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D Y Leung, … , A E Trumble, M Kotb

Research Article

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Evidence for a Streptococcal Superantigen–driven Process in Acute Guttate Psoriasis

Donald Y. M. Leung,*‡ Jeffrey B. Travers,‖ Ralph Giorno,§ David A. Norris,‖ Robert Skinner,¶ Jacob Aelion,¶
Leslie V. Kazemi,* Michael H. Kim,** Anne E. Trumble,* Malak Kotb,¶ and Patrick M. Schlievert**

*Department of Pediatrics, The National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado 80206;
Departments of ‡Pediatrics, §Immunology, and ‖Dermatology, University of Colorado Health Sciences Center, Denver, Colorado 80262;
¶Departments of Medicine and Microbiology, University of Tennessee, Memphis, Tennessee 38163 and the Veteran's Administration
Medical Center, Memphis, Tennessee 38104; and **Department of Microbiology, University of Minnesota, Minneapolis,
Minnesota 55455

Abstract

Recent studies have suggested that T cells play a critical role in the pathogenesis of psoriasis. Guttate psoriasis is a well-defined form of psoriasis frequently associated with streptococcal throat infection. This study tested the hypothesis that T cells in acute guttate psoriasis skin lesions may be activated by streptococcal superantigens. Peripheral blood as well as lesional and perilesional skin biopsies were analyzed for T cell receptor Vβ repertoire using monoclonal antibodies against 10 different Vβ families. Skin biopsies from all patients with acute guttate psoriasis, but not skin biopsies from patients with acute atopic dermatitis or inflammatory skin lesions induced in normal subjects with sodium lauryl sulfate, demonstrated selective accumulation of Vβ2+ T cells ($P < 0.05$). The expansion of Vβ2+ T cells occurred in both the CD4+ and the CD8+ T cell subsets. Sequence analysis of T cell receptor β chain genes of Vβ2-expressing T cells from skin biopsies of patients with guttate psoriasis showed extensive junctional region diversity that is more compatible with a superantigen rather than a conventional (nominal) antigen-driven T cell response. All streptococcal isolates from patients with guttate psoriasis secreted streptococcal pyrogenic exotoxin C, a superantigen known to stimulate marked Vβ2+ T cell expansion. These data support the concept that acute guttate psoriasis is associated with superantigenic stimulation of T cells triggered by streptococcal superantigen(s). (J. Clin. Invest. 1995. 96:2106–2112.) Key words: psoriasis • superantigens • T cells • skin • Streptococci

Introduction

Psoriasis is a chronic inflammatory skin disorder associated with significant morbidity and occupational disability, affecting nearly 2% of the general population. The characteristic skin lesion in psoriasis consists of persistent erythematous plaques due to the infiltration of inflammatory cells and increased proliferation of keratinocytes (1). Immune activation, particularly of

T cells, is thought to play a critical role in the pathogenesis of psoriasis. This concept is supported by the following observations: First, histologic examination of early skin lesions has demonstrated that epidermal hyperplasia is preceded by the infiltration of T lymphocytes and macrophages into the skin (2). Furthermore, T lymphocyte clones grown from psoriatic lesions release growth factors that induce keratinocyte proliferation (3). Second, certain subtypes of psoriasis exhibit a strong association with particular HLA types (4). Third, immunosuppressive drugs which inhibit T cell activation and cytokine secretion such as anti-CD3, corticosteroids, and cyclosporine A are very effective in treating psoriasis (5).

Despite increasing data supporting the concept that psoriasis is immunologically mediated, the actual early events that trigger this common skin condition are poorly understood. Guttate psoriasis is a well-defined form of psoriasis which erupts suddenly and is frequently associated with a preceding history of streptococcal throat infection or a rise in serum antistreptococcal titers (6, 7). Due to the acute nature of guttate psoriasis and its strong association with streptococcal throat infection, this disease offers a unique opportunity to link the nature of the T cell activation in this subtype of psoriasis with a potential etiologic agent. Recent studies demonstrating that streptococcal M proteins and streptococcal pyrogenic exotoxins (SPE)[1] act as superantigens provide a potential mechanism by which streptococci could mediate the activation of skin-infiltrating T cells and monocytes in at least a subset of patients with psoriasis (8–10).

