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.

Acknowledgements

We thank Ms. Y. Yoshimura, Department of Neurology, Graduate School of Medicine, Kyushu University for technical support, and Ms. N. Kinukawa, Department of Medical Information Science, Kyushu University Hospital for help with the statistical analysis. This work was supported in part by grants from the Ministry of Education, Science, Sports and Culture of Japan, a Neuroimmunological Disease Research Committee and from the Ministry of Health and Welfare of Japan for Research on Brain Science.

References

Bell, J.I., Lathrop, G.M., 1996. Multiple loci for multiple sclerosis. Nat. Genet. 13, 377–378.

Bell, R., Collier, D.A., Rice, S.Q.J., Roberts, G.W., MacPhee, C.H., Kerwin, R.W., Price, J., Gloger, I.S., 1997. Systematic screening of the LDL-PLA2 gene for polymorphic variants and case-control analysis in schizophrenia. Biochem. Biophys. Res. Commun. 241, 630–635.

Callea, L., Arese, M., Orlandini, A., Bargnani, C., Priori, A., Bussolino, F., 1999. Platelet activating factor is elevated in cerebral spinal fluid and plasma of patients with relapsing–remitting multiple sclerosis. J. Neuroimmunol. 94, 212–221.

Chataway, J., Feakes, R., Coraddu, F., Gray, J., Deans, J., Fraser, M., Robertson, N., Broadley, S., Jones, H., Clayton, D., Goodfellow, P., Sawcer, S., Compston, A., 1998. The genetics of multiple sclerosis: principles, background and updated results of the United Kingdom systematic genome screen. Brain 121, 1869–1887.

Chihara, J., Maruyama, I., Yasuba, H., Yasukawa, A., Yamamoto, T., Kurachi, D., Mouri, T., Seguchi, M., Nakajima, S., 1992. Possible induction of intracellular adhesion molecule-1 (ICAM-1) expression on endothelial cells by platelet-activating factor (PAF). J. Lipid. Mediat. 5, 159–162.

Confavreux, C., Vukusic, S., Moreau, T., Adeleine, P., 2000. Relapses and progression of disability in multiple sclerosis. N. Engl. J. Med. 343, 1430–1438.

Fukazawa, T., Yabe, I., Kikuchi, S., Sasaki, H., Hamada, T., Miyasaka, K., Tashiro, K., 1999. Association of vitamin D receptor gene polymorphism with multiple sclerosis in Japanese. J. Neurol. Sci. 166, 47–52.

Fukazawa, T., Yamasaki, K., Ito, H., Kikuchi, S., Minohara, M., Horiuchi, I., Tsukishima, E., Sasaki, H., Hamada, T., Nishimura, Y., Tashiro, K., Kira, J., 2000. Both the HLA-DPB1 and -DRB1 alleles correlate with risk for multiple sclerosis in Japanese: clinical phenotypes and gender as important factors. Tissue Antigens 55, 199–205.

Hiramoto, M., Yoshida, H., Imaizumi, T., Yoshimizu, N., Satoh, K., 1997. A mutation in plasma platelet-activating factor acetylhydrolase (Val $^{279} \rightarrow$ Phe) is a genetic risk factor for stroke. Stroke 28, 2417–2420.

Ichihara, S., Yamada, Y., Yokota, M., 1998. Association of a $G^{994} \rightarrow T$ missense mutation in the plasma platelet-activating factor acetylhydrolase gene with genetic susceptibility to nonfamilial dilated cardiomyopathy in Japanese. Circulation 98, 1881–1885.

Ikuta, F., Koga, M., Takeda, S., Ohama, E., Takeshita, I., Ogawa, H., Wang, M., 1982. Comparison of MS pathology between 70 American

and 75 Japanese autopsy cases. In: Kuroiwa, Y., Kurland, L.T. (Eds.), Multiple Sclerosis East and West. Kyushu University Press, Fukuoka, pp. 297–306.

Ito, H., Yamasaki, K., Kawano, Y., Horiuchi, I., Yun, C., Nishimura, Y., Kira, J., 1998. HLA-DP-associated susceptibility to the optico-spinal form of multiple sclerosis in the Japanese. Tissue Antigens 52, 179–182.

Kikuchi, S., Fukazawa, T., Niino, M., Yabe, I., Miyagishi, R., Hamada, T., Tashiro, K., 2002. Estrogen receptor gene polymorphism and multiple sclerosis in Japanese patients: interaction with HLA-DRB1*1501 and disease modulation. J. Neuroimmunol. 128, 77–81.

Kira, J., 2003. Multiple sclerosis in the Japanese population. Lancet Neurol. 2, 117–127.

Kira, J., Kanai, T., Nishimura, Y., Yamasaki, K., Matsushita, S., Kawano, Y., Hasuo, K., Tobimatsu, S., Kobayashi, T., 1996. Western versus Asian types of multiple sclerosis: immunogenetically and clinically distinct disorders. Ann. Neurol. 40, 569–574.

Kosaka, T., Yamaguchi, M., Soda, Y., Kishimoto, T., Tago, A., Toyosato, M., Mizuno, K., 2000. Spectrophotometric assay for serum platelet-activating factor acetylhydrolase activity. Clin. Chim. Acta 296, 151–161.

Kosaka, T., Yamaguchi, M., Miyanaga, K., Mizuno, K., 2001. Serum platelet-activating factor acetylhydrolase (PAF-AH) activity in more than 3000 healthy Japanese. Clin. Chim. Acta 312, 179–183.

Kruse, S., Mao, X., Heinzmann, A., Blattmann, S., Roberts, M.H., Braun, S., Gao, P., Forster, J., Keuhr, J., Hopkin, J.M., Shirakawa, T., Deichmann, K.A., 2000. The Ile198Thr and Ala379Val variants of plasmatic PAF-acetylhydrolase impair catalytical activities and are associated with atopy and asthma. Am. J. Hum. Genet. 66, 1522–1530.

Kurtzke, J.F., 1983. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 33, 1444–1452.

Lock, C., Hermans, G., Pedotti, R., Brendolan, A., Schadt, E., Garren, H., Langer-Gould, A., Strober, S., Cannella, B., Allard, J., Klonowski, P., Austin, A., Lad, N., Kaminski, N., Galli, S.J., Oksenberg, J.R., Raine, C.S., Heller, R., Steinman, L., 2002. Gene-microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. Nat. Med. 8, 500–508.

Lublin, F.D., Reingold, S.C., 1996. Defining the clinical course of multiple sclerosis: results of an international survey. Neurology 46, 907–911.

Lucchinetti, C.F., Mandler, R.N., McGavern, D., Bruck, W., Gleich, G., Ransohoff, R.M., Trebst, C., Weinshenker, B., Wingerchuk, D., Parisi, J.E., Lassmann, H., 2002. A role for humoral mechanisms in the pathogenesis of Devic's neuromyelitis optica. Brain 125, 1450–1461.

