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Evidence for Superantigen Involvement in Skin Homing of T cells in Atopic Dermatitis

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The environmental factors that contribute to the homing of T cells in skin disease is unknown. The skin lesions of atopic dermatitis (AD) are frequently colonized with superantigen (SAg), producing strains of *Staphylococcus aureus*. *In vitro*, these superantigens have the capacity to activate and expand T cells expressing specific T cell receptor BV gene segments, and also to increase their skin homing capacity via upregulation of the skin homing receptor, cutaneous lymphocyte-associated antigen (CLA). These activities have been proposed to enhance the chronic cutaneous inflammation of AD, but an *in vivo* role for SAg has not been conclusively demonstrated. In this study, we sought direct evidence for *in vivo* SAg activity by comparing the SAg profiles of *S. aureus* cultured from the skin of AD subjects to the T cell receptor Vβ repertoire of their skin homing (CLA^+) T cells in peripheral blood. SAg secreting *S. aureus* strains were identified in six of 12 AD patients, and all of these subjects manifested significant SAg-appropriate Vβ skewing within the CLA^+ subsets of both their $CD4^+$ and their $CD8^+$ T cells. T cell receptor Vβ skewing was not detectable among the overall $CD4^+$ or $CD8^+$ T cell subsets of these subjects, and was not present within the CLA^+ T cell subsets of five patients with plaque psoriasis and 10 normal controls. T cell receptor BV genes from the presumptively SAg-expanded populations of skin homing T cells were cloned and sequenced from three subjects and, consistent with a SAg-driven effect, were found to be polyclonal. We conclude that SAg can contribute to AD pathogenesis by increasing the frequency of memory T cells able to migrate to and be activated within AD lesions. *Key words: CLA/inflammation/SEB/TSST-1. J Invest Dermatol 112:249–253, 1999*

Atopic dermatitis (AD) is a complex chronic inflammatory skin disorder affecting 10%–15% of children and is a major cause of occupation-related disability caused by skin disease (Schultz-Larsen *et al*, 1986). It is associated with cutaneous erythema, induration, severe pruritus, elevated serum IgE, eosinophilia, and a disposition towards extracutaneous atopic manifestations such as allergic rhinitis and/or asthma (Hanifin and Rajka, 1980). AD lesions are characterized by cellular infiltrates comprised of memory T cells expressing the skin homing receptor cutaneous lymphocyte-associated antigen (CLA), and monocyte/macrophages (Leung, 1995). Recent studies indicate that the T cell activation in AD is biphasic with activation of the Th 2-like cytokines, IL-4, IL-5, and IL-13, during the acute phase of the eruption (Van der Heijden *et al*, 1991; Hamid *et al*, 1994). The chronic inflammatory AD skin lesion, however, is associated with increased expression of the Th 1 cytokines, interferon-gamma and IL-12 (Grewe *et al*, 1994; Hamid *et al*, 1996). Allergens are thought to play a role in the initiation of the skin lesion by promoting Th2-like cytokine gene expression via the activation of T cells and mast cell degranulation (Leung, 1995); however, the mechanisms that contribute to the chronic phase of the skin lesion are unknown.

AD is often associated with skin colonization by *Staphylococcus aureus* (Leyden *et al*, 1974). In many cases these bacteria are known to produce superantigens (SAg) (Leung *et al*, 1993a; McFadden *et al*, 1993), which have the capacity to directly activate T cells bearing appropriate T cell receptor (TCR) Vβ domains (Marrack and Kappler, 1990). SAg can also induce T cell expression of CLA via an IL-12 dependent mechanism (Leung *et al*, 1995). Thus, SAg may contribute to the pathogenesis of AD by activating large cohorts of T cells within lesions (far more than conventional antigens) and by increasing the population of memory T cells capable of efficient extravasation to skin. Such mechanisms may act to maintain T cell activation in skin and thus perpetuate AD lesions even when the initiating allergen may be absent.

