple sclerosis (MS) and 34 healthy controls were enrolled in this study. IgE antibodies to staphylococcal enterotoxins A (SEA) and B (SEB) in sera were measured using a liquid-phase enzyme immunoassay, and the intracellular production of IL-5 and IL-13 in peripheral blood CD4+ and CD8+ T cells was measured by flow cytometry. ***Results:*** The myelitic patients with hyperIgEemia showed significantly higher positive rates of serum SEA/SEB-specific IgE antibodies (41.7 and 62.5%, respectively) than the healthy controls (5.9 and 8.8%), patients with MS (0 and 21.4%) and those with normoIgEemic myelitis (0 and 0%). Moreover, IL-13-producing CD4+ T cells and CD8+ T cells increased significantly in the myelitic patients with hyperIgEemia compared to the controls, while IL-5-producing CD4+ or CD8+ T cells did not. ***Conclusions:*** The IgE response to staphylococcal superantigens is heightened in myelitic patients with atopic diathesis, which might contribute to increases in IL-13-producing T cells and thus the development of myelitis.

Introduction

Superantigens might link infection and the exacerbation of autoimmune diseases, such as multiple sclerosis (MS), that target the myelin in the central nervous system (CNS) [1]. Superantigens can bind to the major histocompatibility complex outside the antigen-binding cleft and

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Correspondence to: Dr. Jun-ichi Kira
Department of Neurology, Neurological Institute
Graduate School of Medical Sciences, Kyushu University
Fukuoka 812-8582 (Japan)
Tel. +81 92 642 5340, Fax +81 92 642 5352, E-Mail kira@neuro.med.kyushu-u.ac.jp

stimulate T cells bearing the specific T cell receptor (TCR) Vβ [1]. In a mouse MS model, experimental autoimmune encephalomyelitis and staphylococcal enterotoxins A and B (SEA and SEB, respectively) reactivated CNS inflammation [2, 3] and induced epitope spreading [4]. In humans, Zhang et al. [5] and Hermans et al. [6] showed that bacterial superantigens (SEA and SEB) could stimulate myelin basic protein (MBP)-reactive T cell clones from MS patients, resulting in the production of proinflammatory cytokines such as IFN-γ. Although a similar reaction has also been observed in MBP-reactive T cell clones from healthy controls [5], differences in the cytokine profile of MBP-reactive T cells have been shown in MS patients and healthy controls. That is, an increased production of IL-2, IL-4, TNF-α, IFN-γ and IL-10 was seen in MS patients, while in MS patients carrying the HLA-DRB1*1501 allele, an especially large increase in TNF-α was seen [6]. Moreover, it was reported that in cases of acute disseminated encephalomyelitis, MBP-reactive T cell clones are activated by streptococcal exotoxins [7]. Therefore, in animal as well as human models, it is considered that superantigens exacerbate the autoimmune inflammation of the CNS through the multiplication of myelin-autoreactive T cells.

On the other hand, several lines of evidence show that staphylococcal superantigens SEA, SEB and SEC are involved in the exacerbation of atopic dermatitis (AD) [4–6]. It is well known that AD patients show a striking susceptibility to infection and colonization of *Staphylococcus aureus*. *S. aureus* colonizes AD skin and secretes enterotoxins with superantigen activity, which then penetrate into the immune system and activate T cells bearing specific TCR Vβ (Vβ3, 12, 14, 15, 17 and 20) [8, 9]. These activated T cells contribute to the persistence and exacerbation of allergic inflammation in AD skin. The Vβ types stimulated by superantigens in AD patients are shared with those of MBP-reactive T cells activated by the superantigens in MS patients. Alternatively, superantigens themselves act as classic allergens, and induce specific IgE antibodies in their hosts, which triggers the release of inflammatory mediators from sensitized mast cells and basophils after crosslinking of IgE receptors. The presence of IgE antibodies against SEA and SEB was demonstrated to be correlated with the severity of skin inflammation in AD patients [10, 11].