Ma, J.J., Nishimura, M., Mine, H., Saji, H., Ohta, M., Saida, K., Ozawa, K., Kawakami, H., Saida, T., Uchiyama, T., 1998. HLA-DRB1 and tumor necrosis factor gene polymorphisms in Japanese patients with multiple sclerosis. J. Neuroimmunol. 92, 109–112.

Martin-Mondiere, C., Charron, D., Spinnewyn, B., Braquet, P., 1987. MHC in central nervous system: modulation of expression by PAF-acether. Prostaglandins 34, 163.

McDonald, W.I., Compston, A., Edan, G., Goodkin, D., Hartung, H.P., Lublin, F.D., McFarland, H.F., Paty, D.W., Polman, C.H., Reingold, S.C., Sandberg-Wollheim, M., Sibley, W., Thompson, A., van den Noort, S., Weinshenker, B.Y., Wolinsky, J.S., 2001. Recommended diagnostic criteria for multiple sclerosis: guidelines from the international panel on the diagnosis of multiple sclerosis. Ann. Neurol. 50, 121–127.

Miwa, M., Miyake, T., Yamanaka, T., Sugatani, J., Suzuki, Y., Sakata, S., Araki, Y., Matsumoto, M., 1988. Characterization of serum platelet-activating factor (PAF) acetylhydrolase. Correlation between deficiency of serum PAF acetylhydrolase and respiratory symptoms in asthmatic children. J. Clin. Invest. 82, 1983–1991.

Niino, M., Fukazawa, T., Yabe, I., Kikuchi, S., Sasaki, H., Tashiro, K., 2000. Vitamin D receptor gene polymorphism in multiple sclerosis and the association with HLA class II alleles. J. Neurol. Sci. 177, 65–71.

Niino, M., Kikuchi, S., Fukazawa, T., Yabe, I., Tashiro, K., 2003. Genetic polymorphisms of osteopontin in association with multiple sclerosis in Japanese patients. J. Neuroimmunol. 136, 125–129.

O'Flaherty, J.T., Wykle, R.L., Miller, C.H., Lewis, J.C., Waite, M., Bass, D.A., McCall, C.E., DeChatelet, L.R., 1981. 1-*O*-alkyl-*sn*-glyceryl-3-phosphorylcholines. A novel class of neutrophil stimulants. Am. J. Pathol. 103, 70–79.

Shibasaki, H., McDonald, W.I., Kuroiwa, Y., 1981. Racial modification of clinical picture of multiple sclerosis: comparison between British and Japanese patients. J. Neurol. Sci. 49, 253–271.

Stafforini, D.M., Satoh, K., Atkinson, D.L., Tjoelker, L.W., Eberhardt, C., Yoshida, H., Imaizumi, T., Takamatsu, S., Zimmerman, G.A., Mclntyre, T.M., Gray, P.W., Prescott, S.M., 1996. Platelet-activating factor acetylhydrolase deficiency. A missense mutation near the active site of an anti-inflammatory phospholipase. J. Clin. Invest. 97, 2784–2791.

Stafforini, D.M., Numao, T., Tsodikov, A., Vaitkus, D., Fukuda, T., Watanabe, N., Fueki, N., McIntyre, T.M., Zimmerman, G.A., Makino, S., Prescott, S.M., 1999. Deficiency of platelet-activating factor acetylhydrolase is a severity factor for asthma. J. Clin. Invest. 103, 989–997.

Tabira, T., Tateishi, J., 1982. Neuropathological features of MS in Japan. In: Kuroiwa, Y., Kurland, L.T. (Eds.), Multiple Sclerosis East and West. Kyushu University Press, Fukuoka, pp. 273–295.

Tanaka, R., Iijima, K., Xu, H., Inoue, Y., Murakami, R., Shirakawa, T., Nishiyama, K., Miwa, M., Shiozawa, S., Nakamura, H., Yoshikawa, N., 1999. Role of platelet-activating factor acetylhydrolase gene mutation in Japanese childhood IgA nephropathy. Am. J. Kidney Dis. 34, 289–295.

Tetta, C., Bussolino, F., Modena, V., Montrucchio, G., Segoloni, G., Pescarmona, G., Camussi, G., 1990. Release of platelet-activating factor in systemic lupus erythematosus. Int. Arch. Allergy Appl. Immunol. 91, 244–256.

Wardlaw, A.J., Moqbel, R., Cromwell, O., Kay, A.B., 1986. Platelet-activating factor. A potent chemotactic and chemokinetic factor for human eosinophils. J. Clin. Invest. 78, 1701–1706.

Yamada, Y., Ichihara, S., Fujimura, T., Yokota, M., 1998. Identification of the $G^{994} \rightarrow T$ missense mutation in exon 9 of the plasma platelet-activating factor acetylhydrolase gene as an independent risk factor for coronary artery disease in Japanese men. Metabolism 47, 177–181.

Yamasaki, K., Horiuchi, I., Minohara, M., Kawano, Y., Ohyagi, Y., Yamada, T., Mihara, F., Ito, H., Nishimura, Y., Kira, J., 1999. HLA-DPB1*0501-associated opticospinal multiple sclerosis. Clinical, neuroimaging and immunogenetic studies. Brain 122, 1689–1696.

Original Paper

International Archives of Allergy and Immunology

Int Arch Allergy Immunol 2004;134:41–48
DOI: 10.1159/000077532

Received: September 2, 2003
Accepted after revision: January 26, 2004
Published online: March 25, 2004