The term "superantigen" refers to a group of microbial antigens that cause the marked expansion of T cells expressing specific T cell receptor (TCR) β chain variable (Vβ) gene segments (for review see reference 10). In this regard, a recent report by Lewis et al. (11) described a marked overrepresentation of Vβ2+ T cells in the acute skin lesions of patients with guttate psoriasis. It is important to note, however, that although these data are consistent with a superantigenic process, it does not exclude the possible involvement of a conventional antigen. An important distinguishing feature between superantigens and conventional peptide antigens is that superantigens stimulate T cells almost solely through the Vβ portion of the TCR and therefore induce an expansion of both CD4+ and CD8+ T cells. Other variable elements (Dβ, Jβ, Vα, Jα) of the TCR are not generally involved in superantigen recognition. This is markedly different from T cells activated by most conventional peptide antigens which frequently results in an expansion of only a subset of CD4+ or CD8+ Vβ-expressing cells with limited

1. Abbreviations used in this paper: SPE, streptococcal pyrogenic exotoxins; SSA, streptococcal superantigen; TCR, T cell receptor; Vβ, variable region of the TCR β chain.

TCR junctional diversity. The present study was therefore carried out to determine whether Vβ2+ T cell infiltration could be confirmed in an independent population of patients with acute guttate psoriasis from whom streptococcal isolates were characterized and to assess whether this increased Vβ2 expression was consistent with other features of superantigen stimulation.

Methods

Patients. 10 adult patients (6 females and 4 males) with acute guttate psoriasis were studied. Their age ranged from 26 to 41 yr and their extent of skin disease ranged from 20 to 70% of their body surface area. Acute guttate psoriasis was defined as the sudden eruption of small (< 2 mm) erythematous macules which evolved into the parakeratotic maculopapules of psoriasis. In the majority of patients, the onset of the rash was preceded by a sore throat associated with either a positive throat culture for group A β-hemolytic streptococcus or elevated serum streptozyme titers. Their guttate psoriasis improved or completely cleared after oral antibiotic therapy. Blood as well as skin biopsies of untreated lesional skin and uninvolved perilesional skin of early acute guttate lesions (< 1 wk old) were taken from 8 of the 10 patients. Perilesional biopsies were obtained 1 cm from the skin lesion. Streptococcal isolates, but not skin biopsies, were obtained from the remaining two patients. As a control for inflammatory skin lesions, lesional and perilesional skin biopsies were procured from six adult patients with atopic dermatitis diagnosed according to the Hanifin and Rajka criteria (12). In addition, biopsies were obtained of irritant skin reactions induced in six normal subjects by sodium lauryl sulfate. In these experiments, 20 μl of 2.5% (vol/vol) solutions of sodium lauryl sulfate (Sigma Immunochemicals, St. Louis, MO) were placed in 8-mm-diameter Finn chambers (Epitest Ltd., Oy, Finland), applied to the volar forearm of six normal volunteers, and biopsied after 48 h. Informed consent was obtained from all patients before performing all studies.

Characterization of streptococcal isolates. Streptococci isolated from the pharynx of patients with guttate psoriasis were typed for M and T proteins by the World Health Organization Collaborating Center for Reference and Research on *Streptococci* at the University of Minnesota using standard techniques (13). The presence of the SPEA and SPEC genes encoding for SPE proteins was determined by PCR as described previously (14). Inasmuch as all group A streptococcal strains harbor the SPEB gene, its detection by PCR would be noninformative. Thus, the expression of SPEB was determined by Ouchterlony immunodiffusion assay as described previously (15).

mAbs. mAb Leu4 (CD3; Peridin chlorophyll-α protein, i.e., PerCP), Leu3a (CD4; FITC, PE), Leu2a (CD8; PE), G1CL (mouse IgG1 control; FITC, PE), and G2GL (mouse IgG2 control; FITC, PE) were obtained from Becton Dickinson Immunocytometry Systems (San Jose, CA). Biotinylated or unconjugated mAbs specific for TCR Vβ2, Vβ13.1, Vβ17, and Vβ19 were obtained from Immunotech (Westbrook, ME); mAbs directed to TCR Vβ3, Vβ5.1, Vβ5.2, Vβ6.7, Vβ8.1, and Vβ12 were obtained from T Cell Sciences (Cambridge, MA). For tissue staining, an IgG anti-Vβ2 antibody was used (kind gift from Prof. Arthur Boylston, University of Leeds, Leeds, United Kingdom).

