Although an altered cytokine balance has been reported to occur in MS and its pathologic role has been hypothesized, few investigations have been carried out into the immune balance before and during IFNβ therapy, especially in the early stages, as well as long after the initiation of therapy. Only Furlan et al. [6] reported a decrease in IFN-γ- and IL-4-secreting $CD4^+$ cells as well as $CD8^+$ cells from 1 month to 9 months after initiation of IFNβ in Caucasian patients with MS. Moreover, while several studies have been performed to address the cytokine-modulating effects of IFNβ in Caucasian MS patients, the results remain inconclusive [7], and such a study has not been undertaken in Asian MS patients.

There are two distinct MS subtypes of Japanese MS, conventional MS (C-MS) and opticospinal MS (OS-MS). We have shown that OS-MS is immunogenetically distinct from C-MS, since C-MS is associated with HLA-DRB1*1501, as seen in Caucasians, whereas OS-MS is associated with HLA-DPB1*0501 [8,9].

This prompted us to investigate whether IFNβ has an effect on the cytokine balance in Japanese MS patients, and thus we simultaneously examined intracellular Th1 and Th2 cytokine production from $CD4^+$ and $CD8^+$ T cells in the peripheral blood of Japanese MS patients before and during the first week of IFNβ-1b (Betaferon®) therapy, and analyzed alterations in the cytokine balance. Further, to determine the long-term effects of IFNβ on the cytokine balance, we analyzed longitudinally for up to 1 year of therapy.

2. Materials and methods

2.1. Patients

Twenty-two Japanese patients (12 women and 10 men; mean age ± S.D. =39.6 ± 11.3 years) with relapsing-remitting MS, diagnosed according to the revised diagnostic criteria for MS [10], were included in this study. At the time of their enrollment, none of the patients were experiencing an acute attack or had been under immunosuppressive treatment for at least the previous 3 months. The demographic and clinical characteristics of the patients are shown in Table 1. The patients were clinically classified into two subtypes: OS-MS (6 patients; 45.7 ± 15.7 years) and C-MS (16 patients; 37.3 ± 8.8 years), as described previously [8]. Briefly, patients who had both optic nerve and spinal cord involvement without any clinical evidence of disease in either the cerebrum or the cerebellum were considered to have OS-MS. Those with minor brainstem signs, such as transient double vision or gaze nystagmus, were included. All other patients showing disseminated involvement of the CNS were considered to have C-MS. The disease duration and Expanding Disability Status Scale (EDSS) [11] at the baseline were not significantly different between the two subtypes. All

Table 1
Demographic and clinical characteristics of the 22 MS patients before and during IFNβ-1b treatment

	Total	C-MS	OS-MS
Number of patients	22	16	6
Sex (male/female)	10:12	9:7	1:5
Age at baseline (mean ± S.D.)[a]	39.6 ± 11.3	37.3 ± 8.8	45.7 ± 15.7
Disease duration at baseline (mean ± S.D.)[a]	5.8 ± 4.1	6.1 ± 4.2	5.0 ± 4.0
EDSS at baseline (mean ± S.D.)	3.9 ± 1.9	3.8 ± 2.0	4.3 ± 1.6
Relapse rate during 2 years before IFNβ-1b (mean ± S.D.)	1.7 ± 0.9	1.6 ± 1.0	2.1 ± 0.5
Relapse rate during 1 year of IFNβ-1b (mean ± S.D.)	0.8 ± 0.9	0.5 ± 0.7	1.5 ± 0.5

MS, multiple sclerosis; C-MS, conventional multiple sclerosis; OS-MS, opticospinal multiple sclerosis; EDSS, Expanded Disability Status Scale of Kurtzke.
[a] Years.

patients were treated with IFNβ-1b (Betaferon®, Shering) 8×10^6 units given subcutaneously every other day over a period of 1 year. Fifteen patients were given the full dose from the beginning while seven were initiated at half-dose and then increased to the full dose after a few days (after 1 day in one patient, 2 days in two patients and 3 days in four patients). Antipyretics were used in 13 patients at the beginning, and flu-like symptoms were observed in 14 patients.

2.2. Flow cytometric analysis

Intracellular cytokines were studied by flow cytometry, as described previously [12]. IFN-γ was studied as a Th1 cytokine while IL-4, IL-5 and IL-13 were studied as Th2 cytokines. Peripheral blood mononuclear cells from MS patients were collected before treatment (baseline samples, $n=22$), and consecutively after 1 ($n=22$), 2 ($n=22$), 4 ($n=20$), 12 ($n=22$), 24 ($n=20$), and 48 ($n=20$) weeks of IFNβ-1b therapy. Cells were treated for 4 h with 25 ng/ml phorbol 12-myristate 13-acetate (Sigma, St. Louis, MO), 1 μg/ml of ionomycin (Sigma) in the presence of 10 μg/ml brefeldin A (Sigma). Monoclonal antibodies used in this study included: PC5-conjugated anti-CD4 (13B8.2; Becton Dickinson), PC5-conjugated anti-CD8 (B9.11; Becton Dickinson), FITC-conjugated anti-IFN-γ (25723.11; Becton Dickinson), PE-conjugated anti-IL-4 (3010.211; Becton Dickinson), PE-conjugated anti-IL-5 (JES1-39D10; PharMingen, San Diego, CA), and PE-conjugated anti-IL-13 (JES10-5A2; PharMingen). The percentage of cytokine-positive $CD4^+$ or $CD8^+$ T cells was determined as the % cytokine-positive $CD4^+$ population/$CD4^+$ population or % cytokine-positive $CD8^+$ population/$CD8^+$ population.

2.3. Statistical analysis

Statistical analyses for comparing cell percentages before and after treatment were performed using the Bonferroni/Dunn test with a repeated-measure one-way ANOVA. A p value of below 0.05 was considered significant. Comparisons between patients with and without relapse, and those with and without flu-like symptoms were also performed using a repeated-measure ANOVA.

3. Results

3.1. Clinical response to IFNβ therapy

During the 48-week observation period, nine patients suffered thirteen relapses, i.e. one patient experienced three relapses (at 5, 33 and 45 weeks), two experienced two relapses (at 14 and 20 weeks, and at 16 and 24 weeks, respectively) and six experienced one relapse (at 2, 10, 26, 35, 41 and 44 weeks, respectively). During this period, five of six OS-MS patients (83%) relapsed compared to only four of sixteen C-MS patients (25%), and thus the frequency of relapsed patients was significantly higher in OS-MS than in C-MS ($p=0.046$).

The annual relapse rate of the total MS patients during the 1-year period of therapy was 0.8 ± 0.9 (mean $\pm$ S.D.) per year. This is significantly lower than that (1.7 ± 0.9) during the 2-year period before therapy ($p=0.008$). OS-MS showed higher relapse rates before and during therapy than C-MS. Although the annual relapse rate reduced after therapy in both C-MS (69% reduction) and OS-MS (29% reduction), the decrease was only significant in C-MS ($p=0.014$). That the IFNβ-induced reduction in the relapse rate in OS-MS did not reach statistical significance is possibly due to the small sample size.

3.2. Intracellular cytokines of $CD4^+$ T cells

The percentage of intracellular IFN-γ^- IL-4^+ $CD4^+$ T cells was significantly increased at 1 week after the initiation of therapy and persisted until the value returned to baseline after 48 weeks of therapy ($p<0.01$ at each point) (Fig. 1). A similar significant augmentation pattern was observed for the percentage of intracellular IL-13^+ $CD4^+$ T cells ($p<0.01$ at each point). A significant reduction in the percentage of intracellular IFN-γ^+ IL-4^- $CD4^+$ T cells was observed with a later onset, i.e. after 24 weeks of therapy, and persisted thereafter ($p<0.01$ at each point). A decline in the intracellular IFN-γ/IL-4 ratio in $CD4^+$ T cells was apparent after 1 week of therapy and was maintained throughout the 48-week treatment period ($p<0.01$ at each point). No significant IFNβ-induced changes were observed for the percentage of intracellular IL-5^+ $CD4^+$ T cells.

3.3. Intracellular cytokines of $CD8^+$ T cells

No significant IFNβ-induced changes were observed for the percentages of intracellular IFN-γ^- IL-4^+, IFN-γ^+ IL-4^- and IFN-γ^+ IL-4^+ $CD8^+$ T cells, except for a significant reduction in the FN-γ^+ IL-4^- $CD8^+$ T cell percentages at 48 weeks of therapy ($p<0.01$) (Fig. 2), which reflected the gradual decrease in IFN-γ^+ IL-4^- $CD8^+$ T cells over time. The intracellular IFN-γ/IL-4 ratio in the $CD8^+$ T cells gradually reduced during the therapy, and the reduction reached significance at 24 weeks of therapy ($p<0.01$). This effect persisted at 48 weeks of therapy ($p<0.01$). The percentage of either intracellular IL-13^+ or IL-5^+ $CD8^+$ T cells did not show any significant changes after therapy.

3.4. Difference in the cytokine production pattern by clinical subtype

When the cytokine production pattern was compared between OS-MS and C-MS patients, there were no significant differences in the alterations in cytokine production between the two subtypes, although a reduction in the IFN-γ^+ IL-4^- $CD4^+$ T cell percentages after 24 weeks compared with the pretreatment values was only statistically significant in C-MS ($p<0.0001$) and such a decrease was not evident in OS-MS (Fig. 3).

3.5. Clinical response and cytokine production pattern

When intracellular cytokine expression patterns were compared between patients who relapsed ($n=9$) and those who did not ($n=13$) during the 48-week observation period, the only difference found was that those with relapse showed significantly higher IL-13^+ $CD4^+$ T cell percentages than those without, especially from 1 to 4 weeks of IFNβ ($p<0.05$) (none of the other cytokines showed any significant changes between patients with or without relapse in either the $CD4^+$ T or $CD8^+$ T cells) (Fig. 3). However, when pretreatment values were compared between those with and those without relapse during IFNβ therapy, the former showed a significantly higher intracellular IFN-γ/IL-4 ratio in $CD8^+$ T cells ($p<0.05$) than the latter at baseline. Compared with the pretreatment values, patients with relapse showed significantly higher IL-13^+ $CD4^+$ T cell percentages throughout the observation period ($p<0.0001$) while the mild increase in IL-13^+ $CD4^+$ T cell percentages in those without relapse did not reach statistical significance at any time point. In addition, a significant decrease in the IFN-γ^+ IL-4^- $CD4^+$ T cell percentages during 24–48 weeks of the therapy compared with the pretreatment values was only seen in patients without relapse ($p<0.01$), and not in those with. There were no significant differences in intracellular cytokine expression patterns between patients with or those without flu-like symptoms (data not shown).