A role for SAg in AD pathogenesis is supported by observations showing antibiotics to be an effective treatment of AD (Lever *et al*, 1988) and that SAg can induce eczematoid skin reactions in patch test models (Strange *et al*, 1996); however, because only about 50% of *S. aureus* are SAg producing, the pathogenic role of SAg in this disease remains a source of controversy. In this study, we sought direct evidence for SAg involvement in AD by determining whether the presence of a particular SAg in skin lesions of AD subjects would predict an expansion of appropriate (SAg-associated) TCR-Vβ bearing T cells in peripheral blood, especially in the CLA^+ skin-homing memory T cell subset.

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Abbreviations: AD, atopic dermatitis; CLA, cutaneous lymphocyte-associated antigen; PBMC, peripheral blood mononuclear cell; SAg, superantigen; SEB, Staphylococcal enterotoxin B; TSST-1, toxic shock syndrome toxin-1.

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MATERIALS AND METHODS

Patients Twelve AD patients were recruited for study (mean age = 36), all of whom fulfilled the criteria of Hanifin and Rajka (1980). Four of the AD patients had *S. aureus* cultures positive for Staphylococcal enterotoxin B (SEB), two had *S. aureus* cultures positive for toxic shock syndrome toxin-1 (TSST-1), and the remaining six patients were positive for *S. aureus*, but negative for any known SAg (analyzed by enzyme-linked immunosorbent assay of staphylococcal skin culture supernatants, Toxin Technologies, Sarasota, FL). Normal healthy volunteers (n = 10, mean age = 32) and plaque psoriasis patients (n = 5, mean age = 35) with no history of atopy were used as control groups. All patients had greater than 20% skin involvement and were not receiving prednisone at the time of study.

Preparation of peripheral blood mononuclear cells (PBMC) Heparinized venous blood was layered on a Ficoll-Paque density gradient (Pharmacia, Piscataway, NJ) and centrifuged for 25 min at 400 × *g* at room temperature. The PBMC layer was aspirated and washed in Hanks balanced salt solution (Gibco-BRL, Grand Island, NY). Cells were resuspended in Hanks balanced salt solution and counted. PBMC were always >95% viable, as determined by a trypan blue (Sigma, St. Louis, MO) exclusion assay.

Immunofluorescense staining for Vβ-fluorescence-activated cell sorter (FACS) analysis Staining for TCR Vβ expression and analyses was carried out as we previously described (Abe *et al*, 1993). Briefly, PBMC were washed in phosphate-buffered saline (PBS) and resuspended at 10×10^6 cells per ml in a staining solution [PBS with 5% fetal calf serum (Gibco), 1% immunoglobulin (Alpha Therapeutic, Los Angeles, CA), 0.02% sodium azide (Sigma)]. Cells were stained in 96 well, round bottomed plates with a panel of biotinylated monoclonal antibodies against human Vβ2, 3, 5.1, 8, 12, 13.1, 13.2, and 17, then incubated for 30 min at 37°C in the dark as previously described (Abe *et al*, 1993). After the incubation period, cells were washed twice with washing buffer [PBS, 5% fetal calf serum (Gibco), 0.02% sodium azide (Sigma)] by centrifugation at 300 × *g* for 5 min at 4°C. Cell pellets were resuspended in staining solution and incubated with anti-CD3 allophycocyanin, anti-CD4 peridinin chlorophyll protein (Becton Dickinson, San Jose, CA), CLA-fluorescein isothiocyanate (Immunotech, Maine, FL) and a streptavidin phycoerythrin conjugate (Immunotech) for 30 min at 4°C. Stained cells were again washed twice in washing buffer and once in 1 × PBS by centrifugation at 300 × *g* for 5 min at 4°C. Finally, the cells were fixed in 300 μl of 1% (vol/vol) formaldehyde (Polysciences, Warrington, PA) in 1 × PBS. Analysis was performed using four color flow cytometry (FACSCalibur, Becton Dickinson).

