

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

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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.

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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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stimulate T cells bearing the specific T cell receptor (TCR) V β [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 V β (V β 3, 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 V β 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 V β 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 (V β 12 or V β 17) [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 V β 12 or V β 17.

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 $\pm$ SD 33.6 $\pm$ 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 $\pm$ 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 $\pm$ 10.7 years; serum IgE: median 50 IU/ml, range 5–1,123 IU/ml), and 34 healthy controls (33.7 $\pm$ 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 $\pm$ 9.9 years; serum IgE: median 6,535 IU/ml, range 460–30,100 IU/ml) and 13 did not (33.1 $\pm$ 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-

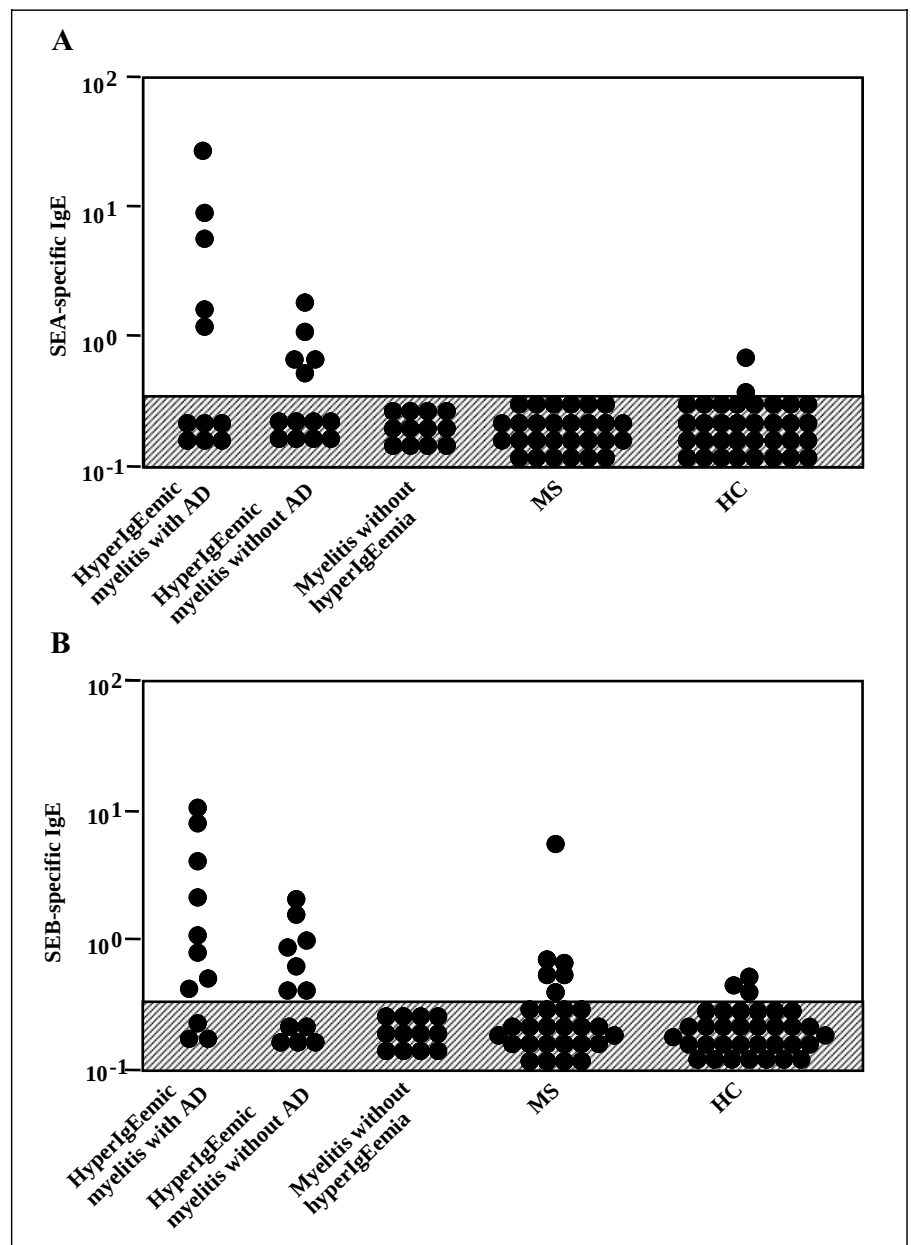


Fig. 1. Distributions of serum SEA- (**A**) and SEB-specific IgE levels (**B**). HC = Healthy controls. The optimal cutoff level for the definition of serum SEA- and SEB-specific IgE positivity was 0.34 IU/ml.

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).