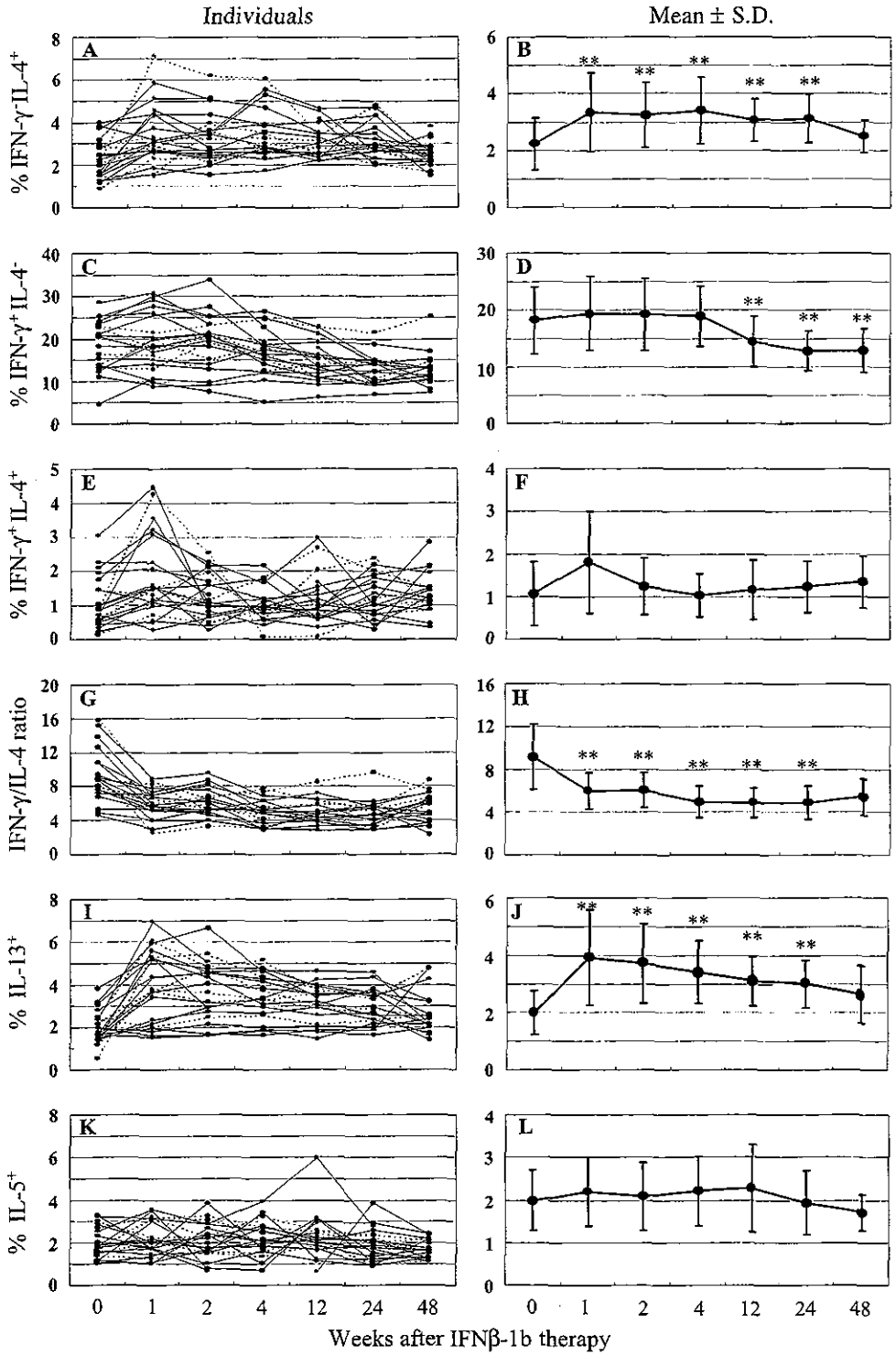

Fig. 1. Changes in the percentage of intracellular cytokine-producing $CD4^+$ T cells. (A, B) IFN-γ^- IL-4^-; (C, D) IFN-γ^+ IL-4^-; (E, F) IFN-γ^+ IL-4^+; (G, H) IFN-γ/IL-4 ratio; (I, J) IL-13^+; (K, L) IL-5^+. (A, C, E, G, I, K) Changes in individual patients. Data obtained from the same patient at different times are connected with a line. A solid line is used for patients with conventional multiple sclerosis and a dashed line for those with opticospinal multiple sclerosis. (B, D, F, H, J, L) Mean ± S.D. of the total multiple sclerosis patients. Repeated measure one-way ANOVA followed by Bonferroni test was used for statistical analysis. $^{**}p<0.01$.

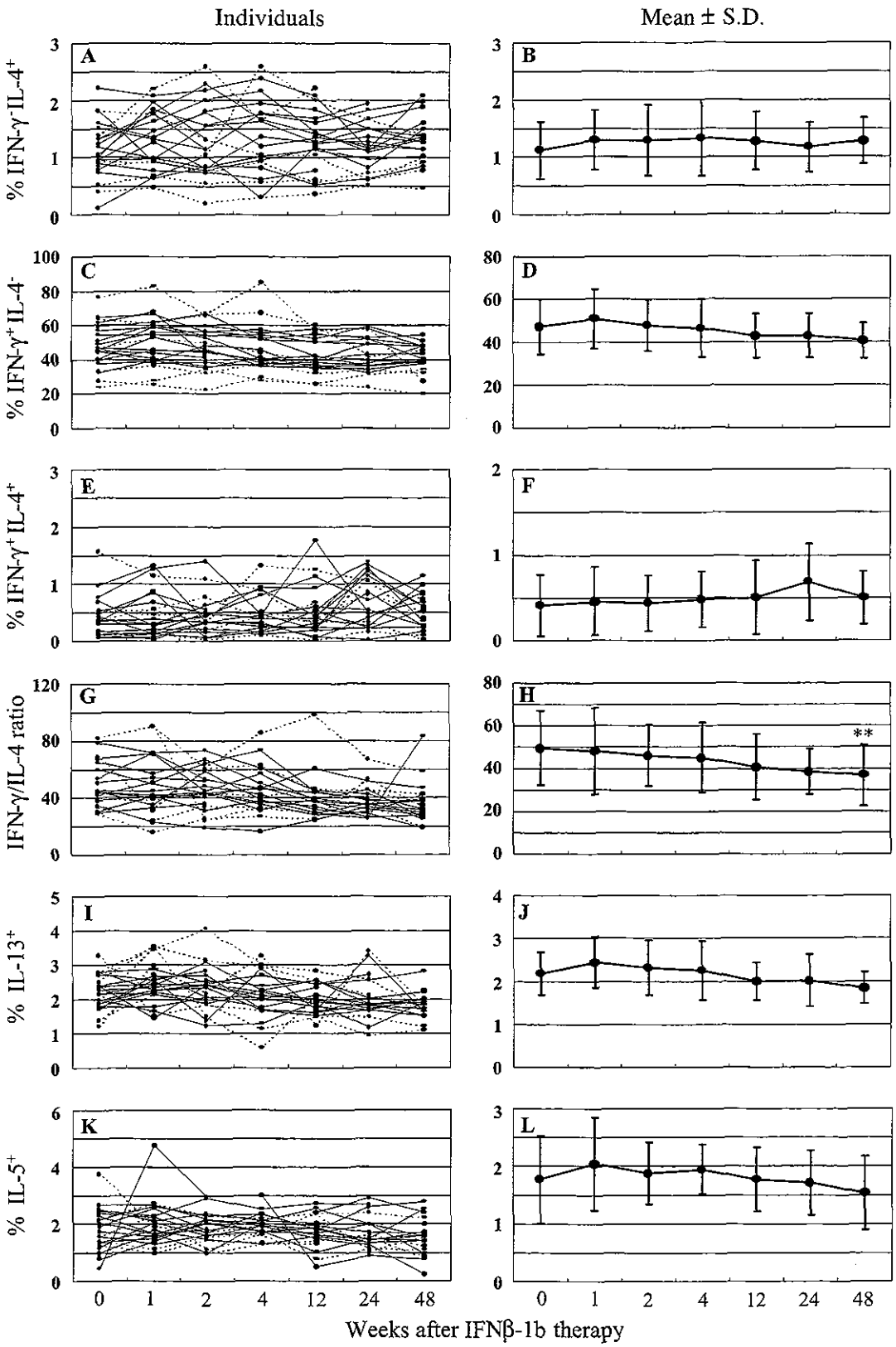

Fig. 2. Changes in the percentage of intracellular cytokine-producing $CD8^-$ T cells. (A, B) IFN-γ^- IL-4^+; (C, D) IFN-γ^+ IL-4^-; (E, F) IFN-γ^+ IL-4^+; (G, H) IFN-γ/IL-4 ratio; (I, J) IL-13^+; (K, L) IL-5^+. (A, C, E, G, I, K) Changes in individual patients. Solid or dashed lines are used as in Fig. 1. (B, D, F, H, J, L) Mean ± S.D. of the total multiple sclerosis patients. $^{**}p < 0.01$.