We recently reported the occurrence of a form of myelitis with distinct clinical features in patients with atopic diathesis; this type was named atopic myelitis [12–14]. A nationwide survey revealed that myelitis with atopic diathesis is widespread among the Japanese population [15]. Individuals with this condition show a longstanding course of myelitic inflammation and myelitic symptoms that fluctuate as the AD is exacerbated. Moreover, we found that spinal cord lesions associated with this disorder biopsied 3 months to 2 years after the onset of myelitis were active eosinophilic inflammations [16, 17] and similar to allergic inflammations in AD skin. These observations suggest that allergic mechanisms might be partly involved in atopic myelitis. On the other hand, the TCR Vβ types stimulated by staphylococcal superantigens and carried on the T cells infiltrating into the *S. aureus*-colonized skin in AD patients [18, 19] are in part common to those of the MBP-reactive T cells activated by bacterial superantigens in MS patients and healthy controls (Vβ12 or Vβ17) [5, 6]. Therefore, in patients with atopic myelitis, autoimmune mechanisms might also be involved in which staphylococcal superantigens activate T cells bearing the homing receptor to skin, cutaneous lymphocyte antigen, on the one hand, and those reactive to MBP on the other through stimulation of TCR Vβ12 or Vβ17.

Since staphylococcal superantigens can act as classic allergens as well as T cell stimulants, their involvement in atopic myelitis was considered interesting. As a first step in clarifying the role of superantigens in atopic myelitis, we determined whether superantigens act as classic allergens in atopic myelitic patients with or without AD. It was hoped that this would shed light on the role of bacterial superantigens in CNS inflammation. In addition, since IL-13 is a critical regulator of the allergic response and IL-5 is a potent mediator of eosinophilic inflammation, IL-5- and IL-13-producing T cells in the peripheral blood of atopic myelitic patients were measured.

Subjects and Methods

Subjects

The subjects consisted of 24 myelitic patients with atopic diathesis (with hyperIgEemia and mite antigen-specific IgE antibodies) as described previously [12–15] (mean age ± SD 33.6 ± 10.4 years; serum IgE: median 720 IU/ml, range 298–30,100 IU/ml), 12 myelitic patients without atopic diathesis (without either hyperIgEemia or mite antigen-specific IgE; 39.4 ± 14.5 years; serum IgE: median 50 IU/ml, range 31–172 IU/ml), 28 patients with clinically definite MS based on the criteria of Poser et al. [20] (37.8 ± 10.7 years; serum IgE: median 50 IU/ml, range 5–1,123 IU/ml), and 34 healthy controls (33.7 ± 7.1 years; serum IgE: median 67.5 IU/ml, range 5.9–760 IU/ml). Among the myelitic patients with hyperIgEemia and mite antigen-specific IgE, 11 had AD (34.2 ± 9.9 years; serum IgE: median 6,535 IU/ml, range 460–30,100 IU/ml) and 13 did not (33.1 ± 11.2 years; serum IgE: median 662.2 IU/ml, range 298–782 IU/ml). The clinical features of the myelitic patients with hyperIgEemia have been described previously [12–15]. Of the 28 MS patients, 4

had hyperIgEemia (36.5 ± 8.2 years; serum IgE: median 342 IU/ml, range 256–1,123 IU/ml) while the other 24 did not (38.0 ± 11.2 years; serum IgE: median 39.5 IU/ml, range 5–213 IU/ml). The controls comprised 34 healthy hospital workers. Of these, 11 had hyperIgEemia (30.6 ± 4.9 years; serum IgE: median 470 IU/ml, range 250–760 IU/ml) while the other 23 did not (35.1 ± 7.5 years; serum IgE: median 35 IU/ml, range 5.9–210 IU/ml). None of the healthy controls had any apparent allergic disorders at the time of examination.

Determination of Allergen-Specific Serum IgE

Serum allergen-specific IgE antibodies were measured with an AlaSTAT assay (Sankojunyaku, Tokyo, Japan), which is a liquid-phase enzyme immunoassay for allergen-specific IgE antibodies, according to MacSharry et al. [21]. As allergens, SEA, SEB and two mite antigens *(Dermatophagoides pteronyssinus* and *Dermatophagoides farinae)* were used. The lower limit of sensitivity of this assay was 0.34 IU/ml; thus, IgE-AlaSTAT levels higher than 0.34 IU/ml were considered positive.