Assessment of peripheral blood T cell repertoire. PBMC were isolated from heparinized blood by Ficoll-Hypaque density gradient centrifugation. TCR Vβ repertoire on freshly isolated PBMC were assessed by two- or three-color immunofluorescence on a FACScan® flow cytometer (Becton Dickinson Immunocytometry Systems) using FITC, PE, and PerCP as the three fluorescent parameters as described previously (16–18). List mode multiparameter data files (each file with forward scatter, side scatter, and three fluorescent parameters) were analyzed using the LYSIS II Windows program. Negative control reagents were used to verify the staining specificity of antibodies. The percentage of T cells bearing each TCR Vβ phenotype was expressed as a percentage of total T (CD3+) cells.

Immunohistochemistry. Skin biopsies placed in OCT compound (Miles, Inc., Elkhart, IN) were flash frozen in liquid nitrogen and stored

at −80°C. 4 mm cryostat sections were cut, dehydrated in acetone for 10 min, and air dried. Sections were incubated for 30–60 min at room temperature with the respective anti-Vβ mAb or anti-CD mAb. Sections were then washed and stained by the labeled avidin biotin method as described previously (19, 20) using peroxidase-labeled streptavidin (Dako Corp., Carpinteria, CA). Afterwards, sections were counterstained with hematoxylin and mounted. For two-color staining, the sequential staining method developed by Wagner and Worman (21) was used with anti-Vβ staining demonstrated by fast blue BB-naphthol AS-MX, and anti-CD4 or CD8 staining demonstrated by fast red TR-naphthol AS-BI phosphate. Control antibodies were tested on step sections of the same tissue specimens. The negative staining controls were isotype-specific mouse immunoglobulins with irrelevant specificity. Normal human tonsil sections were used as positive controls for Vβ T cell staining and CD antibodies. All lymphocyte counts of stained sections were performed without knowledge of the subject's identity or condition. Two different investigators evaluated the skin biopsies. Interobserver variation was < 5%. Results are expressed as a percentage of CD3, CD4, or CD8 reactive T cells.

Cloning and sequencing of TCR β chain junctional regions. Total RNA (5–10 μg) was extracted from homogenized skin biopsies by using Ultraspec RNA isolation solution (Biotecx Laboratories Inc., East Houston, TX). Reverse-transcribed total RNA (0.2 μg; cDNA) was amplified by PCR using primers specific for the Vβ2 TCR family and for the 3′ Cβ primer as previously described (16, 17). The sequences of the primers were 5′-GGGAATTCTCATCAACCATGCAAGCCT-GACCT-3′ for Vβ2, and 5′-GGGATCCTTCTGATGGCTCAAACAC-3′ for Cβ. The Vβ and Cβ primers carried EcoRI or BamHI sites, respectively (underlined). After 35 cycles of PCR, amplified β chain fragments were extracted with phenol-chloroform and precipitated with ethanol. Purified fragments were then digested with EcoRI and BamHI, ligated into plasmid pTZ18R (Pharmacia LKB Biotechnology Inc., Piscataway, NJ), and cloned. Double-stranded plasmid DNAs were prepared by alkali-lysis method and acid phenol extraction and were sequenced by Sequenase Ver2.0 kit (United States Biochemical, Cleveland, OH) following the manufacturer's recommendations (22).

Statistical analysis. Since the distribution of TCR Vβ expression often exhibited departure from normality, analyses were based on the natural logarithm of the T cell scores. Site (lesion versus perilesion) dependent differences in T cell expression for the guttate psoriasis group for each Vβ family and comparisons between the three study groups on lesion and perilesion site differences were made using repeated-measures ANOVA. Fisher's protected least significant difference was used to evaluate significant differences between pairs of means. The proportionate difference in SPEC positive-production between the present guttate psoriasis *streptococci* and toxic shock syndrome *streptococci* was assessed with a χ^2 test. *P* values of < 0.05 were considered statistically significant.