Separation of CLA^+ and CLA^- PBMC populations by FACS Freshly isolated PBMC (100×10^6) were incubated with 1 ml of a 20% dilution of fluorescein isothiocyanate conjugated rat anti-human CLA monoclonal antibody in 500 μl of staining solution, for 30 min at 4°C as previously described (Abernathy-Carver *et al*, 1995). Cells were resuspended and washed twice in 5 ml of PBS by centrifugation at 300 × *g* for 10 min at 4°C. Cells were resuspended in PBS and sorted into CLA^+ and CLA^- populations by FACS (Coulter Epics). CLA^+ T cells were always greater than 98% pure.

Preparation of RNA and reverse transcriptase-polymerase chain reaction (PCR) Total RNA was prepared from CLA^+ and CLA^- PBMC using a commercially available kit according to the manufacturer's instructions (RNAid Plus, Bio 101, La Jolla, CA). First strand cDNA was synthesized at 37°C for 1 h in a 20 μl reaction mixture containing 1 μl of 50 ng per μl random hexamers (Gibco-BRL), 4 μg RNA, 10 U RNAse inhibitor (Promega, Madison, WI), 1 μl 10 mM dNTP (Gibco-BRL), and 200 U Superscript reverse transcriptase (Gibco-BRL). After the synthesis period, the reverse transcriptase was denatured for 10 min at 95°C. cDNA was PCR amplified in a 50 μl reaction mixture containing 5 μl of 10 × PCR buffer II (Perkin Elmer, Branchberg, NJ), 5 μl of 25 mM $MgCl_2$, 8 μl of 10 mM dNTP (Gibco-BRL), 1.5 U Taq-gold DNA polymerase (Perkin Elmer), 4 μl cDNA, and 2.5 μl of each primer (6 μM). The primer sequences were as follows:

TCRBV2, 5′-TCA TCA ACC ATG CAA GCC TGA CCT-3′
TCRBV17, 5′-CAG ATA GTA AAT GAC TTT CAG-3′
TCRBC, 5′-TTC TGA TGG CTC AAA CAC-3′

The reaction mixture was denatured for 1 min at 95°C, annealed for 1 min at 55°C, and extended for 1 min at 72°C, for 35 cycles on a DNA thermal cycler (Perkin Elmer).

Sequencing of PCR products encoding TCRBV genes PCR products were visualized on a 2% agarose (Boehringer, Indianapolis, IL), 0.05% ethidium bromide (Sigma), 1 × tris borate electrophoresis buffer gel. Products of the correct size were ligated overnight at 14°C in a 10 μl reaction mixture containing 1 μl PCR product, 1 μl of 10 × ligation buffer (Invitrogen, Carlsbad, CA), 4 U T4 DNA ligase (Invitrogen), 0.05 μg pCR-II plasmid vector (Invitrogen), and 5 μl sterile water. The supercompetent bacterial strain, *Epicurean coli*, XL-1 Blue (Stratagene, La Jolla, CA) was transformed with the ligated plasmids. In brief, 40 μl aliquots of bacteria were treated with 2 μl of 0.5 M β-mercaptoethanol (Invitrogen) for 10 min at 4°C. Two microliters of individual ligation products were added to separate bacterial suspensions for 30 min at 4°C. The bacteria were heat shocked for 50 s at 42°C, and grown for 1 h at 37°C in SOC medium (Invitrogen). Bacteria were plated on agar plates (1.5% agar, Difco, Detroit, MI), 1% Bactopeptone (Difco), 1% yeast extract (Difco), 0.5% sodium chloride (Sigma), 50 μg kanamycin (Solopak, Elk, IL) per ml, 20 μl of 0.2 M isopropylthiogalactoside (IPTG, Gibco-BRL), 40 μl of 10% 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal, Gibco-BRL), and incubated overnight at 37°C. Positive colonies were incubated overnight in 5 ml Luria-Bertani medium at 37°C, then the plasmids were purified using a commercial purification system (Qiagen, Santa Clarita, CA). Five microliters of each plasmid preparation was digested with 20 U of EcoRI (Gibco-BRL) in 1 × REact III buffer (Gibco-BRL) for 1 h at 37°C. Digests were then electrophoresed on a 2% agarose (Boehringer), 0.05% (vol/vol) ethidium bromide (Sigma), 1 × tris borate electrophoresis buffer gel. Products of the correct size were sequenced using an automated sequencing system. In brief, a reaction mixture was composed of 1.6 μl of a 6 μM sequencing primer (TCRBC, –5′-CGA CCT CGG GTG GGA ACA-3′), 4 μl plasmid DNA, and 4 μl of dye terminator mix (Perkin Elmer). The reaction mixture was denatured for 30 s at 95°C, annealed for 15 s at 50°C, and extended for 4 min at 60°C, for 25 cycles on a DNA thermal cycler (Perkin Elmer). Reaction products were electrophoresed on a 4.5% (wt/vol) acrylamide (Biorad, Hercules, CA), 6 M urea (Sigma), 1 × tris borate electrophoresis buffer gel, and analyzed using an automated sequencing system (Applied Biosysytems/Perkin Elmer).