Flow Cytometric Analysis

Flow cytometric analysis of IL-5- and IL-13-producing T cells was conducted as described previously [22]. Peripheral blood-derived mononuclear cells were treated for 4 h with 25 ng/ml phorbol 12-myristate 13-acetate (Sigma, St. Louis, Mo., USA) and 1 μg/ml ionomycin (Sigma) in the presence of 10 μg/ml brefeldin A (Sigma). After washes with phosphate-buffered saline containing 0.1% bovine serum albumin (0.1% BSA-PBS), antibodies to the cell surface markers were added in the dark for 15 min at room temperature. The cells were washed twice, permeabilized with FACS permeabilizing solution (Becton Dickinson, San Jose, Calif., USA) and incubated with antibodies against human cytokines or isotype-matched controls for 30 min. Finally, the cells were washed with 0.1% BSA-PBS and resuspended in 1% paraformaldehyde to be analyzed by two-color flow cytometry using Epics XL System II (Coulter, Hialeah, Fla., USA). Ten thousand events per lymphocyte were acquired and analyzed. As a negative control, intracellular isotype-matched controls were used. Analysis gates were set on the lymphocytes according to forward- and side-scatter properties. The CD8+ T cells were divided into $CD8^{high}$ and $CD8^{low}$ cells, because many $CD8^{low}$ cells also expressed CD16 and CD56, and were considered natural killer cells. On the other hand, virtually all $CD8^{high}$ cells expressed CD3 but not CD16 (data not shown). $CD8^{high}$ cells were therefore defined as CD8+ T cells and analyzed further. The monoclonal antibodies used in this study included the following: PC5-conjugated anti-CD4 (mouse IgG1; 13B8.2, Becton Dickinson), FITC-conjugated anti-CD8 (mouse IgG1; B9.11, Becton Dickinson), PE-conjugated anti-IL-5 (rat IgG2a; JES1-39D10, PharMingen, San Diego, Calif., USA), PE-conjugated anti-IL-13 (rat IgG1; JES10-5A2, PharMingen), PE-conjugated rat IgG2a (R35-95, PharMingen) and PE-conjugated rat Ig-G1 (R3-34, PharMingen).

Statistics

The Mann-Whitney U test was used for statistical analysis of serum IgE levels. Statistical analysis of the frequency of hyperIgEemia and specific IgE antibodies against SEA and/or SEB was performed with the χ^2 test, or when the criteria for the χ^2 test were not fulfilled, the Fisher's exact test. The correlation between serum IgE and antigen-specific IgE levels was determined using Spearman's rank correlation.

Table 1. Positive rates of serum SEA/SEB-specific IgE antibodies in studied subjects

	SEA IgE >0.34 IU/ml	SEB IgE >0.34 IU/ml
HyperIgEemic myelitis		
Total (n = 24)	10/24 (41.7)	15/24 (62.5)
With AD (n = 11)	5/11 (45.5)	8/11 (72.7)
Without AD (n = 13)	5/13 (38.5)	7/13 (53.8)
NormoIgEemic myelitis (n = 12)	0/12 (0)	0/12 (0)
Multiple sclerosis		
Total (n = 28)	0/28 (0)	6/28 (21.4)
With hyperIgEemia (n = 4)	0/4 (0)	3/4 (75)
Without hyperIgEemia (n = 24)	0/24 (0)	3/24 (12.5)
Healthy controls		
Total (n = 34)	2/34 (5.9)	3/34 (8.8)
With hyperIgEemia (n = 11)	1/11 (9.1)	2/11 (18.2)
Without hyperIgEemia (n = 23)	1/23 (4.3)	1/23 (4.3)

Figures in parentheses represent percentages.

Results

Specific IgE Antibodies to Staphylococcal Enterotoxins (SEA and SEB)

The positive rates of serum SEA- and SEB-specific IgE in the myelitic patients with hyperIgEemia (41.7 and 62.5%, respectively) were significantly higher than in the healthy controls (5.9 and 8.8%; $p = 0.0028$ and $p < 0.0001$), patients with MS (0 and 21.4%; $p = 0.0001$ and $p = 0.0026$), and myelitic patients with normoIgEemia (0 and 0%; $p = 0.0085$ and $p = 0.0003$) (table 1). Among the patients with hyperIgEemic myelitis, those with AD had significantly higher levels of total IgE than those without AD ($p = 0.0092$). However, there was no significant difference in the positive rates of serum SEA/SEB-specific IgE between the two subgroups with hyperIgEemic myelitis. Patients with hyperIgEemic myelitis but without AD had higher positive rates of serum SEA/SEB-specific IgE (38.5 and 53.8%) than the healthy controls with hyperIgEemia (9.1 and 18.2%) ($p = 0.085$ and $p = 0.066$, respectively), although this difference was not statistically significant due to the small sample size. There was no significant difference in the positive rates of either SEA- or SEB-specific IgE between the MS patients and controls, or between the normoIgEemic myelitic patients and controls.