Results

To determine whether acute guttate psoriasis is associated with the expression of a particular TCR Vβ family, T lymphocytes in peripheral blood and frozen skin sections from eight patients with guttate psoriasis were analyzed for reactivity with mAbs specific for 10 TCR Vβ families (Fig. 1). The T cell repertoire in the peripheral blood of these patients was similar to our previously published values on normal subjects (16, 17) and therefore did not demonstrate any evidence for expansion of T cell populations expressing particular TCR Vβ gene segments.

As compared with peripheral blood T cell repertoire, however, a marked accumulation of Vβ2+ T lymphocytes was observed in either the perilesional or acute skin lesions of all patients with guttate psoriasis. A representative photomicrograph of anti-Vβ2 and anti-CD3 staining is shown in Fig. 2. Of interest in six of the eight patients, Vβ2+ T cell expression was greater in perilesional skin as compared with acute skin
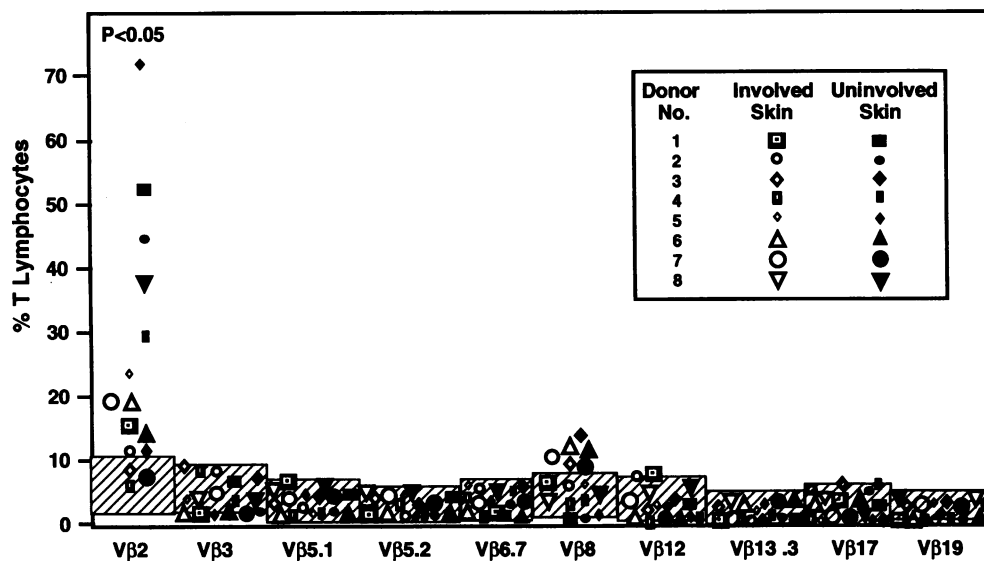
Figure 1. TCR Vβ expression in lesional versus perilesional skin of patients with guttate psoriasis. The cross-hatched areas represent the normal range (mean$\pm$2 SD) of Vβ families in the peripheral blood of patients with guttate psoriasis. Vβ2 was the only Vβ family that demonstrated a significant increase in lesional ($P < 0.05$) or perilesional skin ($P < 0.05$) as compared with peripheral blood.

lesions. In two of the patients > 50% of the infiltrating T cells were Vβ2+, suggesting an important role for these T cells in the pathogenesis of guttate psoriasis. Occasional elevations were observed in the Vβ8 family (three patients). However, as a group, only the Vβ2 family demonstrated a significant expansion ($P < 0.05$).

To determine the specificity of Vβ2 expansion in guttate psoriasis, we analyzed the expression of Vβ2 in skin biopsies from patients with acute atopic dermatitis or inflammatory reactions induced by 48-h sodium lauryl sulfate skin tests on normal individuals. As shown in Fig. 3, biopsies of perilesional and lesional skin of patients with acute guttate psoriasis demonstrated significantly higher ($P < 0.01$) percentages of Vβ2+ T cells than biopsies of perilesional and lesional skin of patients with atopic dermatitis or sodium lauryl sulfate–induced irritant skin reactions.

Superantigens generally stimulate T cells that bear a particular Vβ element independent of other cell surface proteins. Thus, superantigens usually stimulate TCR Vβ expansion in both the CD4+ and CD8+ T cell subsets. We therefore analyzed the Vβ repertoire in CD4+ and CD8+ T cells by a two-color alkaline phosphatase staining technique in frozen skin biopsies

from patients with guttate psoriasis. As compared with peripheral blood, there was an increased percentage of Vβ2+ T cells in both the CD4 and the CD8+ T cell subsets of the skin infiltrate (Table I). Overrepresentation of Vβ2+ T cells was observed in both the epidermis and the dermis.

