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Expression of T Cell Receptor Vβ Chain in Lesional Skin of Atopic Dermatitis

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Atopic dermatitis is a chronic, relapsing inflammatory skin disorder characterized by local infiltration of T cells. To date, numerous reports have shown that *Staphylococcus aureus* may exacerbate atopic dermatitis, and superantigens produced by this organism are thought to be one of the major causative factors in atopic dermatitis. The purpose of this study was to evaluate the role of staphylococcal superantigen in atopic dermatitis by observing expression of the variable region of β chain of T cell receptor (TCR Vβ) in the inflammatory cells infiltrating cutaneous lesions of atopic dermatitis. Fourteen patients with atopic dermatitis were enrolled. Punch biopsy specimens were obtained from lesional and normal-appearing skin of all patients. The expression of TCR Vβ was studied by means of immunohistochemical technique using monoclonal antibodies. In 4 out of 14 patients, the tendencies of preferential expression of specific TCR Vβ were found in lesional skin. This study suggested that staphylococcal superantigen and its corresponding T cell subsets may act as causative or pathogenic factors in a subgroup of atopic dermatitis. *Key words: atopic dermatitis; staphylococcal superantigen; immunohistochemistry*.

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Atopic dermatitis (AD) is a chronic, relapsing inflammatory skin disease that arises most commonly during early infancy, childhood, or adolescence (1). Indirect evidences have been accumulated that *Staphylococcus aureus* (*S. aureus*) can exacerbate this skin disease (2, 3). Leyden et al. first demonstrated that *S. aureus* could be isolated from the affected skin of more than 90% of patients with AD (4). About 60% of *S. aureus* strains isolated from the skin of patients with AD secrete exotoxins with superantigenic properties (5–7).

Staphylococcal superantigens (SAgs) secreted at the skin surface could penetrate inflamed skin and engage HLA-DR on epidermal macrophages or Langerhans' cells to stimulate the production of IL-1 and TNF-α, or activate T cells via their variable region of the beta chain of T cell receptor (TCR Vβ) to proliferate and secrete cytokines, which modulate tissue inflammation (8). Moreover, the fact that nearly half of patients with AD have IgE directed to staphylococcal SAgs suggests that they could act as conventional allergens and cause IgE-mediated histamine release (5).

MATERIAL AND METHOD

Patients

Fourteen patients (2 females and 12 males, aged 9–56 years) (Table I) with AD defined by Hanifin & Rajka's criteria (12) were enrolled in this study. They showed moderate to severe clinical severity. Patients who received systemic steroids or immunosuppressive drugs in 1 month prior to this study, or antihistamines or topical steroids in 2 weeks before enrolment were excluded. Informed consent was obtained from all patients.

Skin specimens

Punch biopsy specimens were obtained from lesional and normal-appearing skin of all patients. Specimens were embedded in Tissue-Tek (OCT compound, Miles Inc., Elkhart, IN, USA), snap-frozen in liquid nitrogen, and stored at −70°C.

Table I. *Subject characteristics*

Patient	Age/ sex	Serum IgE (IU/ml)	Lesion site	Normal-appearing site
1	48/M	454	posterior neck lichenified	back
2	56/M	1500	right shin lichenified	right shin
3	13/M	1500	posterior neck lichenified	back
4	13/F	2500	posterior neck lichenified	back
5	11/M	2500	posterior neck lichenified	back
6	24/M	1500	chest erythematous patch	chest
7	25/M	2000	back erythematous papule	back
8	18/M	600	back erythematous papule	back
9	22/M	9800	back lichenified	buttock
10	19/M	4940	posterior neck lichenified	back
11	20/M	3530	posterior neck lichenified	back
12	12/F	ND	left knee erythematous papule	left calf
13	17/M	ND	posterior neck lichenified	back
14	9/M	383	left knee erythematous papule	left calf

M = male, F = female, ND = not determined.