The distributions of serum SEA/SEB-specific IgE levels in each group are shown in figure 1. Among the sub-

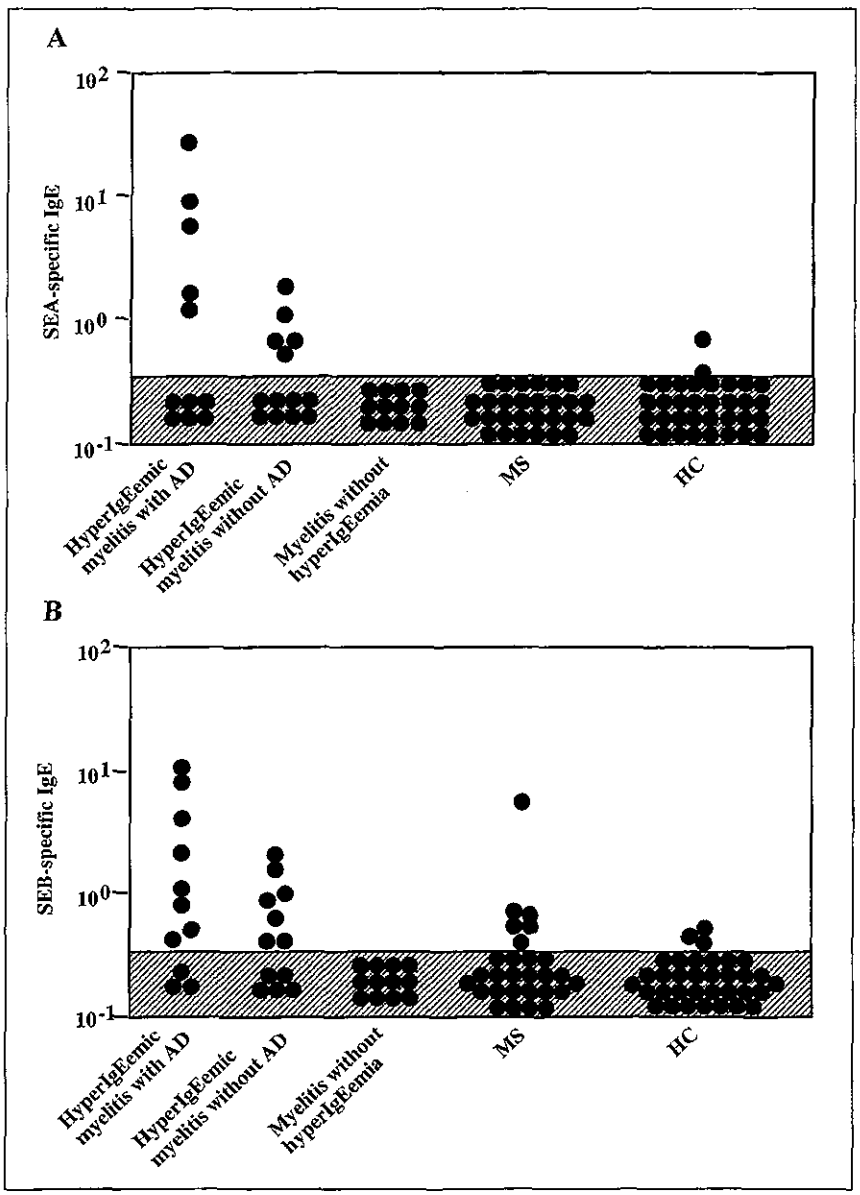

Fig. 1. Distributions of serum SEA- (**A**) and SEB-specific IgE levels (**B**). HC = Healthy controls. The optimal cutoff level for the definition of serum SEA- and SEB-specific IgE positivity was 0.34 IU/ml.

jects sensitized to SEA, those with hyperIgEemic myelitis had higher levels of SEA-specific IgE (median 1.43 IU/ml, range 0.68–27.5 IU/ml) than the SEA-sensitized healthy controls (median 0.53 IU/ml, range 0.37–0.68 IU/ml), although this difference was not statistically significant due to the small sample size. Among the patients with hyperIgEemic myelitis sensitized to SEA, those with AD had significantly higher levels of SEA-specific IgE (median 5.79 IU/ml, range 1.2–27.5 IU/ml) than those without AD (median 0.68 IU/ml, range 0.68–1.83 IU/ml) ($p = 0.0278$). Among the SEB-sensitized subjects, there were no significant differences in the levels of SEB-specific IgE among those with hyperIgEemic myelitis, MS and healthy controls (median 0.99, 0.60 and 0.55 IU/ml, respectively, range 0.4–8.29, 0.38–5.45 and 0.40–0.76 IU/ml, respectively). Among the patients with hyperIgEemic myelitis sensitized to SEB, there were no significant differences in the levels of SEB-specific IgE irrespective of the presence of AD (median 1.62 IU/ml, range 0.50–11.1 IU/ml, and median 0.90 IU/ml, range 0.40–2.07 IU/ml).