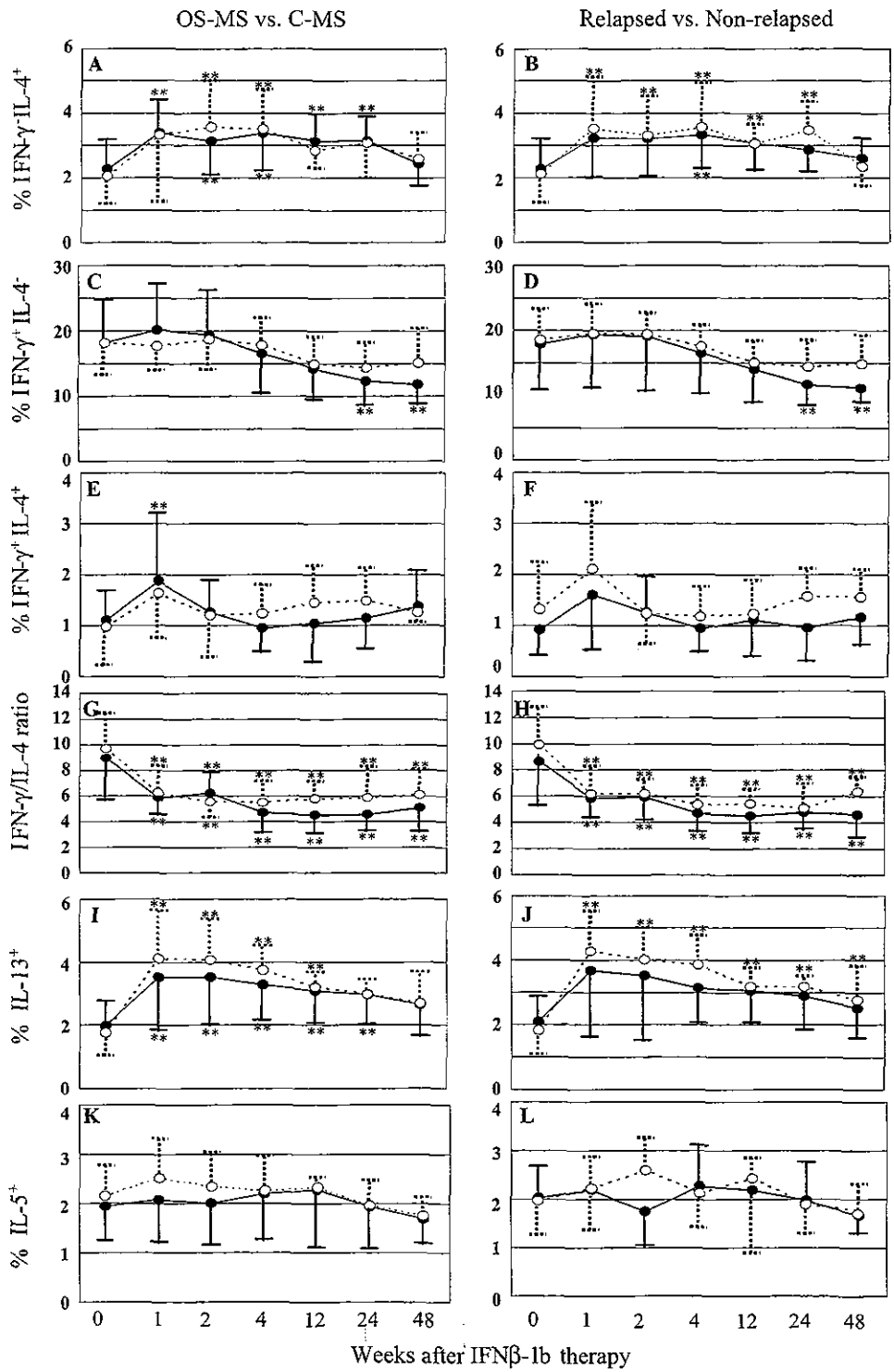

Fig. 3. Comparisons of intracellular cytokine expression patterns between patients with OS-MS (dashed line, open circle, $n=6$) and patients with C-MS (solid line, closed circle, $n=16$) (left column), and between MS patients with (dashed line, open circle, $n=9$) and without (solid line, closed circle, $n=13$) relapse (right column) during the 48-week observation period. (A, B) IFN-γ^- IL-4^-; (C, D) IFN-γ^+ IL-4^-; (E, F) IFN-γ^- IL-4^+; (G, H) IFN-γ/IL-4 ratio; (I, J) IL-13^-; (K, L) IL-5^-. The mean ± S.D. of each group is shown. The cytokine expression pattern is not significantly different between OS-MS and C-MS for any of the cytokines, while only an increase in the IL-13^+ $CD4^-$ T cell percentages is significantly different between the patients with and without relapse ($p<0.05$). $^{**}p<0.01$, compared with the pretreatment value in each subgroup.

4. Discussion

This study revealed that an IFNβ-associated immune deviation toward type 2 was evident soon after the initiation of therapy and persisted for the entire 1 year follow-up period, indicating that this immune deviation may be beneficial to MS patients. Our present results also demonstrate that cytokines varied in their response to IFNβ with time after therapy.

In the early phase, we observed that Th2-like cells such as IFN-γ^- IL-4^+ and IL-13^+ $CD4^+$ T cells increased significantly immediately after the initiation of IFNβ therapy. In addition, although Th1-like cells such as IFN-γ^+ IL-4^- $CD4^+$ T cells were not significantly changed as a whole, 9 of the 22 patients on IFNβ therapy showed transient increases in IFN-γ^+ IL-4^- $CD4^+$ T cells of more than 20% of the baseline value for four weeks. These findings are not consistent with those of Furlan et al. [6] who showed a decrease in IFN-γ- and IL-4-producing cells among $CD4^+$ cells as well as $CD8^+$ cells from 1 through 9 months of IFNβ therapy in Caucasian patients with MS. Such a difference could be attributable to racial differences; however, Furlan et al. [6] did not examine changes in the first few weeks, while our observations in the early phase of IFNβ therapy are in agreement with those of Dayal et al. [4] who reported that IFN-γ-secreting cells increased during the first weeks of IFNβ therapy in some Caucasian patients with MS. Moreover, it has been demonstrated by microarray analysis that IFNβ therapy up-regulates many Th1 genes [13]. Therefore, IFNβ therapy also appears to lead to the production of not only type 2 cytokines in most patients in the early phase, but also type 1 cytokines in some patients. Regarding immune balance; since augmentation of IL-4 production was much larger than that of IFN-γ, the net immune balance, as determined by the intracellular IFN-γ/IL-4 ratio, was toward the Th2 side even in the early phase. Thus, the net immune balance toward Th2 in the early phase may be beneficial to MS, although another mechanism of action could be relevant.

It is of interest that the percentage of IFN-γ^+ IL-4^- $CD4^+$ T cells declined after 6 months and that the IFNβ-induced surge of IL-4 normalized after 6 months of therapy. Thus, the net effect was again toward Th2. Such a late decrease in IFN-γ^+ IL-4^- $CD4^+$ T cells was only seen in patients without relapse during the therapy, further suggesting an important role for the reduction in IFN-γ^+ IL-4^- $CD4^+$ T cells in the reduction in relapse rates by IFNβ. Two recent reports suggest that IFNβ induces apoptosis of activated T cells in the late stage of therapy (after 3 months) [14,15], which is in accord with the late reduction of IFN-γ^+ IL-4^- $CD4^+$ T cells on IFNβ in our MS patients. A reduction in IFN-γ^+ IL-4^- $CD8^+$ T cells in the late phase may thus also be beneficial. In MS brain, $CD8^+$ T cells are outnumbered, most of which bear cytotoxic granules and locate in close proximity to the damaged axons and thus are considered to be cytotoxic cells to transect axons rather than suppressor cells to protect myelin [16]. Therefore, the reduction of Tc1 cells ($CD8^+$ cytotoxic T cells producing IFN-γ but not IL-4), may also contribute to the favorable effect of IFNβ. At baseline, patients who relapsed later during IFNβ therapy had a higher intracellular IFN-γ/IL-4 ratio in $CD8^+$ T cells than those who did not; suggesting that those who tend to relapse even on IFNβ may be in part destined to do so because of their strong tendency toward IFN-γ production.

Among type 2 cytokines; the increase in IL-13^+ $CD4^+$ T cells was significantly higher in patients with relapse during the 48 weeks of therapy than in those without. This observation is consistent to our previous findings that IL-13^+ $CD4^+$ T cells were increased during a relapse, while IL-4- or IL-5-producing T cells were not [12,17]. Genain et al. [18] reported that in a marmoset model of experimental allergic encephalomyelitis (EAE), an non-human primate animal model MS, Th2 cells can exacerbate the disease probably through the production of autoantibodies against myelin. Moreover, even in a rodent model of EAE, myelin basic protein-specific Th2 cells cause EAE in immunodeficient hosts [19]. Therefore, it is possible that IL-13-producing Th2 cells may be related to relapse during IFNβ therapy.

Recent studies reveal that IL-13 plays a crucial role in many aspects of immune regulation; IL-13 has been reported to inhibit the function of NK cells [20,21], which play a major down-regulatory role in EAE [22]. In relapsing-remitting MS, the decrease in NK cell function precedes the onset of clinical attacks [23,24]. Enhanced IL-13 response on IFNβ may thus augment CNS inflammation through down-regulation of NK cells in MS. Moreover, IL-13 up-regulates the expression of major histocompatibility complex class II antigens [25,26] as well as monocyte chemoattractant protein [27] in monocytes/macrophages and microglial cells. IL-13 also up-regulates the expression of vascular cell adhesion molecule-1 in endothelial cells [28,29]. In addition, IL-13 reduces nitric oxide (NO) synthase from monocytes/macrophages and microglial cells [26]. NO inhibits antigen-specific T cell proliferation and induces apoptosis of encephalitogenic T cells [30–33]. IL-13 may thus prevent the death of encephalitogenic T cell proliferation in MS through down-regulation of NO synthesis. All these effects of IL-13 may render patients on IFNβ prone to developing a relapse. To clarify the role of IL-13 in relapse, we considered it important to sequentially analyze IL-13 production by Th2-like cells before, during and after relapse in each patient on IFNβ therapy in a future study.