Another important characteristic of superantigenic stimulation, as compared with stimulation by conventional peptide antigens, is that superantigens stimulate T cells almost exclusively through the Vβ portion of the TCR and therefore induce an expansion of T cells independent of their TCR junctional regions (for review see reference 10). This is different from T cells activated by most conventional peptide antigens which frequently results in an expansion of only a subset of Vβ-expressing cells with limited TCR junctional diversity.

To test the hypothesis that guttate psoriasis is triggered by a superantigen, we cloned and analyzed the sequences of random cDNA clones containing PCR-amplified Vβ2 chain genes of T cells from the skin of three patients with acute guttate psoriasis. In these experiments, RNA was directly extracted from skin biopsies of three patients in whom between 34 and 71% of T cells expressed Vβ2 as determined by mAb reactivity with immunoperoxidase staining (Fig. 1). 60 Vβ2 cDNA clones
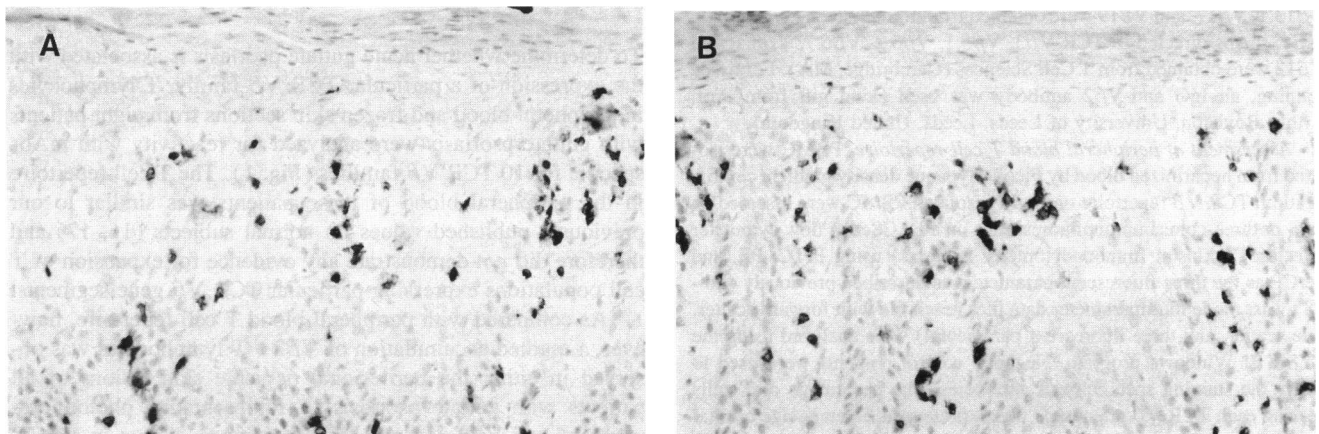

Figure 2. Frozen tissue sections from lesional skin biopsy of patient with guttate psoriasis stained with either monoclonal anti-Vβ2 (*A*) or anti-CD3 (*B*) and counterstained with hematoxylin. Most of the T cells in the epidermal and dermal cell infiltrate reacted with anti-Vβ2. ×256.
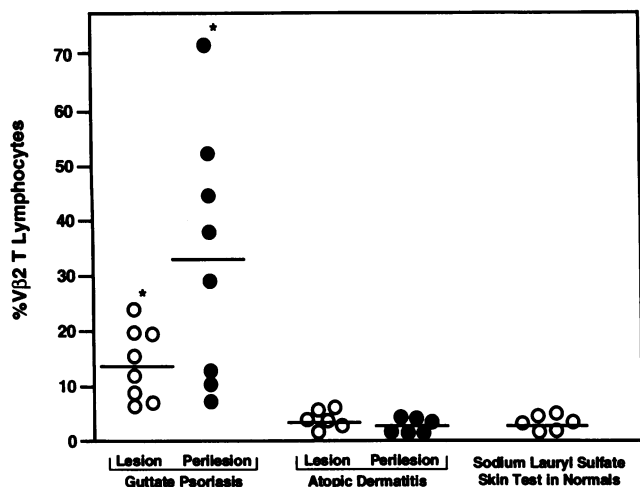
Figure 3. A comparison of T cell receptor Vβ2 expression in lesional versus perilesional skin of patients with guttate psoriasis versus atopic dermatitis, or sodium lauryl sulfate–induced skin test reactions in normal subjects. * As a group the Vβ2 expression in skin biopsies from patients with guttate psoriasis was significantly greater than in biopsies from the other two study groups ($P < 0.01$).