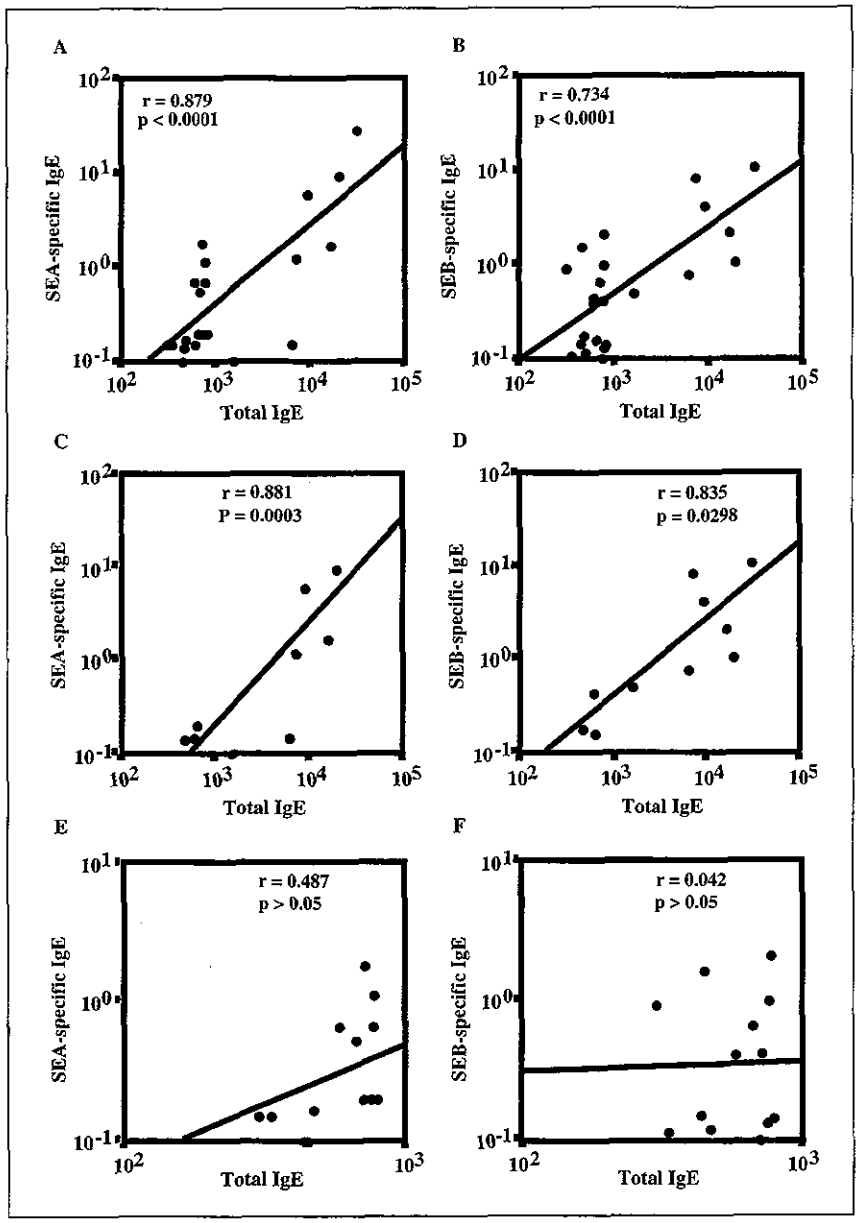

Fig. 2. Scatter plot correlating to total and SEA/SEB-specific IgE levels in all patients with hyperIgEemic myelitis (**A**, **B**), those with hyperIgEemic myelitis and AD (**C**, **D**) and those with hyperIgEemic myelitis but without AD (**E**, **F**). The correlation coefficient was determined by Spearman's rank correlation.

Correlation between SEA- and SEB-Specific IgE Levels in Patients with HyperIgEemic Myelitis

A strong correlation was observed between the levels of SEA- and SEB-specific IgE antibodies in patients with myelitis and atopic diathesis ($r = 0.700$, $p < 0.0001$). On the other hand, there was no correlation between the MS patients and healthy controls because none tested positive for both SEA- and SEB-specific IgE antibodies (data not shown).