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-

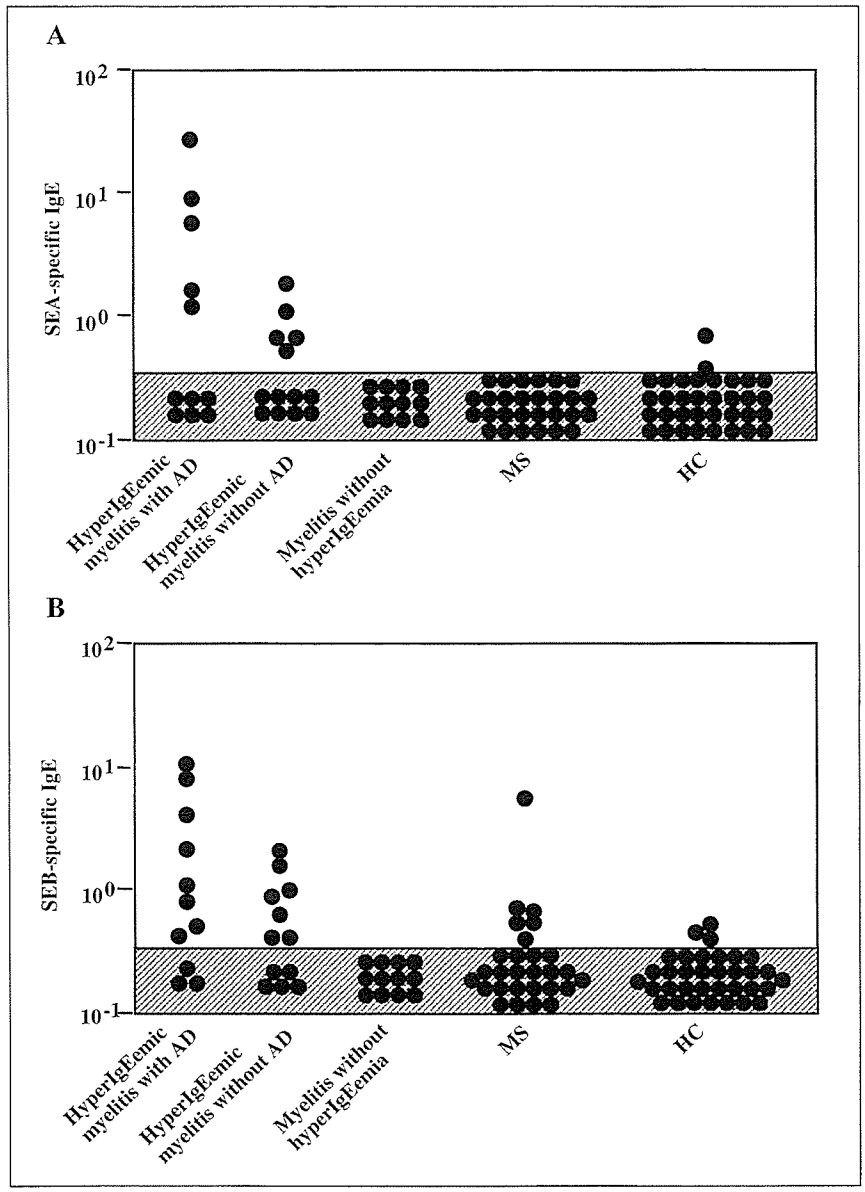

Fig. 1. Distributions of serum SEA- (**A**) and SEB-specific IgE levels (**B**). HC = Healthy controls. The optimal cutoff level for the definition of serum SEA- and SEB-specific IgE positivity was 0.34 IU/ml.

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).

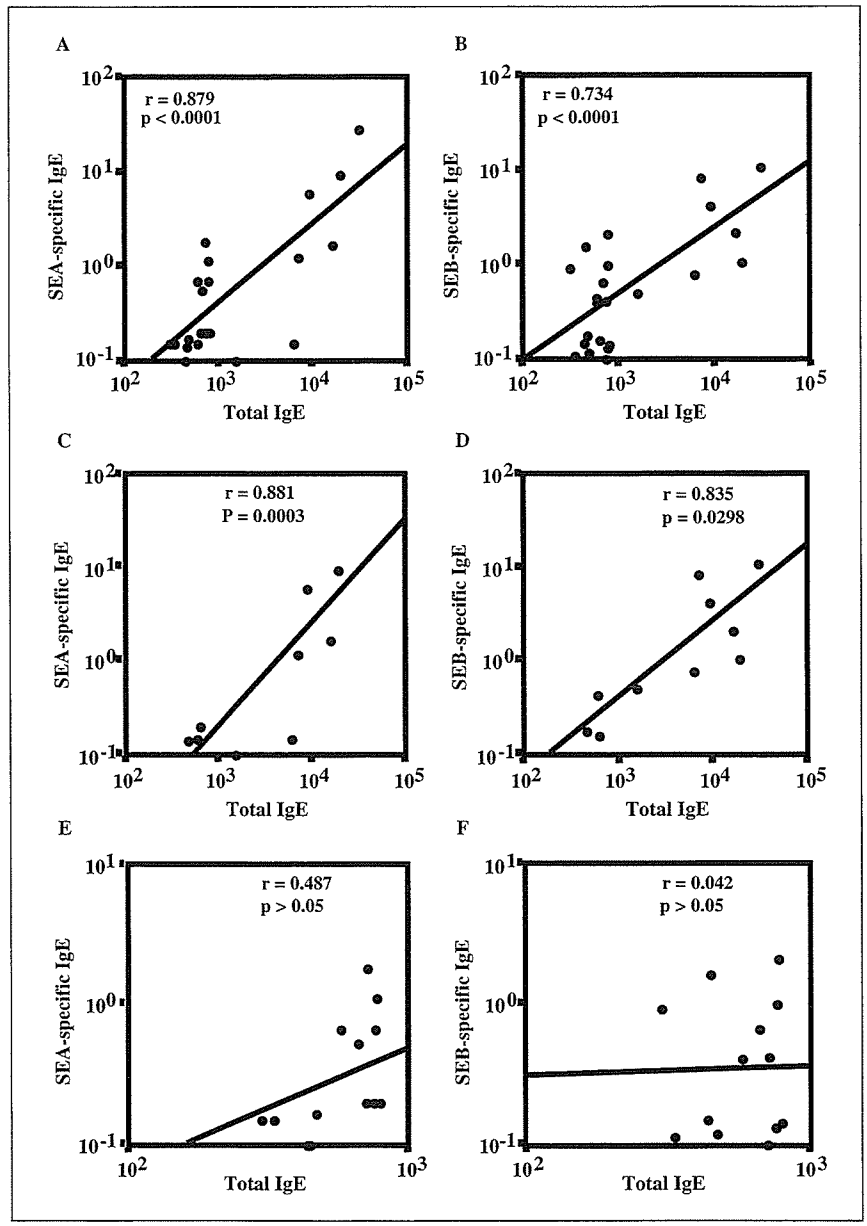

Fig. 2. Scatter plot correlating to total and SEA/SEB-specific IgE levels in all patients with hyperIgEemic myelitis (**A**, **B**), those with hyperIgEemic myelitis and AD (**C**, **D**) and those with hyperIgEemic myelitis but without AD (**E**, **F**). The correlation coefficient was determined by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