Concerning immunologic differences between C-MS and OS-MS; a lower frequency of oligoclonal IgG bands in the cerebrospinal fluid of OS-MS patients compared with C-MS patients is well known [34]. Moreover, in some studies, marked Th1 and Tc1 shifts are present in the peripheral blood throughout relapse and remission phases in OS-MS while a Th1 shift was only evident in the relapse phase in C-MS [15,35,36]. However, in the present study, a reduction in the IFN-γ/IL-4 ratio during IFNβ therapy was commonly seen in both OS-MS and C-MS, suggesting that a common

mechanism may be partly operative in the two MS subtypes, and that the reduction in the intracellular IFN-γ/IL-4 ratio by IFNβ may exert beneficial effects on this process. Contrarily, a decreased in IFN-γ^+ IL-4^- $CD4^+$ T cells in the late phase of IFNβ therapy was only evident in C-MS, and not in OS-MS, which may partly contribute to the higher relapse rates in OS-MS even during IFNβ therapy.

In conclusion, although IFNβ has broad immunomodulatory effects on the cytokine profile, the net immune balance deviation toward the type 2 side may, in part, work beneficially against MS, although the role of IL-13 requires further investigation.

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High frequency of allergic conjunctivitis in myasthenia gravis without thymoma

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Abstract

Objectives: To investigate the frequency of allergic disorders in myasthenia gravis (MG) patients and characterize the features of MG associated with allergic disorders.
Methods: Frequencies of past and present common allergic disorders in 160 MG patients who visited the Department of Neurology, Kyushu University Hospital from April 2000 to July 2003 and in 81 neurological normal controls were studied.
Results: Among various allergic disorders, the frequency of allergic conjunctivitis (AC) was significantly higher in MG patients (39/160, 24.4%, p^{corr}=0.0112), especially with MG without thymoma (36/123, 29.3%, p^{corr}=0.0016), in comparison to the controls (6/81, 7.4%). MG patients with AC showed a significantly higher rate of seronegative MG (43.6% vs. 17.4%, p=0.008) and a higher tendency of ocular MG (43.6% vs. 28.1%, p=0.071). Moreover, MG with AC had significantly lower anti-acetylcholine receptor antibody titers (median 6.8 nmol/l vs. median 23.6 nmol/l, p=0.0359) as well as a lower rate of coexisting thymoma (7.7% vs. 17.4%, p=0.016). The incidence of myasthenic crisis was also lower in MG with AC than without AC, yet the difference was not significant (7.7% vs. 15.7%).
Conclusion: There was a significant association of AC with MG especially for ocular or seronegative MG in cases without thymoma.

Keywords: Myasthenia gravis; Allergy; Allergic conjunctivitis; Thymoma; Anti-acetylcholine receptor antibody

1. Introduction

Myasthenia gravis (MG) is a well-documented autoimmune disease in which autoantibodies such as anti-acetylcholine receptor (AChR) antibodies play an important role in the development of the disease [1]. MG is also known as a heterogeneous disease. The age of onset, for example, varies from infantile to presenile, the clinical type can be either ocular or generalized, thymus pathology shows thymoma or non-thymoma, and anti-AChR antibodies do not always show positive. Although it has been recently suggested that antibodies other than the anti-AChR antibody, for example the anti-MuSK antibody, are associated with some MG cases [2], it is still unclear what causes the differences in the disease.

Previously it was reported that CD23 is over-expressed in the germinal centers of the thymus of MG patients [3]. CD23 is the low affinity receptor for IgE [4,5], and the molecule seems to be involved in various allergic disorders as well as MG [6–10]. Several neurological disorders such as Churg-Strauss syndrome [11], myelitis [12–14], Hopkins syndrome [15], juvenile muscular atrophy of distal upper extremities [16] and migraines [17] have been reported to be associated with various allergies. These observations prompted this study, which examines the frequency of allergic disorders in MG patients in order to give a better insight into the common immunological aberrancies of these conditions.

2. Subjects and methods

The frequency of present and past allergic disorders including bronchial asthma, allergic rhinitis, atopic derma-

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Table 1
Demographic features of 160 MG patients

Sex (M/F)	52:108 (1:2.08)
Mean age at onset (range)	40.4 (0.4–78)
Mean age at examination (range)	52.2 (21–82)
Mean duration of illness (range)	12.7 (0.6–44.7)
Mean anti-AChR antibody titer (range) (nmol/l)	85.8 (0–1200)
Seronegative MG	38/160 (23.8%)
Coexistence of thymoma	37/160 (23.1%)
History of myasthenic crisis	22/160 (13.8%)

titis, urticaria, allergic conjunctivitis, food, drug and metal allergies was studied in 160 consecutive MG patients (108 female and 52 male, mean age 52.2, age range 21–82) who visited the Department of Neurology, Kyushu University Hospital from April 2000 to July 2003; and 81 neurological normal controls (48 female and 33 male, mean age 52.8, age range 16–87) who similarly visited our department during March 1998 and February 2000 and who underwent thorough neurological examinations and laboratory tests and were determined to be neurologically normal. Although bronchial asthma includes both allergic and non-allergic bases, we did not specify 'allergic bronchial asthma' because it is difficult to distinguish the two from a self-administered questionnaire. The diagnosis of MG was established when typical clinical symptoms with diurnal fluctuation and/or easy fatigability were present and at least one of the following tests was positive: (1) Edrophonium test (Tensilon test), (2) repetitive nerve stimulation on the facial and/or median nerves, and (3) measurement of anti AChR antibody through radioimmunoassay. Patients also underwent body CT testing in order to verify thymic abnormalities such as hyperplasia and thymoma. Further, we excluded other diseases that can cause symptoms resembling MG; such as Hashimoto's thyroiditis, Graves' disease, thyroid myopathy, ocular myopathy and mitochondrial diseases.

The patients and controls were requested to complete a questionnaire regarding their past and present history of allergic disorders. The questionnaire set out questions as follows: "Have you ever been diagnosed as having allergic conjunctivitis by your doctor? When did it start and how long did it last? Do you still have the symptom at present? What is the possible cause?" The same questions are repeated for other allergic disorders as well. MG patients and the neurological normal controls completed the questionnaire under the same conditions when they first came to see us.

After completion of the questionnaire, it was noted that there was a high prevalence of AC in the MG patients and so they were divided into two groups, patients with AC (AC+) and those without AC (AC−). Both AC+ and AC− groups were evaluated and compared using clinical features such as the coexistence of thymoma, positive testing for anti-AChR antibodies, rates of seronegative MG, rates of ocular MG, which may include orbicularis oculi muscle weaknesses, and past histories of myasthenic crisis.

The frequency of allergic disorders was statistically compared between MG patients and controls using a chi-square test. The chi-square test was also used to compare the frequency of neurological manifestations, thymoma, seronegative MG and myasthenic crisis between AC+ and AC− patients. Yate's correction method was applied to results when any of the expected number was lower than 5. The uncorrected p values (p^{uncorr}) were multiplied by the number of comparisons (×8) to calculate the corrected p values (p^{corr}). The nonparametric Mann–Whitney U-test was applied to determine the statistical significance of the comparisons of anti-AChR antibody titer levels between AC+ and AC− patients.

3. Results

The demographic features of 160 MG patients are summarized in Table 1. Of the total 160 patients, thymoma was found in 37 (23.1%). Thirty-eight (23.8%) patients were free from anti-AChR antibodies and so were classified as having seronegative MG. A history of myasthenic crisis was present in 22 (13.8%) patients.

The frequencies of the various allergic disorders are summarized in Table 2. Compared with controls, MG patients showed a significantly higher percentage of AC

Table 2
Frequencies of allergic disorders in MG patients

	Control (n=81)	MG total (n=149)	MG	
			Non-thymoma (n=115)	Thymoma (n=34)
Bronchial asthma	4 (4.9)	13 (8.1)	11 (8.9)	2 (5.4)
Allergic rhinitis	11 (13.6)	35 (21.9)	29 (23.6)	6 (16.2)
Atopic dermatitis	4 (4.9)	12 (7.5)	11 (8.9)	1 (2.7)
Urticaria	9 (11.1)	24 (15.0)	21 (17.1)	3 (8.1)
Allergic conjunctivitis	6 (7.4)	39 (24.4)*	36 (29.3)*	3 (8.1)
Food allergy	5 (6.2)	17 (10.6)	13 (10.6)	4 (10.8)
Drug allergy	10 (12.3)	24 (15.0)	19 (15.4)	5 (13.5)
Metal allergy	3 (3.7)	11 (6.9)	8 (6.5)	3 (8.1)

The exact number of patients and the percentages (parenthesis) are shown.
* $p^{corr}<0.05$, as compared with controls.

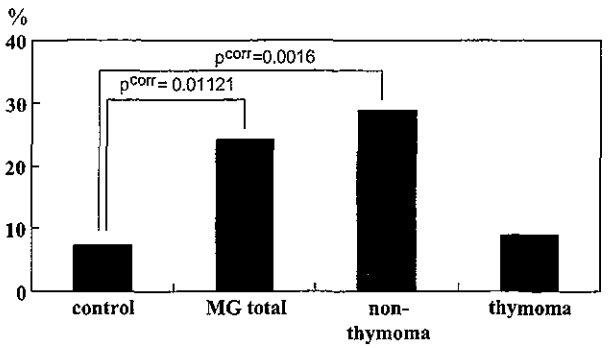

Fig. 1. Frequency of AC in MG patients and controls. MG patients in total have a higher frequency of AC than controls. MG without thymoma has a higher frequency of AC compared to MG with thymoma.

(24.4% in MG and 7.4% in the controls, p^{uncorr}=0.0014, p^{corr}=0.0112) (Table 2). Among MG patients, those with MG and no thymoma showed a significantly higher frequency of AC in comparison with controls (29.3% vs. 7.4%, p^{uncorr}=0.0002, p^{corr}=0.0016) (Fig. 1). There was no significant difference in the frequency of AC between MG cases with thymoma and controls. All other allergic disorders were also present in higher frequencies in MG patients than controls, but the difference was not statistically significant (Table 2).