Correlation between SEA/SEB-Specific IgE and Total Serum IgE Levels in Patients with HyperIgEemic Myelitis

There was a significant correlation between total serum IgE and serum SEA/SEB-specific IgE levels in patients with myelitis and hyperIgEemia, with respective correlation coefficients of 0.879 ($p < 0.0001$) and 0.734 ($p < 0.0001$) (fig. 2A, B). In patients with hyperIgEemic myelitis and AD, serum SEA/SEB-specific IgE levels still

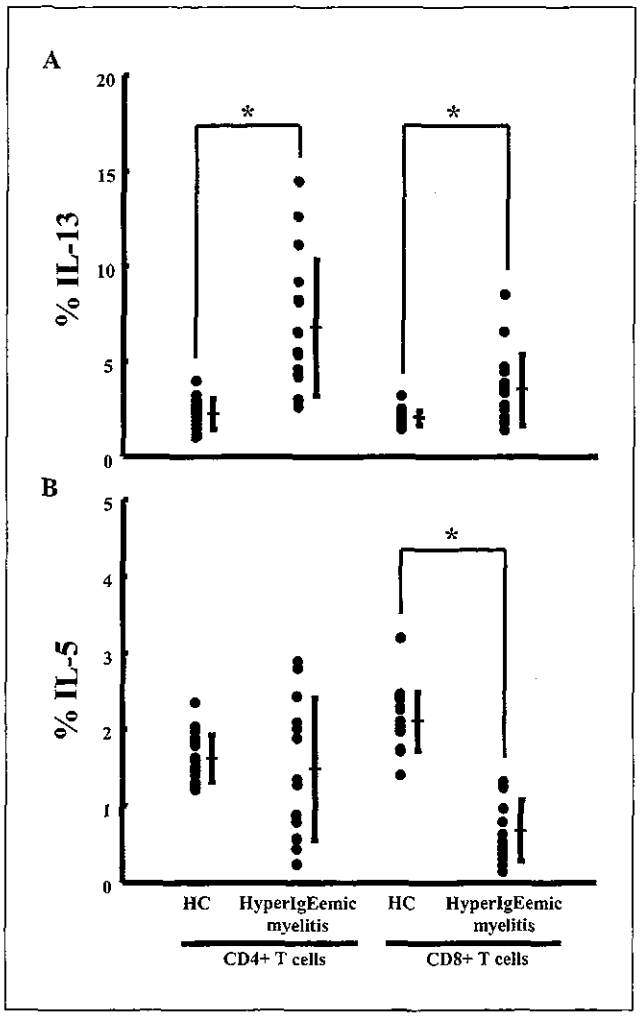

Fig. 3. *Intracellular production of* IL-13 (**A**) and IL-5 (**B**) in peripheral blood CD4+ T cells and CD8+ T cells. HC = Healthy controls.

correlated significantly with total serum IgE levels (r = 0.881, p = 0.0003, and r = 0.835, p = 0.0298, respectively) (fig. 2C, D). However, in patients with hyperIgEemic myelitis but without AD, there was no significant correlation between SEA/SEB-specific IgE and total serum IgE levels (r = 0.487, p = 0.0927, and r = 0.042, p = 0.8126, respectively) (fig. 2E, F).

Correlation between Mite Antigen- and SEA/SEB-Specific IgE Levels in Patients with HyperIgEemic Myelitis

There was no significant correlation between the serum levels of SEA/SEB- and *D. pteronyssinus*-specific IgE antibodies (r = 0.328, p = 0.1247, and r = 0.227, p = 0.3020, respectively). Similarly, there was no significant correlation between the serum levels of SEA/SEB- and *D. farinae*-specific IgE antibodies (r = 0.252, p = 0.1078, and r = 0.193, p = 0.3826, respectively).

Intracellular IL-5 and IL-13 Production

In patients with myelitis and atopic diathesis, percentages of IL-13-producing cells were significantly higher in both CD4+ and CD8+ T cell fractions (6.81 ± 3.55 vs. 2.31 ± 0.77% in CD4+ T cells, $p < 0.0001$; 3.55 ± 1.85 vs. 2.11 ± 0.38% in CD8+ T cells, p = 0.0026) (fig. 3). Percentages of IL-5-producing cells in CD8+ T cells were significantly lower in patients with myelitis and atopic diathesis than in healthy controls (0.68 ± 0.40 vs. 2.11 ± 0.38%, $p < 0.0001$), but they did not differ significantly between the two groups in CD4+ T cells (1.49 ± 0.92 vs. 1.62 ± 0.31%, $p > 0.1$).