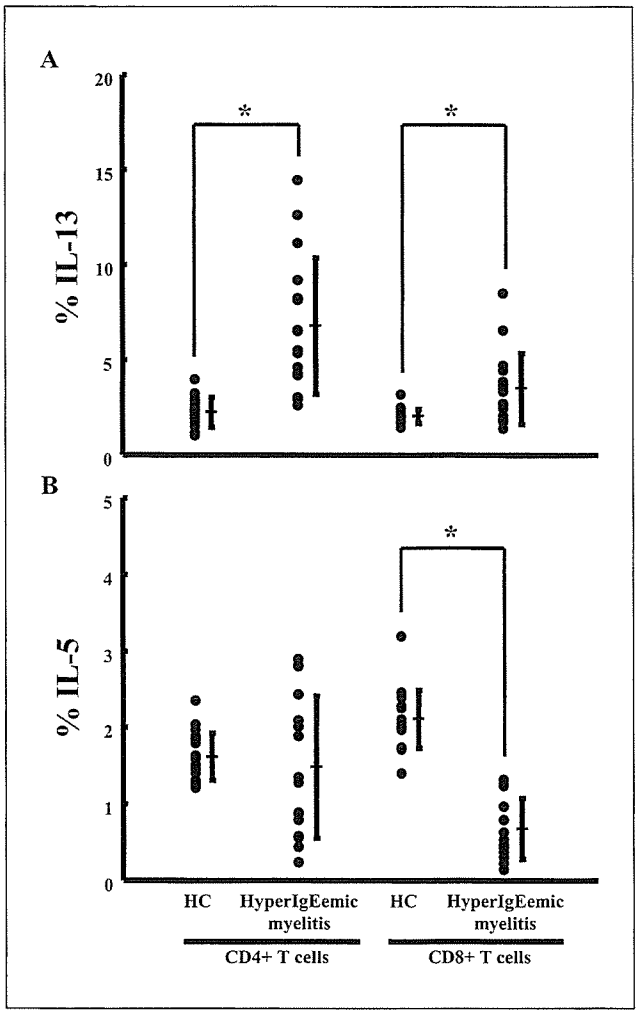

Fig. 3. Intracellular production of IL-13 (**A**) and IL-5 (**B**) in peripheral blood CD4+ T cells and CD8+ T cells. HC = Healthy controls.

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. $2.31 \pm 0.77\%$ in CD4+ T cells, $p < 0.0001$; 3.55 ± 1.85 vs. $2.11 \pm 0.38\%$ 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. $2.11 \pm 0.38\%$, $p < 0.0001$), but they did not differ significantly between the two groups in CD4+ T cells (1.49 ± 0.92 vs. $1.62 \pm 0.31\%$, $p > 0.1$).

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.

Acknowledgments

This work was supported in part by a Neuroimmunological Disease Research Committee grant and a Research on Brain Science grant from the Ministry of Health and Welfare, Japan, and Grants-in-Aid 12470142, 12557060 and 12877097 from the Ministry of Education, Science, Sports and Culture, Japan.

References

1 Murray DL, Ohlendorf DH, Schlievert PM: Staphylococcal and streptococcal superantigens: Their role in human diseases. ASM News 1995;61:229–235.

2 Brocke S, Gaur A, Piercy C, Gautam A, Gijbels K, Fathman CG, Steinman L: Induction of relapsing paralysis in experimental autoimmune encephalomyelitis by bacterial superantigens. Nature 1993;365:642–644.

3 Schiffenbauer J, Johnson HM, Butfiloski EJ, Wegrzyn L, Soos JM: Staphylococcal enterotoxins can reactivate experimental allergic encephalomyelitis. Proc Natl Acad Sci USA 1993;90:8543–8546.

4 Soos JM, Mujtaba MG, Schiffenbauer J, Torres BA, Johnson HM: Intramolecular epitope spreading induced by staphylococcal enterotoxin superantigen reactivation of experimental allergic encephalomyelitis. J Neuroimmunol 2002;123:30–34.

5 Zhang J, Vandevyver C, Stinissen P, Mertens N, Van den Berg-Loonen E, Raus J: Activation and clonal expansion of human myelin basic protein-reactive T cells by bacterial superantigens. J Autoimmun 1995;8:615–632.

6 Hermans G, Stinissen P, Hauben L, Van den Berg-Loonen E, Raus J, Zhang J: Cytokine profile of myelin basic protein-reactive T cells in multiple sclerosis and healthy individuals. Ann Neurol 1997;42:18–27.

7 Jorens PG, VanderBorght A, Ceulemans B, Van Bever HP, Bossaert LL, Ieven M, Goossens H, Parizel PM, Van Dijk H, Raus J, Stinissen P: Encephalomyelitis-associated antimyelin autoreactivity induced by streptococcal exotoxins. Neurology 2000;54:1433–1441.

8 Michie CA, Davis T: Atopic dermatitis and staphylococcal superantigens. Lancet 1996;347: 324.

9 Strange P, Skov L, Lisby S, Nielsen PL, Baadsgaard O: Staphylococcal enterotoxin B applied on intact normal and intact atopic skin induces dermatitis. Arch Dermatol 1996;132:27–33.

10 Lin YT, Yang H, Hwang YW, Tsai MJ, Tsao PN, Chiang BL: Comparison of serum specific IgE antibodies to staphylococcal enterotoxins between atopic children with and without atopic dermatitis. Allergy 2000;55:641–646.

11 Leung DY, Harbeck R, Bina P, Reiser RF, Yang E, Norris DA, Hanifin JM, Sampson HA: Presence of IgE antibodies to staphylococcal enterotoxins on the skin of patients with atopic dermatitis: Evidence for a new group of allergens. J Clin Invest 1993;92:1374–1380.

12 Kira J, Yamasaki K, Kawano Y, Kobayashi T: Acute myelitis associated with hyperIgEemia and atopic dermatitis. J Neurol Sci 1997;148: 199–203.

13 Kira J, Kawano Y, Yamasaki K, Tobimatsu S: Acute myelitis with hyperIgEemia and mite antigen-specific IgE: Atopic myelitis. J Neurol Neurosurg Psychiatry 1998;64:676–679.

14 Kira J, Kawano Y, Horiuchi I, Yamada T, Imayama S, Furue M, Yamasaki K: Clinical, immunological and MRI features of myelitis with atopic dermatitis (atopic myelitis). J Neurol Sci 1999;162:56–61.

15 Osoegawa M, Ochi H, Minohara M, Murai H, Umehara F, Furuya H, Yamada T, Kira J: Myelitis with atopic diathesis: A nationwide survey of 79 cases in Japan. J Neurol Sci 2003; 209:5–11.

16 Kikuchi H, Osoegawa M, Ochi H, Murai H, Horiuchi I, Takahashi H, Yamabe K, Iwaki T, Mizutani T, Oda M, Kira J: Spinal cord lesions of myelitis with hyperIgEemia and mite antigen specific IgE (atopic myelitis) manifest eosinophilic inflammation. J Neurol Sci 2001; 183:73–78.

17 Osoegawa M, Ochi H, Kikuchi H, Shirabe S, Nagashima T, Tsumoto T, Tamura Y, Yamabe K, Takahashi H, Iwaki T, Kira J: Eosinophilic myelitis associated with atopic diathesis: A combined neuroimaging and histopathological study. Acta Neuropathol (Berl) 2003;105:289–295.