The 160 MG patients were then subdivided into an AC+ group (39 patients) and an AC− group (121 patients). In the AC+ group, AC preceded MG in 17 cases, MG preceded AC in 15 cases, and both occurred at the same time in four cases, whilst results were unknown in three cases. In the AC+ group, there was a significantly low percentage (7.7%) of thymoma-related MG, in contrast with 28.1% in the AC− group (p=0.016, Fig. 2A). The percentage of seronegative MG was significantly higher in the AC+ group (43.6%) in comparison with the AC− group (17.4%) (p=0.008, Fig. 2B). Anti-AChR antibody titers of seropositive cases were significantly lower in the AC+ group (median 6.8 nmol/l) compared to the AC− group (median 23.6 nmol/l) (p=0.0359, Fig. 2C). The rate of ocular MG tended to be higher in the AC+ group (43.6%) in comparison to the AC− group (28.1%) (p=0.071, Fig. 2D). The incidence of myasthenic crisis was lower in the AC+ group (7.7%) compared with the AC− group (15.7%), but the difference was not statistically significant (Fig. 2E).

4. Discussion

In this study, it was found that the frequency of AC is significantly higher in MG patients, especially in cases of MG without thymoma, in comparison to control patients, and that MG cases with AC have a significantly higher frequency of seronegative MG and lower counts of anti-AChR antibody titers.

The present study has methodological limitations in respect to the accuracy of diagnoses of allergic disorders since such diagnoses are based upon only the questionnaire. However, the types of allergic disorders in the questionnaire investigated were essentially the same as those previously reported in two epidemiological surveys of allergic disorders [18,19], and the phrases of the questions in our study followed one of them [18]. There should be no selection bias because the neurological normal controls and MG patients all completed the same questionnaire in the same setting when they came to our out-patient clinic for their first visit. Moreover, in MG patients, the increase of only AC among eight allergic disorders investigated was highly significant in non-thymoma patients (p^{corr}=0.0016) but not in thymoma patients (p^{corr}>0.05). In addition, the frequency of AC differed significantly between seronegative and

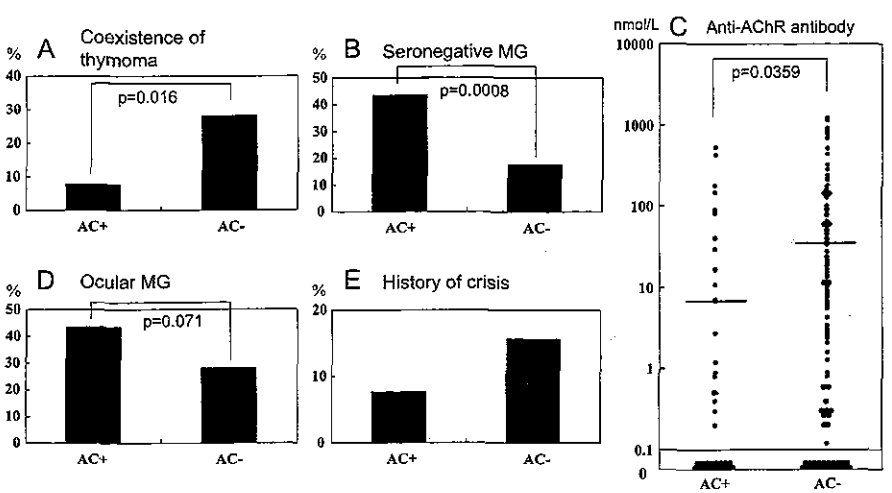

Fig. 2. Comparison of clinical features between MG with AC and MG without AC. (A) Percentage of thymoma associated MG was significantly higher in AC negative cases. (B) Percentage of seronegative MG was significantly higher in AC positive patients. (C) Anti-AChR antibody titer was significantly higher in AC negative patients. Bar indicates median value of each group excluding seronegative cases. (D) Patients without AC had a higher rate of ocular MG. (E) Patients without AC had a higher incidence rate of myasthenic crisis.

seropositive MG patients (p=0.0008). These findings would be difficult to explain if MG patients' concerns about medical problems were the primary cause. Rather, we consider that it reflects some biological features of seronegative non-thymomatous MG patients.

Seronegative MG is frequently encountered in ocular MG cases. As shown in this study, the frequency of ocular MG in the MG patients with AC tended to be higher when compared to the MG cases without AC. This may in part explain the low incidence of crisis in MG patients with AC. The lower rate of thymoma-related MG in the AC+ group also appears to have contributed to a favorable course, since thymoma-related MG was shown to have more severe generalized symptoms than non-thymomatous MG [20]. The rate of seronegative MG in the present study was 23.8%, which is nearly identical to the rate of seronegative MG reported in a nationwide epidemiological survey carried out in Japan in 1987 (23.9% of 963 cases) [21], but somewhat higher than the reported rate of seronegative MG in Caucasians (10–20%) [1,22,23]. Although the reason for such a racial difference of seronegative MG frequency remains to be elucidated, an association of AC with seronegative MG may have some relevance since it was reported that Asians tend to develop atopic tendency more often than Caucasians when living in the same environmental conditions [24].

There are no previous studies that have investigating the association of allergic disorders with MG. In this study, among various allergic disorders, only an increase in AC was statistically significant, yet all other allergic disorders investigated tended to be higher in MG patients than in controls. One possible mechanism for the reasons behind the association of AC with MG is that as ocular manifestations including orbicularis oculi weaknesses are common in MG patients, the conjunctivae may be heavily exposed to such allergens as pollens and house dust mites which could then trigger AC. The increased rate of ocular MG in the AC+ group supports this notion. However, since AC preceded MG in 44% of the MG with AC cases, local exposure to environmental allergens cannot be the sole reason for this association.

It is interesting to note that AC is associated only with MG without thymoma, and not with MG with thymoma. In hyperplastic thymus cases, numerous germinal centers are seen, where CD23 as well as bcl-2 are found to be over-expressed [3,25,26]. These molecules play important roles in preventing germinal center B cells from entering apoptosis, which thereby results in autoantibody production. In allergic disorders, aberrant lymphocyte development in the thymus is considered to play an important role in the occurrence of atopic dermatitis, in which the suppression of lymphocyte apoptosis also leads to the development of allergic reactions [27,28]. Therefore, the second hypothesis for an association between MG and AC is that MG without thymoma and AC may have common disease backgrounds originating from thymic alterations, distinct from that of MG with thymoma lacking germinal centers. This notion is also supported by observations that the frequencies of other allergic disorders also tended to be higher in MG without thymoma than in the controls. Moreover, CD23, a low affinity receptor for IgE, is profoundly related to allergic disorders. The soluble form of CD23 is elevated in allergic diseases as well as in MG [6–10], which also supports the common immunological aberrancy between MG and certain allergic diseases. The production of autoantibodies in thymoma-related MG is likely to be independent from the germinal center, and have an alternative pathway.

To summarize, it was found that there is a significant association of AC with certain forms of MG in cases without thymoma. However, this study should be considered a preliminary one, and an epidemiological survey accompanied by laboratory testing for allergies will be required in the future to clarify such an association. Nonetheless, MG may predispose patients to AC through the heavy exposure of allergens to the conjunctivae due to orbicularis oculi weaknesses, in which case special care in respect to air-borne allergens appears warranted.

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Effect of immunotherapy in myelitis with atopic diathesis

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Abstract

Objective: A recent nationwide survey of myelitis with atopic diathesis in Japan disclosed that the disease frequently shows a chronic persistent course. A neuropathological study of the spinal cord also revealed chronic active inflammation. Since the effects of various immunotherapies have not been studied extensively in this condition, we evaluated the efficacies of various immunotherapies in patients with myelitis with atopic diathesis.

Patients and methods: Forty-two treatments in 26 patients with myelitis with atopic diathesis were retrospectively analyzed. One of the following therapies was administered: (1) corticosteroids (CS) (pulse therapy followed by oral administration with gradual tapering); (2) intravenous immunoglobulin (IVIG) (400 mg/kg/day for 5 consecutive days); (3) plasma exchanges (PE); or (4) PE followed by IVIG or CS (PE+IVIG/CS). The therapeutic efficacies were evaluated by thorough neurological examination and laboratory tests including MRI, somatosensory evoked potentials (SEPs) and motor evoked potentials (MEPs).

Results: Objective neurological findings improved in 89% of the PE group and 90% of the PE+IVIG/CS group, compared with only 72% of the CS and 60% of the IVIG groups. Improvement determined by laboratory tests was seen in 57% of the PE and 57% of the PE+IVIG/CS groups, compared with only 15% of the CS and none of the IVIG groups. Thus, the improvement rate determined by laboratory tests was significantly greater for therapies including PE than for those without PE ($p=0.0187$).

Conclusions: These data suggest that immunotherapy is effective in myelitis with atopic diathesis despite a chronic persistent course, and that PE is the most beneficial immunotherapy.

Keywords: Myelitis; Atopy; Treatment; Corticosteroid; Plasma exchange; IVIG

1. Introduction

We have previously reported the occurrence of myelitis with atopic diathesis in Japanese patients [1–3]. Following our reports, several similar cases have been reported in the Japanese literature [4–6], and a recent nationwide survey disclosed the widespread occurrence of myelitis with atopic diathesis (atopic myelitis) throughout Japan [7].

Atopy is generally considered to be a helper type 2 (Th2) disease, in which type 2 cytokines, such as IL-4, IL-5, IL-6 and IL-13, play critical roles in the induction of inflammation [8]. Pathological studies on biopsied specimens of the spinal cord from patients with atopic myelitis revealed eosinophil infiltration as well as deposition of activated eosinophil products, such as eosinophil cationic protein (ECP), in the lesions [9,10]. Moreover, amounts of IgE in the cerebrospinal fluid (CSF) were found to be increased in

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this condition [7]. These findings suggest that an inflammatory mechanism driven by Th2 cells may be operative, even in the central nervous system (CNS), similar to other atopic disorders.