Statistical analysis Vβ FACS data were log-transformed prior to analysis in order to produce more normally distributed residuals. The mean Vβ expression between study groups was then compared using a one-way ANOVA test. Association between specific Vβ skewing and the SAg being produced was assessed using a standard χ^2-test.

RESULTS

The TCR Vβ repertoire of PBMC from AD patients with SEB-producing *S. aureus* (n = 4), TSST-1-producing *S. aureus* (n = 2), or non-SAg-producing *S. aureus* (n = 6) were analyzed by FACS. Plaque psoriasis patients (n = 5) and nonatopic healthy individuals (n = 10) served as controls. Within the CLA^+, skin homing population (**Fig 1**), an elevation of T cells bearing the SEB-reactive TCR, Vβ17, was observed in patients with SEB-producing *S. aureus*. In patients with TSST-1-producing *S. aureus*, there was an elevation of $V\beta2^+$ T cells (**Fig 1**). This is a TCR known to be stimulated by TSST-1. This elevation of $V\beta2^+$ and $V\beta17^+$ T cells in AD patients with SAg-producing *S. aureus* was observed within both the $CD4^+$ and the $CD8^+$ subpopulations (**Fig 2*a*, *b***, respectively), consistent with a SAg driven response. It is of note that a single patient with SEB-producing *S. aureus* did show an elevation in the $V\beta2$ $CD4^+$ T cell population (**Fig 2*a***); however, this elevation was not observed in the $CD8^+$ population, indicating this was more likely to be an antigen-driven, rather than a SAg-driven expansion. No statistical difference was found in the percentage of CLA^+ T cells between the three goups (AD with SAg-producing *S. aureus*, mean = 9.8% ± 2.3; AD with non-SAg-producing *S. aureus*, mean = 8.9% ± 1.5; psoriasis patients, mean = 11.3% ± 2.6). These data show that, within the CLA^+ skin homing T cell population, there was an expansion of SAg reactive T cells which was specific to the SAg being produced in each patient. Moreover, this correlation was found to be statistically significant ($p < 0.0001$).

CLA^+ T cells from three AD patients were isolated by FACS cell sorting and examined for TCR-Vβ chain junctional region and NDβN region diversity. From a single patient with SEB-producing *S. aureus* and $V\beta17^+$ T cells (**Table I**) increased to 2.9 times the median value for normals, 47 clones were sequenced. If