18 Bunikowski R, Mielke MEA, Skarabis H, Worm M, Anagnostopoulos I, Kolde G, Wahn U, Renz H: Evidence for a disease-promoting effect of *Staphylococcus aureus*-derived exotoxins in atopic dermatitis. J Allergy Clin Immunol 2000;105:814–819.

19 Skov L, Olsen JV, Giorno R, Schlievert PM, Baadsgaard O, Leung DYM: Application of Staphylococcal enterotoxin B on normal and atopic skin induces up-regulation of T cells by a superantigen-mediated mechanism. J Allergy Clin Immunol 2000;105:820–826.

20 Poser CM, Paty DW, Scheinberg L, McDonald WI, Davis FA, Ebers GC, Johnson KP, Sibley WA, Silberberg DH, Tourtellotte WW: New diagnostic criteria for multiple sclerosis. Ann Neurol 1983;13:227–231.

21 MacSharry C, McKay IC, Lewis C: Methods for quantifying allergen-specific IgE antibodies: Solid-phase and liquid-phase techniques. J Clin Immunoassay 1993;16:153–158.

22 Ochi H, Osoegawa M, Wu XM, Minohara M, Horiuchi I, Murai H, Furuya H, Kira J: Increased IL-13 but not IL-5 production by CD4-positive T cells and CD8-positive T cells in multiple sclerosis during relapse phase. J Neurol Sci 2002;201:45–51.

23 Akdis M, Simon H-U, Weigl L, Kreyden O, Blaser K, Akdis CA: Skin homing (cutaneous lymphocyte-associated antigen-positive) CD8+ T cells respond to superantigen and contribute to eosinophilia and IgE production in atopic dermatitis. J Immunol 1999;163:466–475.

24 Wakugawa M, Hayashi K, Nakamura K, Tamaki K: Evaluation of mite allergen-induced Th1 and Th2 cytokine secretion of peripheral blood mononuclear cells from atopic dermatitis patients: Association between IL-13 and mite-specific IgE levels. J Dermatol Sci 2001; 25:116–126.

25 Aleksza M, Lukács A, Antal-Szalmás P, Hunyadi J, Szegedi A: Increased frequency of intracellular interleukin (IL)-13 and IL-10, but not IL-4, expressing CD4+ and CD8+ peripheral T cells of patients with atopic dermatitis. Br J Dermatol 2002;147:1135–1141.

26 Zimmermann N, Hershey GK, Foster PS, Rothenberg ME: Chemokines in asthma: Cooperative interaction between chemokines and IL-13. J Allergy Clin Immunol 2003;111:227–242.

27 Sironi M, Sciacca FL, Matteucci C, Conni M, Vecchi A, Bernasconi S, Minty A, Caput D, Ferrara P, Colotta F, Mantovani A: Regulation of endothelial and mesothelial cell function by interleukin-13: Selective induction of vascular cell adhesion molecule-1 and amplification of interleukin-6 production. Blood 1994;84: 1913–1921.

28 Bochner BS, Klunk DA, Sterbinsky SA, Coffman RL, Schleimer RP: IL-13 selectively induces vascular cell adhesion molecule-1 expression in human endothelial cells. J Immunol 1995;154:799–803.

29 Taskapan MO, Kumar P: Role of staphylococcal superantigens in atopic dermatitis: From colonization to inflammation. Ann Allergy Asthma Immunol 2000;84:3–10.

30 Desreumaux P, Capron M: Eosinophils in allergic reactions. Curr Opin Immunol 1996;8: 790–795.

31 Denburg JA: Microenvironmental influences on inflammatory cell differentiation. Allergy 1995;50(suppl 25):25–28.

32 Abernathy-Carver KJ, Sampson HA, Picker LJ, Leung DY: Milk-induced eczema is associated with the expansion of T cells expressing cutaneous lymphocyte antigen. J Clin Invest 1995;95:913–918.

33 Reekers R, Busche M, Wittmann M, Kapp A, Werfel T: Birch pollen-related foods trigger atopic dermatitis in patients with specific cutaneous T cell responses to birch pollen antigens. J Allergy Clin Immunol 1999;104:466–472.

34 Beyer K, Castro R, Feidel C, Sampson HA: Milk-induced urticaria is associated with the expansion of T cells expressing cutaneous lymphocyte antigen. J Allergy Clin Immunol 2002; 109:688–693.

35 David TJ, Cambridge G: Bacterial infection and atopic eczema. Arch Dis Child 1986;61: 20–23.

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Journal of the Neurological Sciences 222 (2004) 65–73

Journal of the Neurological Sciences

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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

Received 3 October 2003; received in revised form 9 April 2004; accepted 12 April 2004
Available online 1 June 2004

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).

0022-510X/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jns.2004.04.012

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 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.

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.

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).