Clinical studies on myelitis with atopic diathesis have revealed that it frequently shows a chronic persistent course, and that MRI lesions also tend to persist for years despite their small size [1–3,7,10]. These clinical and neuroimaging features as well as neuropathological findings suggest that the mechanism for myelitis with atopic diathesis is possibly distinct from the autoimmune mechanism proposed for multiple sclerosis (MS). Thus, the response to various immunotherapies in this condition may also be distinct from those in MS. Since there are no reports that extensively examine the effects of immunotherapy in this condition, we retrospectively evaluated the treatment responses in patients with atopic myelitis by neurological and laboratory examinations.

2. Patients and methods

2.1. Patients

Myelitis with atopic diathesis (atopic myelitis) was defined as myelitis of unknown cause with either (1) hyperIgEemia and allergen-specific IgE positivity against common allergens, such as *Dermatophagoides pteronyssinus* (*D. pteronyssinus*), *Dermatophagoides farinae* (*D. farinae*) and cedar pollen, or (2) coexistent atopic diseases, as described previously [7]. Atopic disorders such as atopic dermatitis, bronchial asthma, allergic rhinitis and food allergies, which involve atopic mechanisms, were regarded as coexistent atopic diseases [11]. Cases showing multiple brain white matter lesions which met McDonald's diagnostic criteria for multiple sclerosis [12] were excluded from the study.

Twenty-six patients with atopic myelitis who had been admitted to the Department of Neurology at Kyushu University Hospital from 1992 to 2003 were enrolled in this study. The study was approved by the Institutional Review Board of Kyushu University Hospital. The demographic features of the patients are summarized in Table 1. The patients consisted of 13 males and 13 females (1:1). The mean age at treatment was 36.6±11.2 years (mean± S.D.) (range, 15–58), and the mean duration of the disease at treatment was 25.8±26.8 months (mean±S.D.) (range, 1–132). The mode of onset and disease course before any treatment of the 26 patients were: acute and persistent in 7 (27.0%), acute and fluctuating in 6 (23.1%), acute and step-wise progression in 4 (15.4%), subacute and fluctuating in 2 (7.7%), subacute and step-wise progression in 1 (3.8%) and slowly progressive in 6 (23.1%).

Atopic disorders were present in 80.8% of patients: allergic rhinitis in 10/26 (38.5%), atopic dermatitis in 9/26 (34.6%), bronchial asthma in 4/26 (15.4%) and a food allergy in 5/26 (19.2%). Eosinophilia was found in 11/26 patients (42.3%) and hyperIgEemia in 19/26 (73.1%) at the time of the initial treatment. Twenty-four of the twenty six patients (92.3%) had specific IgE against *D. pteronyssinus*, 22/26 (84.6%) against *D. farinae* and 18/26 (69.2%) against cedar pollen. CSF examination revealed cell counts of 1.0±1.3/μl (mean±S.D.) (range, 0–15/μl) and a total protein level of 35.6±17.9 mg/dl (mean±S.D.) (range, 16–106 mg/dl). CSF pleocytosis (>5/μl) was found in 6/26 patients (23.1%), and an increased protein level (>40 mg/dl) was found in 8/26 (30.8%).

2.2. Treatments

One of the following treatments was administered after informed consent was obtained from each patient, and the efficacies of the treatments were evaluated by clinical examinations as well as laboratory tests, and analyzed retrospectively: (1) corticosteroids (CS); (2) intravenous immunoglobulin (IVIG); (3) plasma exchanges (PE); or (4) PE followed by either IVIG or CS (PE+IVIG/CS). CS were administered by intravenous injection of methylprednisolone 1000 mg/day for 3 days followed by oral prednisolone (initial dose of 60 mg/day) with gradual tapering. Patients assigned to IVIG treatment received intravenous infusions of gammaglobulin (Kenketsu Glovenin-I-Nichiyaku, Takeda Chemical Industries) at a dose of 400 mg/kg/day for 5 consecutive days. PE was performed three times using Spectra (COBE) at 2- to 3-day intervals, and the entire procedure was carried out in a closed circuit. Briefly, the patient's blood was obtained from a forearm vein and delivered to a single-stage channel where centrifugation separated the blood into plasma and blood cells. The blood cells were then returned to the patient's vein together with a replacement solution consisting of 2.3% albumin. Plasma (40 ml/kg) was replaced in each procedure. In the patients who received PE+IVIG or PE+CS treatment, either IVIG or CS was performed 1–3 weeks after the PEs had been completed. The procedures for each treatment were the same as described above.

2.3. Evaluation of treatments

The clinical effects of the treatments were evaluated objectively by neurological examination by expert neurologists before and 2–4 weeks after each treatment. Improvement in any neurological sign was considered to be an indication of effectiveness. Kurtzke's expanded disability status scale score (EDSS) [13] and the Scripps neurological rating scale (NRS) [14] were also evaluated. MRI examinations including a dual-echo, proton density, T2-weighted spin-echo series (TR=2000 ms, TE=90 ms) and T1-weighted spin-echo series (TR=600 ms, TE=20 ms) with and without gadolinium enhancement were performed before and after the treatments as described previously [3], and the treatment effects were evaluated by expert neuroradiologists without knowledge of the treatment modality.

Table 1
Demographic features of atopic myelitis patients

Case	Age	Sex	Atopic diathesis									SL	CSF		Tx	Improvement	
			AD	BA	AR	FA	Eo	IgE	DP	DF	CP		Cell	Prot		NE	Lab
1	25	F	–	–	+	–	–	+	+	+	+	MT	1	21	PE	+	+
2	21	M	–	+	–	–	+	+	+	+	–	C5	2	25	CS	+	–
3	58	F	–	–	+	–	+	+	+	+	+	C1	1	16	CS	+	Wor
4	43	M	–	+	–	+	–	–	+	+	+	C5	4	51	PE	+	–
5	32	M	+	–	–	–	–	–	+	+	+	L1	0	43	PE	+	–
6	33	F	+	–	+	–	–	+	+	–	+	C5	0	26	PE+IVIG	+	–
7	27	M	–	–	+	+	+	+	+	+	–	C3	2	20	CS	+	–
	28											C3	15	56	PE+IVIG	+	–
	30											UT	ND	ND	IVIG	–	ND
	31											UT	ND	ND	PE+IVIG	+	–
	32											LT	0	70	PE+CS	–	+
8	38	F	+	–	–	–	+	+	+	+	+	C6	1	25	PE	–	+
9	48	F	–	–	+	+	–	–	–	–	+	C6	ND	ND	CS	+	–
10	48	M	–	–	+	–	+	+	+	+	–	C6	1	106	CS	+	–
11	24	F	+	–	–	+[a]	–	–	–	–	–	C5	3	17	CS	+	–
12	22	F	–	+	–	–	–	–	+	+	+	L5	3	52	CS	–	ND
	23											L5	1	52	IVIG	–	–
13	31	M	+	–	–	–	+	–	+	+	+	C5	0	20	CS	+	–
14	48	M	+	–	–	–	+	+	+	+	+	LT	5	24	IVIG	+	–
	48											LT	1	24	PE	+	+
	46											MT	0	29	CS	+	+
15	50	F	+	–	–	–	–	+	+	+	+	C6	1	31	CS	+	ND
16	51	M	–	+	+	–	–	+	+	+	–	C3	ND	ND	IVIG	+	–
	51											C3	1	28	PE	+	–
17	34	F	–	–	+	–	+	+	+	+	+	LT	10	35	CS	+	ND
	37											MT	1	27	PE+IVIG	+	–
	39											MT	0	38	PE+IVIG	+	ND
18	54	F	–	–	+	–	–	+	+	+	+	C4	2	35	PE+IVIG	–	–
	54											C4	9	49	PE+CS	+	+
19	41	F	–	–	+	+	–	–	+	+	+	C3	ND	ND	PE	+	Wor
	41											C3	5	26	CS	–	–
20	18	F	+	–	–	–	+	+	+	+	+	C4	8	19	CS	+	–
21	35	F	+	–	–	–	–	+	+	+	–	C6	9	25	PE+CS	–	+
22	37	M	–	–	–	–	+	+	+	+	+	C3	5	30	CS	+	ND
	40											C3	ND	ND	PE	+	ND
23	42	M	–	–	–	–	+	+	+	+	+	C6	1	53	CS	+	+
	43											C6	12	39	PE	+	+
	44											C6	ND	ND	IVIG	+	–
24	33	M	–	–	–	–	–	+	+	+	+	UT	3	29	CS	–	–
25	15	M	–	–	–	–	–	+	+	–	–	C5	1	46	CS	–	–
	16											C5	0	32	PE+IVIG	+	+
26	25	M	–	–	–	–	–	+	+	+	–	LT	1	26	CS	+	ND

Age=age at treatment; AD=atopic dermatitis; BA=bronchial asthma; AR=allergic rhinitis; FA=food allergy; Eo=eosinophilia (>4%); IgE=cases of hyperIgEemia in which total IgE exceeded the normal limit (*N*: <240 IU/ml); DP=*Dermatophagoides pteronyssinus*-specific IgE (N: <0.34 IU/ml); DF=*Dermatophagoides farinae*-specific IgE (*N*: <0.34 IU/ml); CP=cedar pollen-specific IgE (*N*: <0.34 IU/ml); SL=clinically estimated level of the spinal cord lesion: C=cervical, UT=upper thoracic, MT=middle thoracic, LT=lower thoracic, L=lumbar; CSF=cerebrospinal fluid: Cell=number of cells (/μl), Prot=protein concentration (mg/dl); Tx=treatment: CS=corticosteroids, IVIG=intravenous immunoglobulin, PE=plasma exchanges; Improvement=improvement detected in each patient: NE=improvement detected by neurological examination, Lab=improvement supported by MRI and/or electrophysiological studies, +=improved, –=unchanged, ND=not done, Wor=worsened.

[a] This patient was allergic to egg white and positive for allergen-specific IgE against egg white protein.