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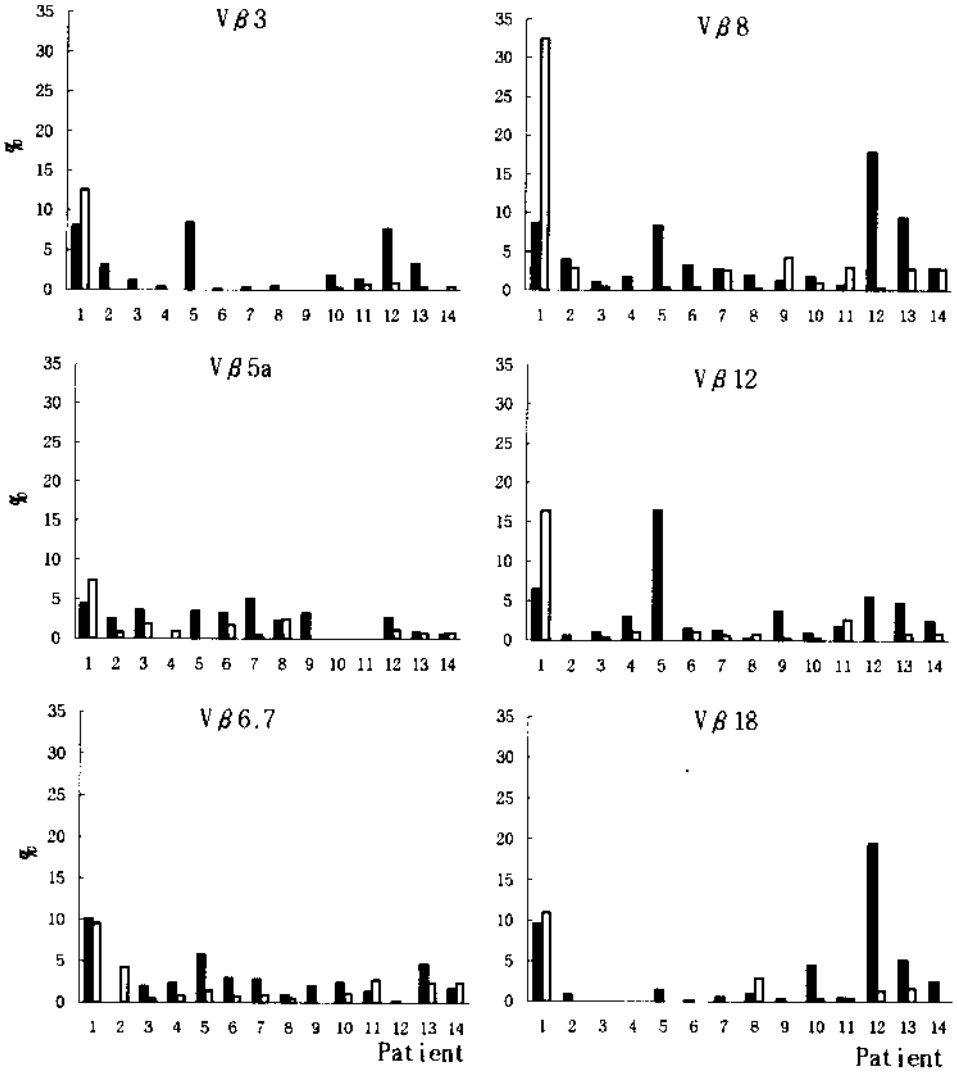

Fig. 1. Percentage of cells expressing Vβ among the perivascular infiltrating total cells in lesional (■) and normal-appearing skin (□).

Monoclonal antibodies

Monoclonal antibodies (MoAbs) specific for TCR Vβ; TCR Vβ3, Vβ8, Vβ18 (Immunotech, Marseille, France) and TCR Vβ5, Vβ6.7, Vβ12 (Endogen, Woburn, MA, USA) were used. Each of these six Vβ panels are related with SEA (Vβ5, 6.7), SEB (Vβ3, 12, 18), SEC (Vβ3, 12), SED (Vβ5, 12) and SEE (Vβ6.7, 8, 18) (13). Because Vβ5.1 MoAb is related with only SEE and Vβ13 MoAb, SEC2 and SEC3, these 2 MoAbs were not included in our study. Also Vβ19 MoAb is not related with staphylococcal SAg. Vβ2-expressing T cells were revealed to be insignificant in Leung et al.'s (9) and our preliminary study (data not shown).