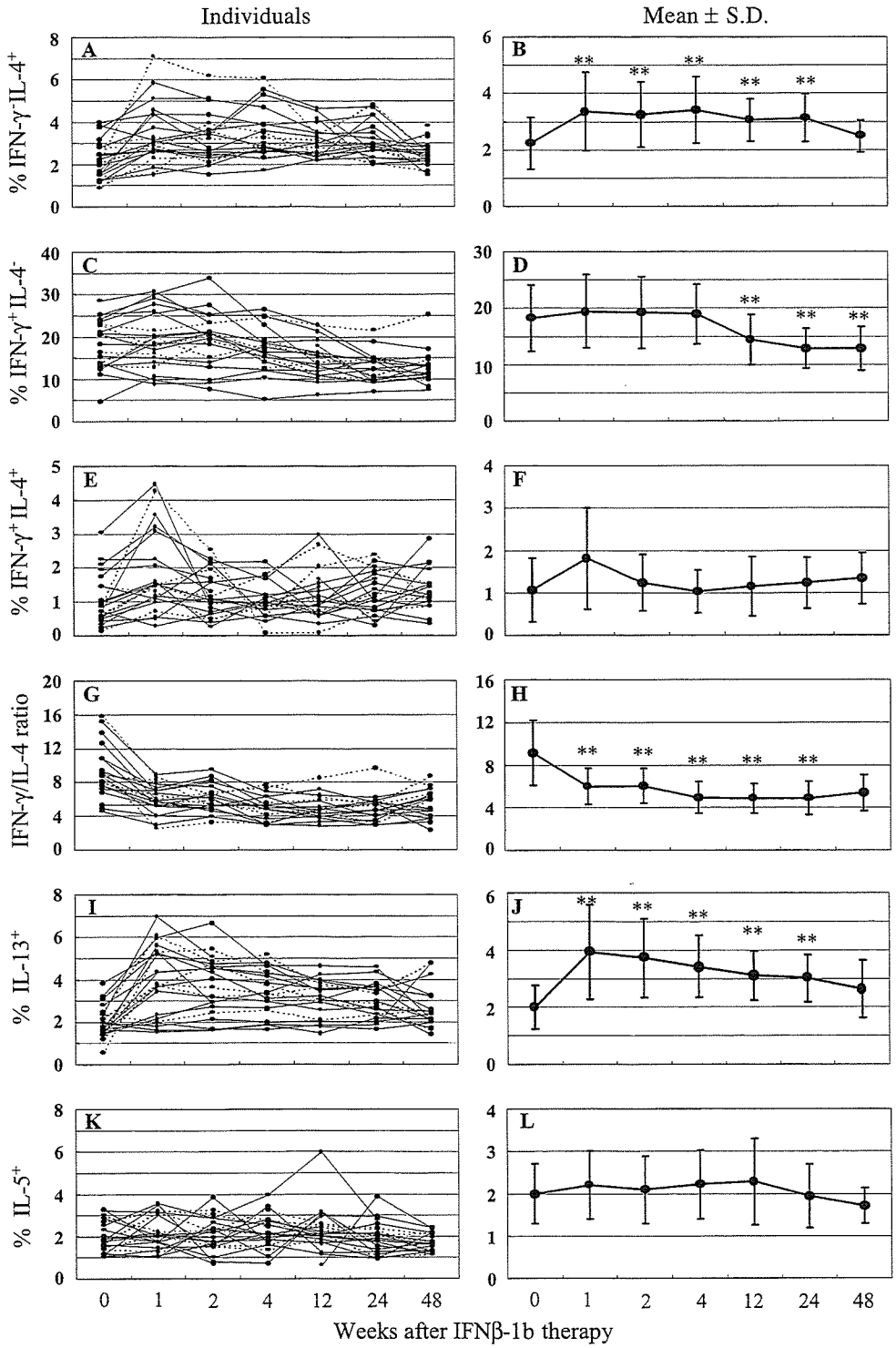

Fig. 1. Changes in the percentage of intracellular cytokine-producing $CD4^+$ T cells. (A, B) IFN-γ^- IL-4^+; (C, D) IFN-γ^+ IL-4^-; (E, F) IFN-γ^+ IL-4^+; (G, H) IFN-γ/IL-4 ratio; (I, J) IL-13^+; (K, L) IL-5^+. (A, C, E, G, I, K) Changes in individual patients. Data obtained from the same patient at 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. $^{**}p < 0.01$.

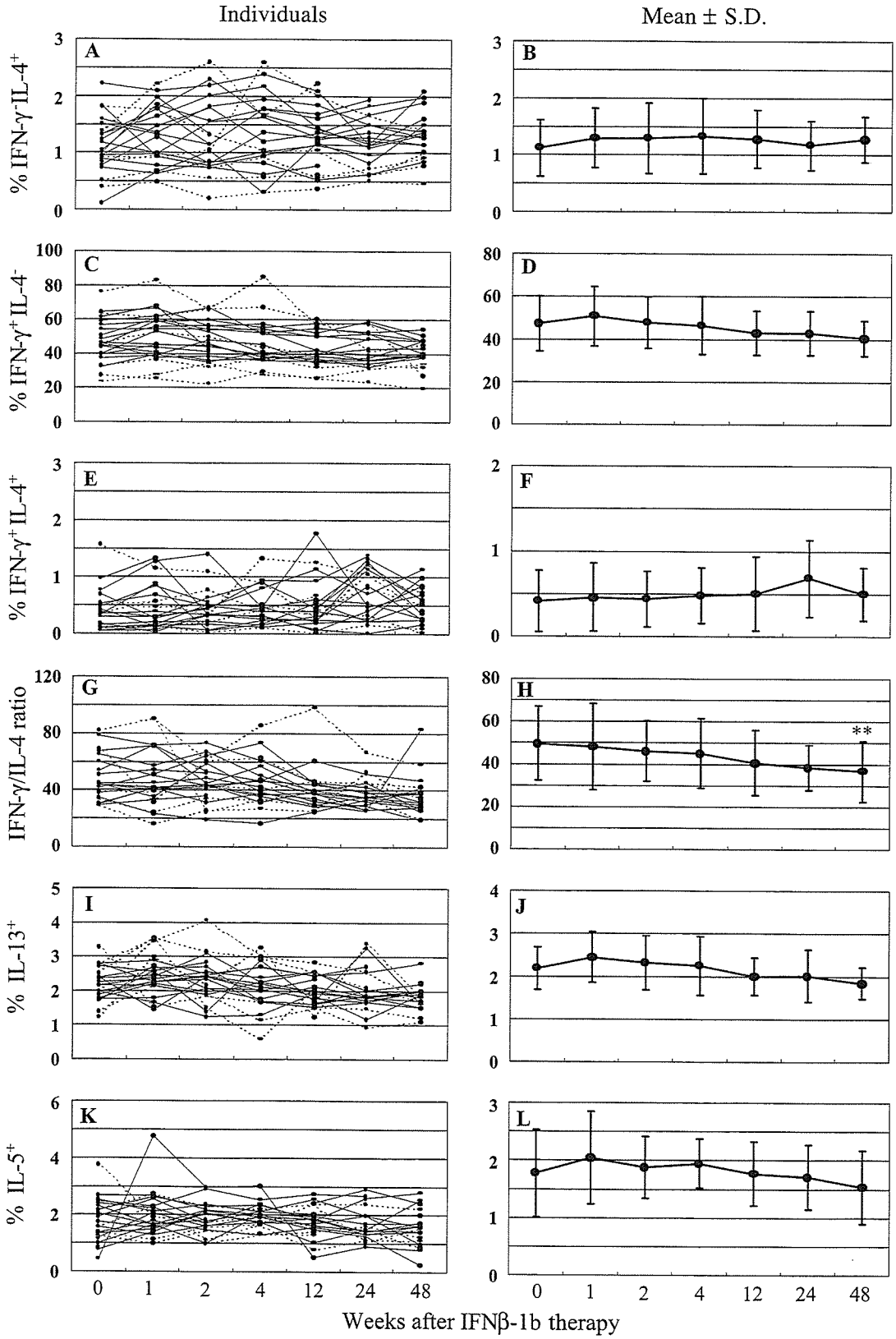

Fig. 2. Changes in the percentage of intracellular cytokine-producing $CD8^+$ T cells. (A, B) IFN-γ^- IL-4^+; (C, D) IFN-γ^+ IL-4^-; (E, F) IFN-γ^+ IL-4^+; (G, H) IFN-γ/IL-4 ratio; (I, J) IL-13^+; (K, L) IL-5^+. (A, C, E, G, I, K) Changes in individual patients. Solid or dashed lines are used as in Fig.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