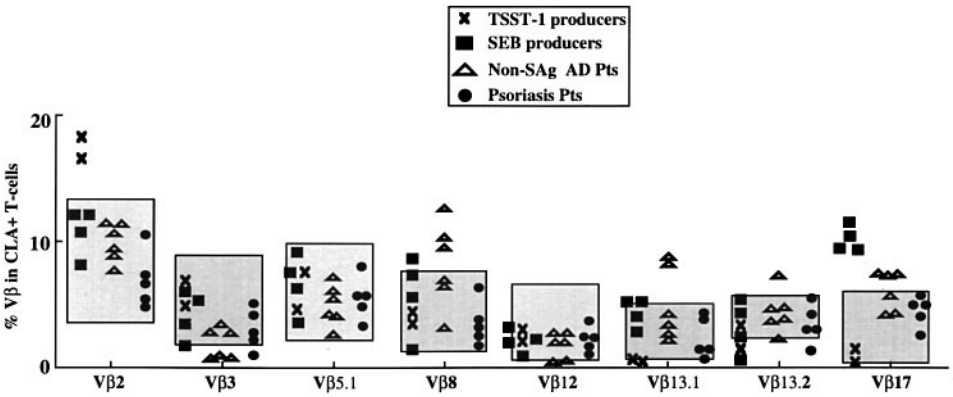

Figure 1. AD patients with SAg-producing *S. aureus* show an elevation in SAg-reactive, CD3$^+$ skin homing T cells. Immunophenotyping of the T cell repertoire within the skin homing, CLA$^+$/CD3$^+$ T cell population was performed. The hatched boxes represent the 2.5th to 97.5th percentile values for normal individuals. Elevations in Vβ2$^+$ and Vβ17$^+$ T cells were observed in patients with TSST-1- (n = 2) and SEB- (n = 4) producing *S. aureus*, respectively.

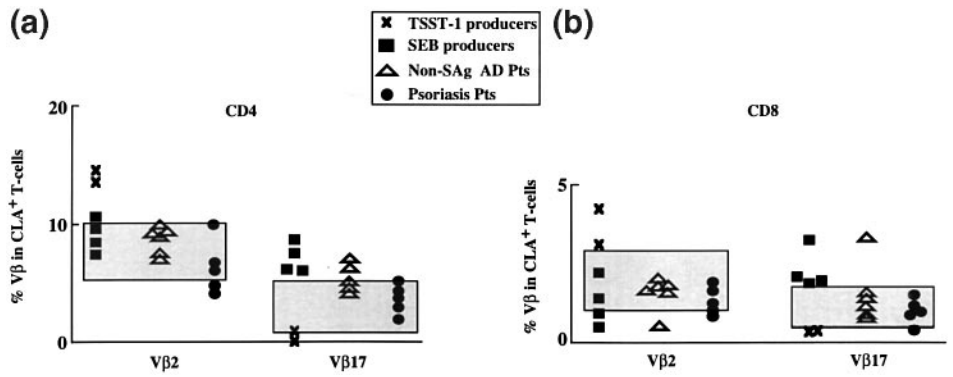

Figure 2. Expansion of SAg-reactive T cells in both CD4 and CD8 subpopulations. (*a*) AD patients with SAg-producing *S. aureus* show an elevation in SAg-reactive, CD4$^+$ skin homing T cells. Immunophenotyping of the T cell repertoire within the CLA$^+$/CD4$^+$ T cell population was performed. Elevations in Vβ2$^+$ and Vβ17$^+$ T cells were observed in patients with TSST-1- and SEB-producing *S. aureus*, respectively. (*b*) AD patients with SAg-producing *S. aureus* show an elevation in SAg-reactive, CD8$^+$ skin homing T cells. Immunophenotyping of the T cell repertoire within the CLA$^+$/CD8$^+$ T cell population was performed. Elevations in Vβ2$^+$ and Vβ17$^+$ T cells were observed in patients with TSST-1 and SEB producing *S. aureus*, respectively.

Table I. The expanded population of SAg-reactive, skin homing T cells from AD patients with SAg-producing *S. aureus*, comprise a polyclonal population

Patient	SAg produced	BV elevation (fold increase)	No. of clones sequenced	No. of identical sequences
LM	SEB	BV17 (×2.9)	47	2
RG	TSST-1	BV2 (×2.1)	33	0
KC	TSST-1	BV2 (×2.0)	21	0

this was an antigen-driven response one would predict that 67% of the sequences obtained would be the same; however, of the sequences obtained, only two of the 47 were found to be identical. From the two patients with TSST-1-producing *S. aureus* and elevated Vβ2$^+$ T cells (**Table I**), all sequences were different. These data demonstrate that the increased expression of SAg reactive T cells in the skin homing population was polyclonal, again indicative of a SAg-driven response.