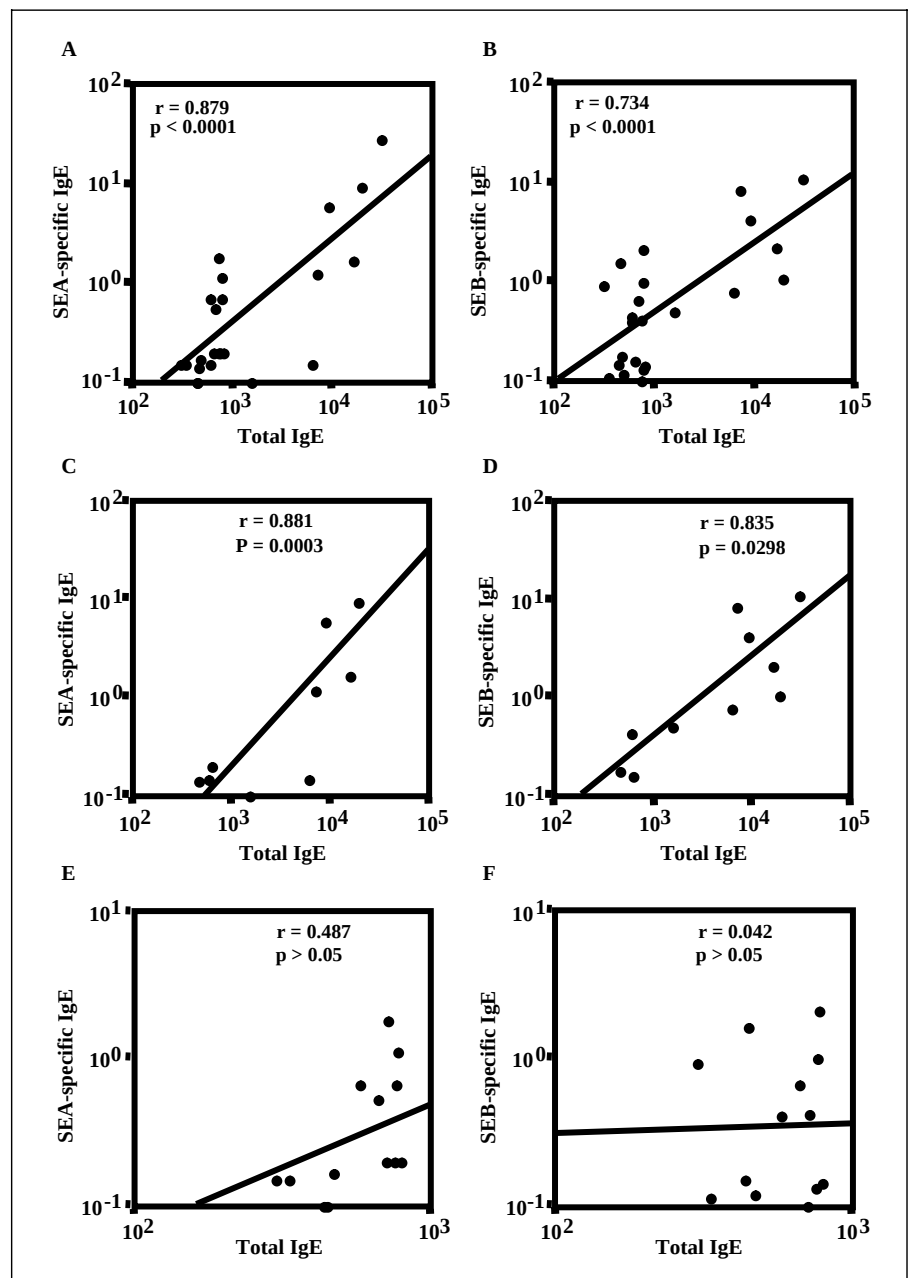


Fig. 2. Scatter plot correlating to total and SEA/SEB-specific IgE levels in all patients with hyperIgEemic myelitis (**A, B**), those with hyperIgEemic myelitis and AD (**C, D**) and those with hyperIgEemic myelitis but without AD (**E, F**). The correlation coefficient was determined by Spearman's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

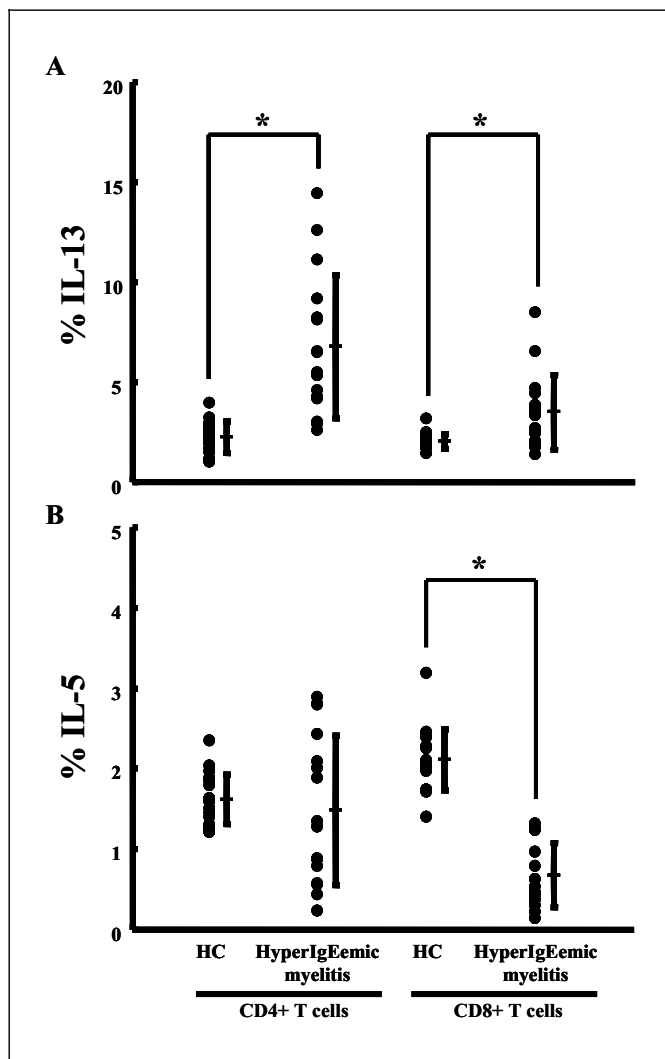


Fig. 3. Intracellular production of IL-13 (**A**) and IL-5 (**B**) in peripheral blood CD4+ T cells and CD8+ T cells. HC = Healthy controls.

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 V β , V β 12 and V β 17, in toxigenic *S. aureus*-colonized skin [18, 19], and promotes cutaneous inflammation. Similarly, SEB activates MBP-reactive T cells with TCR V β 12 or V β 17 to secrete a proinflammatory cytokine, IFN- γ , in MS patients [5, 6]. Therefore, in atopic myelitic patients, it seems possible that staphylococcal enterotoxins might activate T cells bearing TCR V β 12 or V β 17, 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 V β usage of these patients should be examined in the future to disclose whether T cells bearing toxin-reactive TCR V β 12 or V β 17 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

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