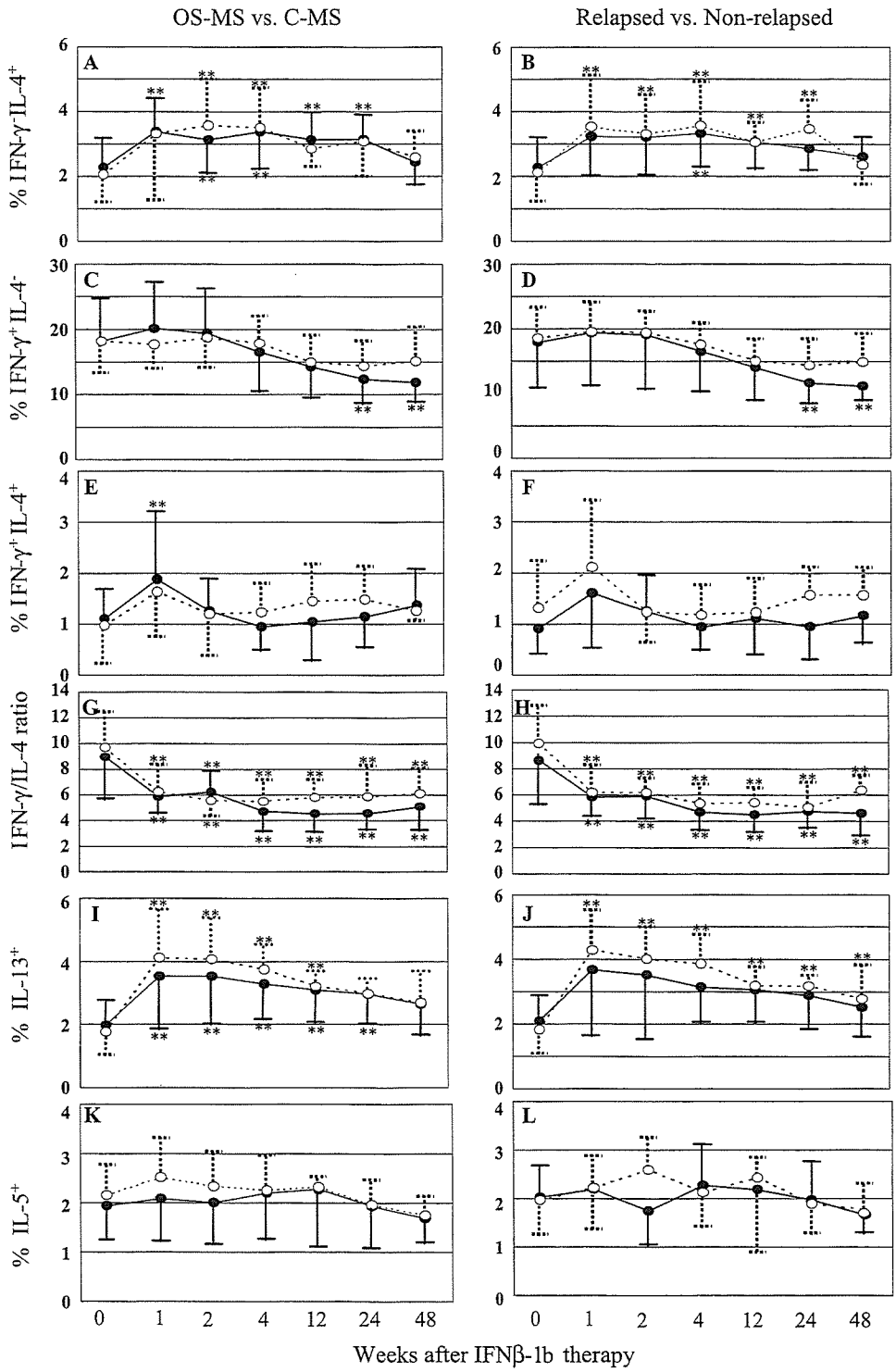

Fig. 3. Comparisons of intracellular cytokine expression patterns between patients with OS-MS (dashed line, open circle, $n=6$) and patients with C-MS (solid line, closed circle, $n=16$) (left column), and between MS patients with (dashed line, open circle, $n=9$) and without (solid line, closed circle, $n=13$) 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 IFNγ/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.

Acknowledgements

This study was supported in part by a Neuroimmunological Disease Research Committee grant and a Research on Brain Science grant from the Ministry of Health and Welfare, Japan, and Grants-in-Aid 12470142, 12557060 and 12877097 from the Ministry of Education, Science, Sports and Culture, Japan.