Discussion

This is the first study to find that myelitis with atopic diathesis shows a strong IgE antibody response to bacterial superantigens as well as a significant increase in IL-13-producing CD4+ T cells and CD8+ T cells compared with healthy controls.

Although myelitis with atopic diathesis showed high levels of total serum IgE, it is considered less likely that the high frequency of IgE antibodies against staphylococcal enterotoxins is due to nonspecific IgE binding. First, this is because there was no significant correlation between the serum levels of staphylococcal enterotoxin- and mite antigen-specific IgE antibodies. Second, although a significant correlation between total IgE and staphylococcal enterotoxin-specific IgE levels was observed in patients with hyperIgEemic myelitis and AD, there was no such correlation in patients with hyperIgEemic myelitis but without AD, in whom positive rates of anti-SEA/SEB-specific IgE did not significantly differ from those in patients with AD. The lack of such correlations suggests the presence of a specific IgE response to bacterial superantigens in atopic myelitis patients, at least in those without AD.

It is noteworthy that patients with hyperIgEemic myelitis had higher positive rates of serum IgE antibodies against staphylococcal enterotoxins, regardless of the presence or absence of AD. This observation suggests that patients with hyperIgEemic myelitis are not only susceptible to the colonization of superantigen-secreting *S. aureus*

but also that they tend to respond immunologically to such superantigens.

Bacterial superantigens have been reported to induce the production of IL-13 by CD4+ T cells as well as CD8+ T cells in atopic patients [23]. The present study revealed an increase in IL-13-producing CD4+ T and CD8+ T cells in myelitic patients with atopic diathesis. IL-13 plays a critical role in maintaining prominent IgE production in various atopic disorders [24, 25], because there is no feedback mechanism exerted by IL-13 due to the lack of IL-13 receptors on the T cells. Increased IL-13 production is thus likely to contribute to hyperIgEemia and the induction of allergen-specific IgE. Moreover, IL-13 contributes to eosinophil recruitment at inflammatory sites through induction of eotaxin [26], and also the induction of adhesion molecules [27, 28]. Therefore, it is possible that increased IL-13 production by T cells is one of the key events for maintaining disease activity in myelitic patients with atopic diathesis.

Observations that IgE is elevated in the cerebrospinal fluid of patients with atopic myelitis [15] and that the spinal cord pathology of the disease is eosinophilic inflammation [16, 17] suggest that an allergic mechanism similar to other atopic disorders is operative in the spinal cord inflammation. In AD patients, many studies have indicated that staphylococcal enterotoxins exacerbate skin inflammation through an antitoxin IgE-mediated mechanism [29]. Eosinophils attracted by chemotactic factors released by IgE-activated mast cells or basophils damage tissues through the release of proinflammatory cytokines and activated eosinophil products, such as eosinophil cationic protein, major basic protein and eosinophil-derived neurotoxin [30, 31]. However, in the CNS, as staphylococcal enterotoxins are not usually present, a direct antitoxin IgE-mediated mechanism might not be feasible. Food allergies can induce AD skin lesions through activation of T cells bearing the skin homing receptor, cutaneous lymphocyte antigen [32–34]. This suggests that an allergic mechanism might induce inflammation in areas that are distant from the places where the allergens are exposed to the hosts through activation of lymphocytes bearing specific homing receptors in the distant organs. Such activated lymphocytes might produce proinflammatory cytokines and exacerbate inflammation in the distant organs where relevant antigens do not exist. In atopic myelitic patients, therefore, bacterial superantigens might not be of primary importance as allergens, but be one of the important exacerbating factors contributing to spinal cord inflammation.

On the other hand, SEB induces the expansion and selective accumulation of T cells expressing SEB-reactive TCR Vβ, Vβ12 and Vβ17, in toxigenic *S. aureus*-colonized skin [18, 19], and promotes cutaneous inflammation. Similarly, SEB activates MBP-reactive T cells with TCR Vβ12 or Vβ17 to secrete a proinflammatory cytokine, IFN-γ, in MS patients [5, 6]. Therefore, in atopic myelitic patients, it seems possible that staphylococcal enteroxins might activate T cells bearing TCR Vβ12 or Vβ17, which have an antimyelin autoreactivity and contribute to the exacerbation of spinal cord inflammation. Since at least half of the atopic myelitic patients in this study were sensitized to staphylococcal enterotoxins, as shown by the antitoxin IgE, the TCR Vβ usage of these patients should be examined in the future to disclose whether T cells bearing toxin-reactive TCR Vβ12 or Vβ17 expand in vivo.