Motor evoked potentials (MEPs) with transcranial magnetic stimulation were recorded as previously described [15]. The upper limb MEPs were recorded from the abductor pollicis brevis muscle. To stimulate the hand motor area, the center of the eight-shaped coil was placed over a point 2 cm anterior to either C3 or C4 (International 10–20 System). To stimulate the cervical root, the center of the coil was placed on the posterior neck over the 7th cervical spinous process. The lower limb MEPs were recorded from the abductor hallucis muscle. To stimulate the leg motor area, the coil was positioned over the vertex. Magnetic stimulation to the lumbar root was performed by placing the center of the coil over the 4th lumbar spinous process. Somatosensory evoked potentials (SEPs) were analyzed following electric stimulation of the median nerve and tibial nerve [15]. The peak amplitudes of the far-field

P14 and the cortical N20, and the peak-to-peak amplitude of P25, P30 and N35 were measured. The latency of each peak was also measured. On follow-up studies, the evoked potentials were considered to be improved when unevoked potentials became evoked or when a delayed peak shifted forward to fall within the normal limits.

2.4. Statistical analysis

Wilcoxon signed-ranks test was applied to determine the statistical significance of the changes in the EDSS and NRS scores, the percentage of peripheral eosinophils, and the amounts of total IgE and allergen-specific IgE against *D. pteronyssinus*, *D. farinae* and cedar pollen. Kruskal–Wallis rank test was used for comparison of the EDSS and NRS scores and the disease duration among the four treatment groups. Chi-square test, or Fisher's exact test when the Chi-square test was not fulfilled, was used for statistical analyses of comparison of improvement rates by treatment.

3. Results

3.1. Pretreatment status

The 26 patients received a total of 42 treatments. Disease duration in terms of months at the time of treatment was significantly different among the four treatment groups (CS: 19.2±30.8; IVIG: 25.4±11.9; PE: 20.9±16.4; and PE+IVIG/CS: 42.5±27.5; p=0.0374). Of the 42 events, 40 (95.2%) showed sensory impairment, such as hypesthesia, hypalgesia and a decrease in vibration sense, and 36 (85.7%) had a motor impairment, such as muscle weakness, hyperreflexia and pathological reflexes on neurological examination (details are shown in Table 2). Five patients (11.9%) were diagnosed as having sphincter disturbance by uroflowmetry analysis. The pretreatment EDSS scores were 3.54±0.98 (mean±S.D.) (range, 2.0–7.5), and these differed significantly among the four treatment groups (p=0.0031) (Table 3). The pretreatment NRS scores were 83.12±8.20 (mean±S.D.) (range, 66–94), and these also differed significantly among the four groups (p=0.0151). Clinically estimated main lesions existed in the cervical spinal cord in 27/42 cases (64.3%), in the thoracic spinal cord in 12/42 (28.6%) and in the lumbar spinal cord in 3/42 (7.1%).

On T2-weighted MRI images (T2WI), localized spinal cord lesions of high signal intensity were detected in 22/40 pretreatment scans (55.0%). Gadolinium-enhancement of the lesions was observed in 8 of these 22 pretreatment lesions (36.4%). Cervical spinal cord lesions were most frequently observed among the spinal cord lesions on MRI (18/22, 81.8%), while 8/22 (36.4%) were in the thoracic spinal cord and 0/22 were in the lumbar spinal cord. Abnormalities in MEPs were disclosed in 8/35 cases

Table 2
Effect of each treatment on neurological symptoms and signs

	CS		IVIG		PE		PE+IVIG/CS		Total	
	Pre	Imp	Pre	Imp	Pre	Imp	Pre	Imp	Pre	Imp
Sensory										
Hypesthesia	17	12	4	2	6	6	10	5	37	25
Hypalgesia	5	2	1	0	3	2	3	1	12	5
Decrease in vibration sense	12	7	3	1	4	2	9	3	28	13
Decrease in position sense	2	2	0	0	0	0	2	0	4	2
Pseudoathetosis	0	0	0	0	0	0	0	0	0	0
Girdle sensation	3	2	1	0	1	1	3	2	8	5
Lhermitte sign	2	1	0	0	0	0	0	0	2	1
Sensory total	17	13	5	3	8	7	10	6	40	28
(% of improvement)		76.5		60.0		78.5		60.0		70.0
Motor										
Muscle weakness	13	9	3	1	6	4	10	8	32	22
Muscle atrophy	0	0	0	0	0	0	0	0	0	0
Spasticity	5	2	0	0	1	0	3	0	9	2
Hyperreflexia	8	0	2	0	4	1	6	2	20	3
Hyporeflexia	1	1	0	0	0	0	0	0	1	1
Pathological reflex	6	1	1	1	2	0	4	0	13	2
Gait	5	3	0	0	0	0	7	3	12	6
Motor total	14	9	3	1	6	4	9	8	36	25
(% of improvement)		64.3		33.3		66.7		88.9		69.4
Autonomic										
Sphincter disturbance	2	2	0	0	0	0	3	2	5	4
Autonomic total	2	2	0	0	0	0	3	2	5	4
(% of improvement)		100.0		–		–		66.7		80.0
Total	18	13	5	3	9	8	10	9	42	33
(% of improvement)		72.2		60.0		88.9		90.0		78.6

CS=corticosteroids; IVIG=intravenous immunoglobulin; PE=plasma exchanges. Pre=number of patients who had the symptoms/signs before treatment. Imp=number of patients whose symptoms/signs improved after treatment. *UE=upper extremities*; LE=lower extremities.

Table 3
Effect of each treatment on EDSS and NRS

	CS		IVIG		PE		PE+IVIG/CS		Total	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
EDSS										
Mean	3.44	3.19	3.40	3.40	2.83	2.56	4.40	4.00	3.54	3.27
S.D.	0.55	0.62	0.55	0.55	0.56	0.58	1.43	1.43	0.98	0.98
Rate of improvement (%)	6/18 (33.3)		0/5 (0.0)		3/9 (33.3)		5/10 (50.0)		14/42 (33.3)	
p	0.0235		NA		>0.1		0.0384		0.0007	
NRS										
Mean	85.72	89.50	83.20	84.40	86.22	91.44	75.60	81.30	83.12	87.36
S.D.	7.47	5.24	11.35	12.40	4.74	3.36	6.15	6.57	8.20	7.33
Rate of improvement	12/18 (66.7)		3/5 (60.0)		8/9 (88.9)		10/10 (100.0)		33/42 (78.6)	
p	0.0023		>0.1		0.0115		0.005		<0.0001	

CS=corticosteroids; IVIG=intravenous immunoglobulin; PE=plasma exchanges. Pre=pre-treatment; Post=post-treatment. S.D.=Standard deviation; NA=not applicable.

(22.9%) in the upper limbs and 10/36 (27.8%) in the lower limbs. Abnormal findings in SEPs were observed in 5/33 cases (15.2%) in the upper limbs and 17/32 (53.1%) in the lower limbs.

3.2. Effects of treatments on the neurological symptoms and signs

3.2.1. Overall effects of treatments

Overall improvement seen by neurological examination was demonstrated in 33/42 treatments (78.6%) (Table 2). Among the neurological signs, sensory signs improved in 28/40 treatments (70.0%), motor signs in 25/36 (69.4%) and autonomic signs in 4/5 (80.0%). EDSS scores improved in 14/42 treatments (33.3%) (Table 3). The pretreatment EDSS scores of 3.54 ± 0.98 were significantly reduced to 3.27 ± 0.98 after the treatments ($p=0.0007$). NRS scores improved in 33/42 treatments (78.6%) (Table 3). The pretreatment NRS scores were 83.12 ± 8.20 and they significantly increased to 87.36 ± 7.33 after the treatments ($p<0.0001$).

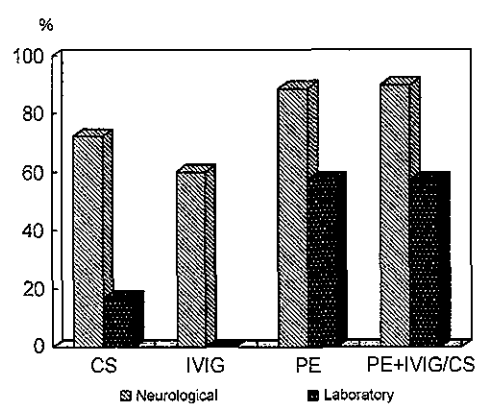

Fig. 1. The efficacy of each treatment for atopic myelitis. The rates of improvement evaluated by neurological examination and laboratory tests are shown. The number of events (treatments) for the neurological examination and laboratory tests were 18 and 13 for CS, 5 and 3 for IVIG, 9 and 7 for PE and 10 and 7 for PE+IVIG/CS, respectively.

3.2.2. Effects of each treatment

Effects of each treatment are summarized in Tables 2 and 3. EDSS score did not change in the IVIG group, but improved significantly in the CS and in PE+IVIG/CS groups. EDSS improvement did not reach statistical significance in just the PE group. NRS scores improved in

Table 4
Overall effect of treatment on MRI and evoked potential findings

MRI changes		
T2-weighted Images (T2WI)		
Disappearance of T2-high intensity lesion	1/13	(7.7%)
Reduction of T2-high intensity lesion	3/13	(23.1%)
Increase of area on T2WI	1/13	(7.7%)
Newly appeared area on T2WI	0	
Gd-enhanced T1-weighted images		
Disappearance of Gd-enhanced lesion	2/5	(40.0%)
Reduction of Gd-enhanced lesion	1/5	(20.0%)
Newly appeared Gd-enhanced lesion	1	
Overall effects		
Improved	6/13	(46.2%)
Unchanged	5/13	(38.5%)
Worsened	2/13	(15.4%)
Evoked potential changes		
MEPs upper limbs		
Improved	2/6	(33.3%)
Unchanged	4/6	(66.7%)
Worsened	0/6	(0.0%)
MEPs lower limbs		
Improved	1/7	(14.3%)
Unchanged	6/7	(85.7%)
Worsened	0/7	(0.0%)
SEPs upper limbs		
Improved	0/2	(0.0%)
Unchanged	2/2	(100.0%)
Worsened	0/2	(0.0%)
SEPs lower limbs		
Improved	3/5	(60.0%)
Unchanged	2/5	(40.0%)
Worsened	0/5	(0.0%)
Overall effects		
Improved	6/8	(75.0%)
Unchanged	2/8	(25.0%)
Worsened	0/8	(0.0%)