Indirect immunoperoxidase staining

The biopsy specimens were cut into 5 μm sections. Skin sections were stained by using the immunoperoxidase technique (Universal DAKO LSAB Kit. DAKO Corp., Carprinteria, CA, USA). All procedures were carried out at room temperature. The sections were air-dried and fixed in cold acetone (10 min). They were incubated sequentially with 3% H2O2 (5 min), blocking reagent (5 min), diluted primary antibody (45 min) and biotinylated anti-mouse antibody (10 min). After incubation with avidin-biotin-horseradish-peroxidase complex for 10 min, the color reaction was developed with 0.01% 3-amino-9-ethylcarbazole (Zymed Laboratory Inc, San Francisco, CA, USA) (5 min). All washings were performed with phosphate-buffered saline. The sections were counterstained with aqueous hematoxylin (Biomeda Corp., Foster, CA, USA) and mounted with universal mount (Research Genetics, Pittsburgh, PA, USA). Five high-power fields (×400) were assessed for each section. Well-stained cells were counted independently by two observers. Each Vβ family was expressed as a percentage of the total cells in the perivascular area. Results were shown as the mean of positive cell counts in five high-power fields.

No staining was observed in controls which consisted of sections incubated with normal mouse IgG or PBS as the primary antibody.

RESULTS

In 4 (patients 1, 5, 12 and 13) of 14 patients with AD, preferential expressions of specific TCR Vβ were found in lesional skin (Fig. 1). Patient 1 showed relatively frequent Vβ6.7+ (10.0%), Vβ18+ (9.6%), Vβ8+ (8.7%) and Vβ3+ (8.1%) (Fig. 2). Patient 5 showed preferential expression of Vβ12+ (16.5%), Vβ3+ (8.4%) and Vβ8+ (8.4%). Patient 12 showed higher usage of Vβ18+ (19.5%), Vβ8+ (17.8%) and Vβ3+ (7.7%). Patient 13 showed frequent expression of Vβ8+ (9.3%).

In normal-appearing skin, patient 1 showed higher expression of Vβ8+ (32.4%), Vβ12+ (16.3%), Vβ3+ (12.6%), Vβ18+ (10.9%) and Vβ5+ (7.4%) (Fig. 1).

Patients 1, 5 and 13 were biopsied from chronic lichenified lesions, and patient 12 from acute erythematous papule (Table I). But we could not conclude that there is a difference in the TCR Vβ expression between acute and chronic lesions with our data.

DISCUSSION

S. aureus, a common human pathogen, produces several enterotoxins, such as staphylococcal enterotoxin A (SEA) through SEE, toxic shock syndrome toxin 1 (TSST-1) and exfoliative toxins (ExFT). These enterotoxins have superantigenic properties that activate T cells by interacting with TCR Vβ and major histocompatibility complex class II molecules on antigen-presenting cells (14 – 16).