To summarize, these results suggest that staphylococcal enterotoxins might be involved in the exacerbation and persistence of a specific form of human CNS inflammation, myelitis with atopic diathesis. Because antimicrobial therapy has been shown to alleviate the skin inflammation of AD patients [33], such therapy might also be helpful in patients with hyperIgEemic myelitis.

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Time-dependent cytokine deviation toward the Th2 side in Japanese multiple sclerosis patients with interferon beta-1b

Hirofumi Ochi, Mei Feng-Jun, Manabu Osoegawa, Motozumi Minohara, Hiroyuki Murai, Takayuki Taniwaki, Jun-ichi Kira*

Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan

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Abstract

To address the immune mechanism sustaining interferon beta (IFNβ) efficacy in multiple sclerosis (MS), we longitudinally analyzed expressions of IFN-γ, IL-4, IL-5 and IL-13 in $CD4^+$ T cells and $CD8^+$ T cells in 22 Japanese MS patients (16 patients with conventional MS and 6 with opticospinal MS) undergoing IFNβ using flow cytometry. During the 48-week observation period, five opticospinal MS patients (83%) relapsed compared to only four conventional MS patients (25%); the frequency of relapsed patients was significantly higher in the former ($p=0.046$). The effects of IFNβ on individual cytokines were time-dependent and altered cytokine productions were particularly evident in $CD4^+$ rather than $CD8^+$ T cells. A decreased intracellular IFN-γ/IL-4 ratio in $CD4^+$ T cells was thus evident soon after the initiation of therapy, and persisted for the entire 1 year follow-up period, regardless of whether or not the patient relapsed ($p<0.01$). IFNβ treatment resulted in a rapid increase in the percentage of IFN-γ^- IL-4^+ and IL-13^+ $CD4^+$ T cells 1 week after the initiation of therapy and high values were sustained for 6 months but declined to the baseline over 1 year. Later, the percentage of IFN-γ^+ IL-4^- $CD4^+$ T cells decreased significantly from weeks 24 through 48 of therapy ($p<0.01$). When comparisons with the pretreatment values were made for each subtype of MS, a significant reduction of IFN-γ^+ IL-4^- $CD4^+$ T cell percentages was shown in conventional MS ($p<0.0001$), but not in opticospinal MS. Moreover, when such a comparison was made by the presence or absence of relapse during therapy, a significant reduction of IFN-γ^+ IL-4^- $CD4^+$ T cell percentages was observed in MS patients without relapse ($p<0.01$). Thus, a reduction of IFN-γ^+ IL-4^- $CD4^+$ T cell percentages in the late phase of therapy is considered important for reducing relapse in conventional MS. When the expression patterns of IFN-γ, IL-4, IL-5 and IL-13 in $CD4^+$ T cells and $CD8^+$ T cells were compared between patients with and without relapse during therapy, the only significant difference was an increase in the IL-13^+ $CD4^+$ T cell percentages in patients with relapse compared to those without ($p<0.05$). The results indicate that in $CD4^+$ T cells IL-4 was preferentially up-regulated in the early course and IFN-γ was down-regulated in the late phase of IFNβ therapy. The net effect of IFNβ on the immune balance was entirely toward type 2 immune deviation, possibly contributing to its beneficial effects on MS.

Keywords: Multiple sclerosis; Interferon beta; IFNγ; IL-4; IL-13; Th1; Th2; Opticospinal MS

1. Introduction

Multiple sclerosis (MS), a chronic inflammatory disease of the central nervous system (CNS), is generally considered to be a Th1-type cell-mediated immune disease triggered by environmental factors in genetically susceptible individuals [1,2]. Treatment of relapsing-remitting MS with interferon beta (IFNβ) reduces the frequency and severity of clinical relapses. Due to cytokine dysregulations between Th1 and Th2 cells in MS, it has been hypothesized that the beneficial effect of IFNβ in MS might be due to the induction of a protective Th2 immune response or inhibition of the Th1 immune response [3]. However, Dayal et al. [4] reported that IFN-γ-secreting cells increased during the first week of IFNβ therapy in some patients, and this may be related to clinical exacerbation, although it was not a uniform response. In a retrospective study, IFNβ-induced exacerbations were not observed during the first 90 days of therapy [5]. Thus, demonstrating that the immune mechanism by which IFNβ acts is not well-understood, especially at the initiation of therapy.

* Corresponding author. Tel.: +81-92-642-5340; fax: +81-92-642-5352.
E-mail address: kira@neuro.med.kyushu-u.ac.jp (J. Kira).

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