Some patients with non-SAg-producing *S. aureus* also showed elevations in Vβ8, Vβ13.1, and Vβ13.2 T cells in the skin homing population (**Fig 1**); however, these elevations occurred in either the CD4$^+$ or the CD8$^+$ only, never in both CD4$^+$ and CD8$^+$ T cell subsets. Within the total CD3$^+$ T cell population a significant expansion of Vβ8$^+$ T cells was observed in the AD group with non-SAg-producing *S. aureus* (**Fig 3**, $p < 0.05$) as compared with the other study groups. This expansion was found to be solely within the CD8$^+$ population and was statistically significant when compared with the other study groups (**Fig 4*b***, $p < 0.05$). Moreover, sequence analysis of TCR-Vβ8$^+$ T cells from peripheral blood T cells of patients with elevated Vβ8, demonstrated an oligoclonal expansion (data not shown) indicative of an antigen-driven process. Two individuals, in the non-SAg-producing AD group, showed an elevation of TCR-Vβ8$^+$ T cells in the CD4$^+$ population (**Fig 4*a***, closed triangles), but this was not observed in their CD8$^+$ population (**Fig 4*b***).

DISCUSSION

This study has demonstrated that subjects with AD and skin colonization with SAg-producing *S. aureus* manifest expanded populations of circulating skin homing (CLA$^+$) memory T cells that express TCR appropriate for the SAg being produced on their skin surface. TSST-1-producing *S. aureus* correlated with expansions of CLA$^+$ T cells bearing the TSST-1-reactive TCR Vβ2, whereas SEB-producing *S. aureus* correlated with expansions of CLA$^+$ T cells bearing the SEB reactive TCR Vβ17. Interestingly, expanded populations of Vβ3- and Vβ12-bearing CLA$^+$ T cells were not observed in subjects with SEB-producing *S. aureus*, despite the fact that SEB also reacts with these TCR (Kotzin *et al*, 1993). This observation may reflect our recent finding that Vβ17 is the most reactive TCR for this SAg (DYM Leung, unpublished observation).

SAg stimulate both CD4$^+$ and CD8$^+$ T cell subsets. Consistent with this property of SAg, elevated frequencies of SAg-reactive T cells were found within both the CD4$^+$ and the CD8$^+$ populations. Further support for these TCR Vβ expansions being SAg driven (as opposed to antigen driven) is provided by our TCR gene sequencing data, which demonstrated that the expanded Vβ2$^+$ and Vβ17$^+$ skin homing T cells were polyclonal. Expansions of SAg-reactive T cell populations could not be discerned among the overall CD4$^+$ and CD8$^+$ T cell populations, indicating the SAg effect is largely, if not completely, restricted to the CLA$^+$ skin homing subset. Taken together, these data strongly support the hypothesis that *S. aureus* secretion of SAg in the lesional skin of AD subjects has definite effects on the repertoire of the recirculating, skin homing memory T cells.

The restriction of the expanded Vβ-restricted T cell populations to the CLA$^+$ memory T cell subset shown here, as well as the general linkage between SAg pathophysiology and skin inflammation, is likely related to the ability of SAg to upregulate CLA expression on the T cells they activate (Leung *et al*, 1995). We have previously shown that CLA upregulation is an indirect effect of SAg. In addition to their T cell activating properties, SAg induce IL-12