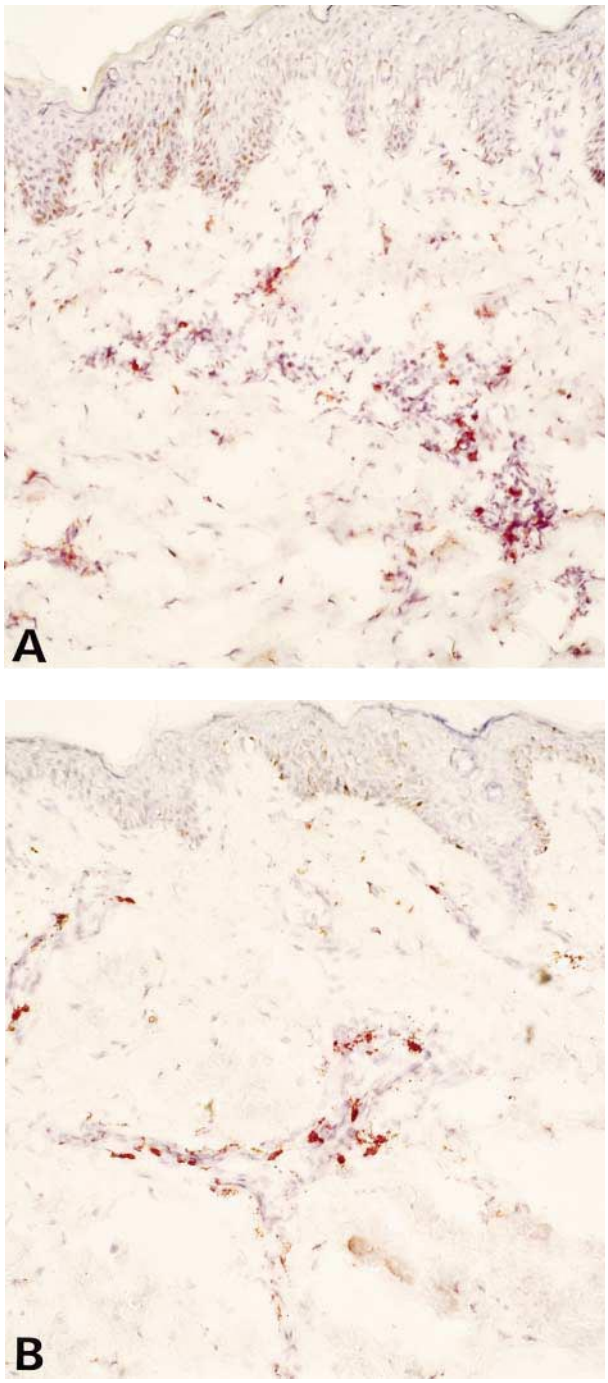

Fig. 2. Frozen tissue sections from lesional (A) and normal-appearing (B) skin biopsy of patient 1, stained with monoclonal anti-Vβ8 (×160).

There have been evidences supporting the roles of staphylococcal SAgs in AD. Eczematous lesions of AD are usually colonized densely with *S. aureus* (4, 17). Density of *S. aureus* was 100 to 1,000 times higher in dermatitic, lichenified and impetiginized lesional skins, compared to normal-appearing skin of patients (18). One or more staphylococcal SAgs have been shown to be produced in *S. aureus* isolated from AD patients (5 – 7). AD patients with either impetiginized or without superinfected lesion show better response to combined treatments with antistaphylococcal antibiotics and topical corticosteroids than to corticosteroids alone (19, 20). Significant levels of IgE to SEA, SEB and TSST-1 were detected in sera of AD patients (5). This result implies the allergenic role as well as the superantigenic action of staphylococcal SAg. Stimulation of peripheral blood mononuclear cells (PBMC) of AD with staphylococcal SAgs displayed significantly greater proliferation and an increase of SEA/SEB specific TCR Vβ T-cells than that of the normal controls (10, 11).

Potential involvement of SAgs in the pathogenesis of AD implies an enrichment for SAg-specific TCR Vβ T cells in lesional skin. Therefore, we examined the Vβ repertoire of TCR in lesional and normal-appearing skin of AD with Vβ3, Vβ5, Vβ6.7, Vβ8, Vβ12 and Vβ18 MoAbs. In our study, the preferential expression of TCR Vβ was observed in 4 of 14 patients. Vβ8 showed most prominent expression and some expression of Vβ18, Vβ12 and Vβ3 was found, but less prominent. The preferential expression of Vβ3, Vβ8 and Vβ12 was consistent with Neuber et al.'s result (10), although Vβ3 was most prominent in their study and Vβ8 in this study.

In patient 1, the expression of Vβ was related with SEA, SEB, SEC and/or SEE, in patient 5, SEB, SEC, SED and/or SEE, in patient 12, SEB, SEC and/or SEE, and in patient 13, SEE. It could be concluded that one or more staphylococcal SAgs may be involved in the development or exacerbation of AD. This is supported by Leung et al.'s study in which AD patients had IgE antibodies directed against at least one staphylococcal SAg.

The pattern of Vβ expression was heterogeneous among individual patients in the present study. This result was consistent with Yudate et al.'s study (11), which showed relatively irregular pattern of TCR Vβ expression induced by stimulation with staphylococcal SAg in AD PMBC.

SAgs are believed to penetrate the skin readily through the cutaneous lesions of AD, where the barrier functions are disrupted (21). *S. aureus* density varies according to the clinical characteristics of skin lesions (18). From these points, it is possible to expect differences in the expression of Vβ between acute erythematous and chronic lichenified lesions. We found that acute lesions are infiltrated with more inflammatory cells than chronic ones, but could not conclude that there is a difference in TCR Vβ expression between acute and chronic lesions with our data.

Our data revealed the preferential expression of some TCR Vβ in a proportion of the patients, but not in all. This result can be explained by the point that AD is a heterogeneous disorder which has subgroups of patients with different pathogenic mechanisms. In this study, selective expansion of specific

TCR Vβ was demonstrated in a subgroup of AD patients and this suggests that staphylococcal SAg may act as a causative or pathogenic factor in this subgroup.

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