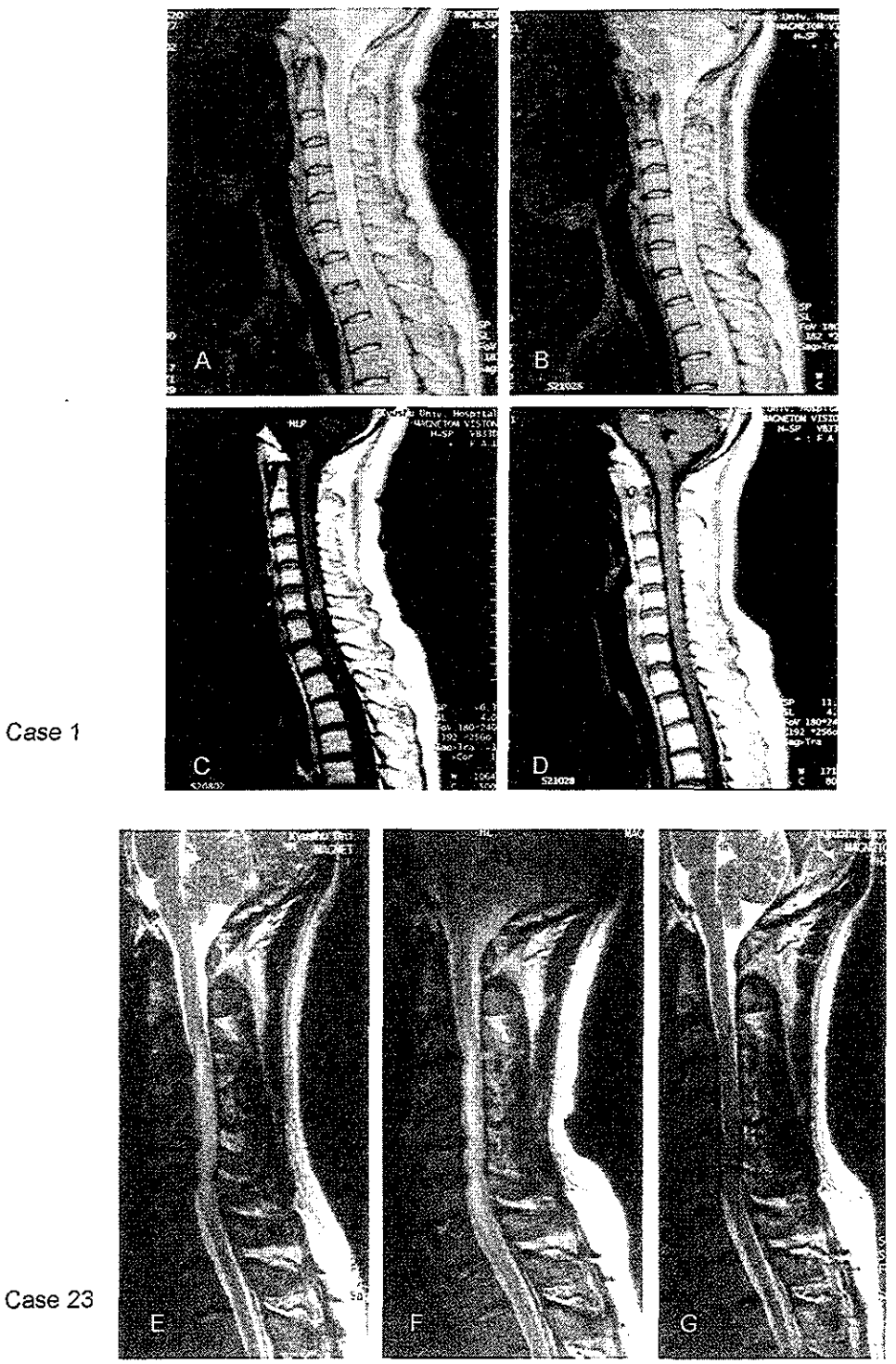

Fig. 2. Representative cases showing improvement on MRI. Case 1: (A) T2-weighted MRI before PE; (B) T2-weighted MRI after PE; (C) T1-weighted MRI with gadolinium enhancement before PE; and (D) T1-weighted MRI with gadolinium enhancement after PE. A T2-high intensity lesion is located at the C6 spine level and is enhanced by gadolinium. After the PE treatment, the enhancement has disappeared although the T2-high intensity lesion is unchanged. Case 23: (E) T2-weighted MRI before CS; (F) T2-weighted MRI after CS and before PE; and (G) T2-weighted MRI after PE. A T2-high lesion is seen at the C3-7 spine levels. After the treatment with CS, the lesion is not improved, but it disappears after performing PE.

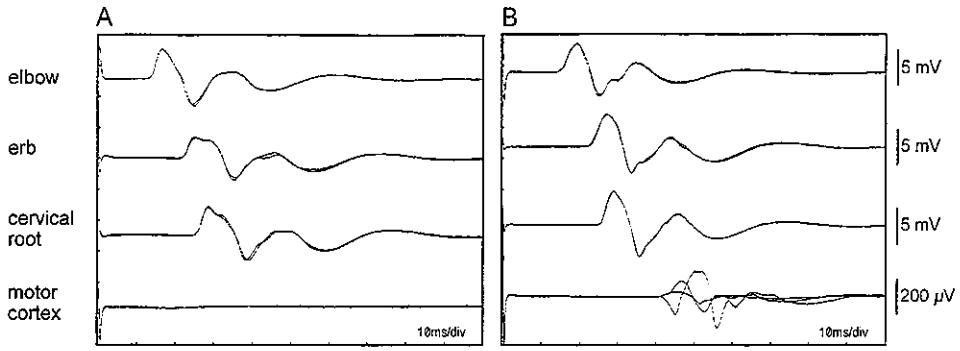

Fig. 3. A representative case showing improvement in the MEP of the left upper limb (Case 8). (A) Before PE treatment; and (B) After PE treatment. The MEP of the motor cortex stimulation that was not evoked before treatment becomes evoked after performing PE.

all four treatment groups but the improvement did not reach the statistical significance in the IVIG group. In summary, objective neurological findings improved in 88.9% of the PE and 90.0% of the PE+IVIG/CS groups, compared with only 72.2% of the CS and 60.0% of the IVIG groups (Fig. 1). The improvement rates on neurological examination and in the EDSS scores were higher for treatments including PE (PE and PE+IVIG/CS groups) than for those without PE (CS and IVIG groups) (17/19, 89.5% vs. 16/23, 69.6%, p=0.118 on neurological findings; 8/19, 42.1% vs. 6/23, 26.1%, p=0.273 in EDSS scores; and 18/19, 94.7% vs. 15/23, 65.2% p=0.0203 in NRS scores). The EDSS scores for treatments including PE (PE group and PE+IVIG/CS group) were significantly reduced from 3.66±0.13 to 3.32±0.13 (p=0.0094), and the NRS scores were significantly elevated from 83.12±8.20 to 87.36±7.33 (p<0.0001).

3.3. Effects of treatments on laboratory findings

3.3.1. Effects of treatments on spinal cord MRI findings

High signal intensity lesions on T2-weighted MRI disappeared in 1/13 treatments (7.7%) and the size was reduced in 3/13 (23.1%) (Table 4). In one treatment (5.9%), T2-weighted MRI revealed an increase in the high signal intensity area. On gadolinium-enhanced T1-weighted images, gadolinium-enhanced lesions disappeared in 2/5 treatments (40.0%) and the lesion size was reduced in 1/5 (25.0%). In one case whose pre-treatment MRI lesion was not enhanced by gadolinium, an enhanced lesion newly appeared after the treatment. Representative cases in which MRI improvements were observed are shown in Fig. 2. Overall, improvement after immunotherapies was observed on MRI in 6/13 (46.2%).

3.3.2. Effects of treatments on the evoked potentials

Electrophysiologically, improvements in MEPs were observed in 2/6 treatments (33.3%) for the upper limbs and 1/7 (14.3%) for the lower limbs (Table 4). Three of five treatments (60%) showed improvements in SEPs for the lower limbs, compared with 0/2 for the upper limbs. A representative case showing MEP improvement is shown in Fig. 3. Overall, improvement in the evoked potentials after immunotherapies was seen in 6/8 (75%).

3.3.3. Effects of each treatment on laboratory examinations (MRI and/or EPs)

Improvements supported by laboratory examinations were seen in 4/7 (57.1%) of the PE group (of these 7 events, neurological improvement was seen in 6) and 4/7 (57.1%) of the PE+IVIG/CS group (of these 7 events, neurological improvement was seen in 6), compared with only 2/13 (15.4%) of the CS group (of these 13 events, neurological improvement was seen in 11) and 0/3 of the IVIG group (of these 3 events, neurological improvement was seen in 2).

Table 5
Effect of each treatment on blood tests

	CS		IVIG		PE		PE+IVIG/CS		Total	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Eosonophil (%)	5.98	1.76	3.17	3.27	3.6	2.99	5.39	4.48	5.04	2.85
p	0.0052		NA		>0.1		>0.1		0.049	
Total IgE (IU/ml)	2058	1024	229	230	2063	2011	833	1202	1355	1115
p	>0.1		NA		>0.1		>0.1		>0.1	
D. pteronyssinus IgE (IU/ml)	53.4	46.3	22.6	21.4	36.4	35.7	45.2	48.0	42.7	43.7
p	>0.1		NA		>0.1		>0.1		>0.1	
D. farinae IgE (IU/ml)	40.7	36.5	20.1	20.7	35.6	35.9	40.3	43.9	38.4	38.9
p	>0.1		NA		>0.1		>0.1		>0.1	
Cedar pollen IgE (IU/ml)	9.6	9.1	<0.34	<0.34	4.8	4.1	29.4	28.0	17.2	16.3
p	>0.1		NA		>0.1		>0.1		>0.1	

CS=corticosteroids; IVIG=intravenous immunoglobulin; PE=plasma exchanges. Pre=pre-treatment; Post=post-treatment. NA=not applicable.