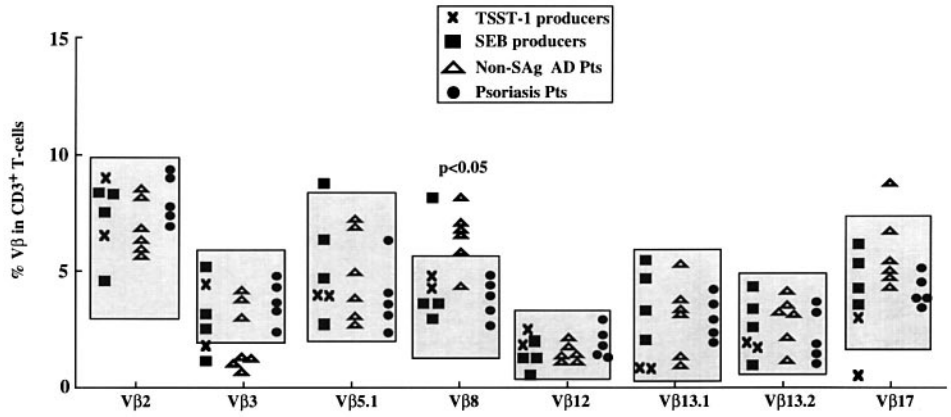

Figure 3. Vβ8$^+$ T cells are expanded within the total peripheral blood T cell population. Immunophenotyping of the CD3$^+$, T cell repertoire when gated on the total, T cell population was performed. Vβ8 was found to be significantly raised in the non-SAg-producing AD group when compared with normals, plaque psoriasis patients, and AD patients with SAg-producing *S. aureus* ($p < 0.05$).

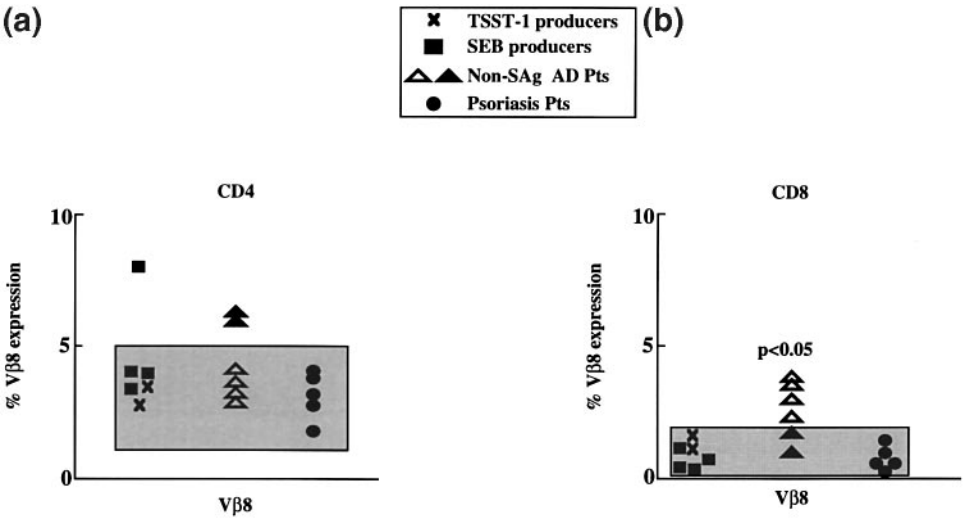

Figure 4. Analysis of Vβ8$^+$ repertoire in the CD4$^+$ and CD8$^+$ T cell subpopulations. (*a*) The expansion of Vβ8$^+$ T cells is not observed within the CD4$^+$ population. Immunophenotyping of the CD4$^+$/Vβ8$^+$, T cell repertoire when gated on the total T cell population was performed. The closed triangles represent two individual AD patients with non-SAg-producing *S. aureus* that showed elevated Vβ8$^+$ T cells in the CD4$^+$ population only. (*b*) The expanded population of Vβ8$^+$ T cells is observed solely within the CD8$^+$ population. Immunophenotyping of the CD8$^+$, T cell repertoire when gated on the total T cell population was performed. Vβ8 was found to be significantly raised in the non-SAg-producing AD group when compared with normals, plaque psoriasis patients, and AD patients with SAg-producing *S. aureus* ($p < 0.05$). The closed triangles represent individual AD patients with non-SAg-producing *S. aureus* who had elevated Vβ8$^+$ T cells in the CD4$^+$ population.

production by non-T accessory cells (Lester, 1995). Along with its effects in promoting IFN-γ production (Wu, 1993), IL-12 is a potent upregulator of CLA expression on activated T cells (Leung *et al*, 1995).