References

[1] Steinman L, Miller A, Bernard CC, Oksenberg JR. The epigenetics of multiple sclerosis: clues to etiology and a rationale for immune therapy. Annu Rev Neurosci 1994;17:247–65.
[2] Martino G, Hartung HP. Immunopathogenesis of multiple sclerosis: the role of T cells. Curr Opin Neurol 1999;12:309–21.
[3] Yong VW, Chabot S, Stuve O, Williams G. Interferon beta in the treatment of multiple sclerosis: mechanisms of action. Neurology 1998;51:682–9.
[4] Dayal AS, Jensen MA, Lledo A, Arnason BGW. Interferon-gamma-secreting cells in multiple sclerosis patients treated with interferon beta-1b. Neurology 1995;45:2173–7.
[5] Khan OA, Hebel JR. Incidence of exacerbations in the first 90 days of treatment with recombinant human interferon beta-1b in patients with relapsing-remitting multiple sclerosis. Ann Neurol 1998;44:138–9.
[6] Furlan R, Bergami A, Lang R, Brambilla E, Franciotta D, Martinelli V, et al. Interferon-β treatment in multiple sclerosis patients decreases the number of circulating T cells producing interferon-gamma and interleukin-4. J Neuroimmunol 2000;111:86–92.
[7] Karp CL, van Boxel-Dezaire AH, Byrnes AA, Nagelkerken L. Interferon-β in multiple sclerosis: altering the balance of interleukin-12 and interleukin-10? Curr Opin Neurol 2001;14:361–8.
[8] Kira J, Kanai T, Nishimura Y, Yamasaki K, Matsushita S, Kawano Y, et al. Western versus Asian types of multiple sclerosis: immunogenetically and clinically distinct disorders. Ann Neurol 1996;40: 569–74.
[9] Yamasaki K, Horiuchi I, Minohara M, Kawano Y, Ohyagi Y, Yamada T, et al. HLA-DPB1*0501-associated opticospinal multiple sclerosis: clinical, neuroimaging and immunogenetic studies. Brain 1999;122: 1689–96.
[10] McDonald WI, Compston A, Edan G, Goodkin D, Hartung HP, Lublin FD, et al. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. Ann Neurol 2001;50:121–7.
[11] Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 1983;33: 1444–52.
[12] Ochi H, Wu XM, Osoegawa M, Horiuchi I, Minohara M, Murai H, et al. Tc1/Tc2 and Th1/Th2 balance in Asian and Western types of multiple sclerosis, HTLV-I-associated myelopathy/tropical spastic paraparesis and hyperIgEaemic myelitis. J Neuroimmunol 2001; 119:297–305.
[13] Wandinger KP, Sturzebecher CS, Bielekova B, Detore G, Rosenwald A, Staudt LM, et al. Complex immunomodulatory effects of interferon-β in multiple sclerosis include the upregulation of T helper 1-associated marker genes. Ann Neurol 2001;50:349–57.
[14] Gniadek P, Aktas O, Wandinger K-P, Bellmann-Strobl J, Wengert O, Weber A, et al. Systemic IFN-β treatment induces apoptosis of peripheral immune cells in MS patients. J Neuroimmunol 2003;137: 187–96.
[15] Ahn J, Feng X, Patel N, Dhawan N, Reder AT. Abnormal levels of interferon-gamma receptors in active multiple sclerosis are normalized by IFN-beta therapy: implications for control of apoptosis. Front Biosci 2004;9:1547–55.
[16] Neumann H, Medana IM, Bauer J, Lassmann H. Cytotoxic T lymphocytes in autoimmune and degenerative CNS diseases. Trends Neurosci 2002;25:313–9.
[17] Ochi H, Osoegawa M, Wu XM, Minohara M, Horiuchi I, Murai H, et al. Increased IL-13 but not IL-5 production by CD4-positive T cells and CD8-positive T cells in multiple sclerosis during relapse phase. J Neurol Sci 2002;201:45–51.
[18] Genain CP, Abel K, Belmar N, Villinger F, Rosenberg DP, Linington C, et al. Late complications of immune deviation therapy in a nonhuman primate. Science 1996;274:2054–7.
[19] Lafaille JJ, van de Keere F, Hsu AL, Baron JL, Haas W, Raine CS, et al. Myelin basic protein-specific T helper 2 (Th2) cells cause experimental autoimmune encephalomyelitis in immunodeficient hosts rather than protect them from the disease. J Exp Med 1997; 186:307–12.
[20] de Vries JE. Molecular and biological characteristics of interleukin-13. Chem Immunol 1996;63:204–18.
[21] de la Barrera S, Finiasz M, Fink S, Ilarregui J, Alemán M, Olivares L, et al. NK cells modulate the cytotoxic activity generated by *Mycobacterium leprae*-hsp65 in leprosy patients: role of IL-18 and IL-13. Clin Exp Immunol 2004;135:105–13.
[22] Zhang B, Yamamura T, Kondo T, Fujiwara M, Tabira T. Regulation of experimental autoimmune encephalomyelitis by natural killer (NK) cells. J Exp Med 1997;186:1677–87.
[23] Baxter AG, Smyth MJ. The role of NK cells in autoimmune disease. Autoimmunity 2002;35:1–14.
[24] Kastrukoff LF, Lau A, Wee R, Zecchini D, White R, Paty DW. Clinical relapses of multiple sclerosis are associated with 'novel' valleys in natural killer cell functional activity. J Neuroimmunol 2003;145: 103–14.
[25] Zurawski G, de Vries JE. Interleukin 13, an interleukin 4-like cytokine that acts on monocytes and B cells, but not on T cells. Immunol Today 1994;15:19–26.
[26] Cash E, Minty A, Ferrara P, Caput D, Fradelizi D, Rott O. Macrophage-inactivating IL-13 suppresses experimental autoimmune encephalomyelitis in rats. J Immunol 1994;153:4258–67.
[27] Szczepanik AM, Funes S, Petko W, Ringheim GE. IL-4, IL-10 and IL-13 modulate A beta(1–42)-induced cytokine and chemokine production in primary murine microglia and a human monocyte cell line. J Neuroimmunol 2001;113:49–62.
[28] Sironi M, Sciacca FL, Matteucci C, Conni M, Vecchi A, Bernasconi S, et al. Regulation of endothelial and mesothelial cell function by interleukin-13: selective induction of vascular cell adhesion molecule-1 and amplification of interleukin-6 production. Blood 1994;84: 1913–21.
[29] Bochner BS, Klunk DA, Sterbinsky SA, Coffman RL, Schleimer RP. IL-13 selectively induces vascular cell adhesion molecule-1 expression in human endothelial cells. J Immunol 1995;154: 799–803.
[30] Zettl UK, Mix E, Zielasek J, Stangel M, Hartung HP, Gold R. Apo-

ptosis of myelin-reactive T cells induced by reactive oxygen and nitrogen intermediates in vitro. Cell Immunol 1997;178:1–8.
[31] Bogdan C. The multiplex function of nitric oxide in (auto)immunity. J Exp Med 1998;187:1361–5.
[32] Willenborg DO, Fordham SA, Staykova MA, Ramshaw IA, Cowden WB. IFN-γ is critical to the control of murine autoimmune encephalomyelitis and regulates both in the periphery and in the target tissue: a possible role for nitric oxide. J Immunol 1999;163:5278–86.
[33] van der Veen RC, Dietlin TA, Dixon Gray J, Gilmore W. Macrophage-derived nitric oxide inhibits the proliferation of activated T helper cells and is induced during antigenic stimulation of resting T cells. Cell Immunol 2000;199:43–9.
[34] Nakashima I, Fujihara K, Misu T, Fujimori J, Sato S, Takase S, et al. A comparative study of Japanese multiple sclerosis patients with and without oligoclonal IgG bands. Mult Scler 2002;8:459–62.
[35] Horiuchi I, Kawano Y, Yamasaki K, Minohara M, Furue M, Taniwaki T, et al. Th1 dominance in HAM/TSP and the optico-spinal form of multiple sclerosis versus Th2 dominance in mite antigen-specific IgE myelitis. J Neurol Sci 2000;172:17–24.
[36] Wu XM, Osoegawa M, Yamasaki K, Kawano Y, Ochi H, Horiuchi I, et al. Flow cytometric differentiation of Asian and Western types of multiple sclerosis, HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) and hyperIgEaemic myelitis by analyses of memory CD4 positive T cell subsets and NK cell subsets. J Neurol Sci 2000;177:24–31.

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Journal of the Neurological Sciences 225 (2004) 27–31

Journal of the Neurological Sciences

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High frequency of allergic conjunctivitis in myasthenia gravis without thymoma

Hiroyuki Murai*, Manabu Osoegawa, Hirofumi Ochi, Jun-ichi Kira

Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan

Received 25 November 2003; received in revised form 9 April 2004; accepted 17 June 2004
Available online 7 August 2004

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-

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

0022-510X/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jns.2004.06.018