SAg activation, and the IL-12 upregulation of CLA, operates on both naïve and memory T cells (Leung *et al*, 1995), suggesting that the expansion of Vβ-specific CLA$^+$ T cells reported here may originate from (i) the activation of existant CLA$^+$ memory T cells in the skin (SAg induced IL-12 acting to maintain and/or upregulate the level of CLA expression on these already CLA$^+$ T cells); (ii) the activation of naïve T cells in draining peripheral lymph node (SAg induced IL-12 inducing CLA on these SAg activated cells, as well as promoting a "Th 1" cytokine synthesis phenotype); and/or (iii) the activation of pre-existent CLA$^+$ and memory T cells in draining peripheral lymph node with similar consequences. Thus, the combined mechanism of SAg-induced proliferation and CLA upregulation may converge to recruit a large cohort of new skin homing, likely Th 1-type, Vβ-restricted T cells to the recirculating, skin homing memory T cell pool. Such cells would be capable of extravasating in any site of superficial skin inflammation where they would be available to contribute to the inflammatory response if an activating ligand were present, and could potentially account for the second phase of the AD inflammatory skin reaction. Obviously, if the appropriate SAg is available on the skin surface, these cells could be activated in bulk, and thereby greatly augment the inflammatory process in such sites.

This hypothesis is supported by other clinical observations in SAg-associated disorders. Michie and Davis (1996) reported that in 14 of 68 patients that had survived toxic shock syndrome caused by TSST-1-producing *S. aureus*, a chronic eczematoid dermatitis developed during the first 2 wk of illness. This eczematoid dermatitis persisted for over 1 y after the acute illness, suggesting that SAg can trigger eczema. In the same study, a control group of patients with gram-negative septic shock did not develop eczema. TSST-1 may induce an upregulation of CLA on T cells present in the mucosal tissue, diverting these cells to the skin and initiating the eczema. Likewise, in Kawasaki syndrome, a disease in which SAg have been linked to its pathogenesis, an increased prevalence of AD has been reported in patients recovering from the illness (Brosius *et al*, 1988; Leung *et al*, 1993b).

The concept that SAg are involved in the pathogenesis of AD is supported by work from several other groups. Neuber *et al* (1996) showed a preferential over-representation of Vβ3, 8, and 12 positive T cells in lesional AD skin. These are T cells known to be SAg reactive. Moreover, the data also suggested a selective stimulation of Th 2 type T cells via SAg stimulation. It is also of interest to note that circulating IgE antibodies directed against SEA and SEB have been reported in several reports (Jozefckz, 1974; Leung *et al*, 1993a). Tada *et al* (1996) reported that up to 80% of AD patients have detectable circulating IgE directed against SAg. Thus, SAg may also act as allergens and trigger mast cell degranulation in AD patients. More recently, Herz *et al* (1998), investigated the role of SAg in allergic skin inflammation, by developing a humanized severe combined immuno-deficient mouse model. In this model, mice were reconstituted with PBMC from *Dermatophagoides pteronyssinus*-sensitive AD patients and nonatopic controls. Epicutaneous challenge with either *Dermatophagoides pteronyssinus* or SEB alone, triggered a modest T cell infiltrate of the dermis;

however, when these mice were challenged with both stimuli simultaneously, a significantly greater inflammatory skin infiltrate was observed. These data suggest that a synergistic mechanism exists between allergen and superantigenic stimulation of the immune system.

In our study, AD subjects without SAg-producing *S. aureus* lacked consistent expansions of T cells bearing SAg-reactive TCR, but did display elevations of T cells bearing other TCR. These elevations were present in either the $CD4^+$ or the $CD8^+$ T cell subsets, but not both. Some were visualized only in the CLA^+ memory T cell subset, whereas others, particularly the prominent expansions of $V\beta8^+$, $CD8^+$ T cells, were discernible within the overall T cell population. Sequence analysis of these Vβ8 expansions demonstrated oligoclonality. Taken together, these data suggest these expansions are antigen driven, and not related to novel SAg. Recent data have indicated that allergen-specific $CD4^+$ T cells are preferentially represented in the CLA^+ memory subset in AD patients (Santamaria-Babi *et al*, 1995), and thus it is possible that the $CD4^+$ CLA^+ subset expansions observed in this study represent allergen stimulated clones. Overall our data illustrate the heterogeneity of agents able to trigger or augment the inflammatory response in this common skin disease with involvement of both allergens and microbial SAg.

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