were derived from these 3 patients. Since there was a 3–7-fold increase in Vβ2+ T cell expression in their skin as compared with peripheral blood, in a clonotypic expansion of Vβ2+ T cells, 67–87% of the clones would be expected to have identical junctional sequences. However, as shown in Table II, none of the Vβ2 clones had the same junctional sequence, and considerable diversity among the sequences within each individual was apparent. The CDR3 regions varied greatly in terms of residue number and sequence, and a conserved motif was not apparent.

Finally, we isolated group A *Streptococci* from seven patients with acute guttate psoriasis. As shown in Table III, there was no common M or T type among these isolates. Furthermore, these isolates did not consistently produce either SPEA or SPEB. However, all seven isolates produced SPEC, a superantigen known to induce the expansion of T cells expressing the Vβ2 gene segment (22). Of interest, several of the isolates also secrete SPEA or SPEB which are known to stimulate Vβ8+ T cell expansion (9, 23). As a comparison, we have previously analyzed 34 consecutive isolates of group A *streptococci* from toxic shock syndrome patients (24) and 75 isolates from streptococcal infections other than toxic shock syndrome (25). The streptococcal toxic shock syndrome isolates predominantly expressed M protein types 1 and 3, and consistently produced SPEA and SPEB, but only occasionally produced SPEC. Using a χ^2 analysis to compare the proportion of SPEC-producing *streptococci* in our guttate psoriasis patients versus the previous group of *streptococci* from toxic shock syndrome patients (24), a significant difference was observed between these two groups of isolates ($P < 0.0001$). Of the 75 isolates from a variety of other streptococcal diseases only 30% made SPEA, 63% made SPEB, and 67% made SPEC (25).

Taken together, these data suggest that *streptococci* isolated from guttate psoriasis versus toxic shock syndrome have significantly different profiles of superantigen expression and that isolates associated with guttate psoriasis are more like isolates from other streptococcal infections. The complete nucleotide sequence of SPEC from one of the guttate psoriasis isolates was also determined (data not shown). The translated amino

acid sequence was the same as that previously seen for SPEC (26).

Discussion

Previous investigations have identified a number of factors which may play a role in the pathogenesis of psoriasis. These include keratinocyte proliferation, vascular endothelial activation, T lymphocyte activation, neutrophil infiltration, and activation of dermal macrophages. Many of these pathologic features, however, likely reflect downstream events which have occurred as the result of immune activation and skin inflammation. Indeed, careful histologic examination of early psoriatic lesions has demonstrated that activation of T lymphocytes, endothelial cells, and macrophages precedes epidermal proliferation (2). Furthermore, immunosuppressive drugs such as cyclosporin and FK506, whose primary action is the selective inhibition of cytokine production by activated T cells, are effective in treating psoriasis (5). Treatment with anti–T cell mAbs has also been found to clear psoriatic lesions (27). Conversely, T cell clones derived from psoriatic skin lesions can promote keratinocyte proliferation in vitro (3).

The mechanisms by which T cells, keratinocytes, and macrophages achieve and maintain their activated state in psoriatic skin lesions are poorly understood. Although multiple factors may contribute to the exacerbation of psoriasis, there are many reports suggesting that bacterial infection can trigger this illness (6, 7, 28, 29). The relationship between bacterial infection and exacerbation of psoriasis is perhaps most clearly demonstrated in guttate psoriasis where there are multiple reports demonstrating that acute exacerbation of guttate psoriasis, in the majority of patients, is preceded by antecedent infection with group A *streptococci* and rises in serum antistreptococcal titers (6, 7). Patients with this form of psoriasis frequently improve with systemic antibiotic therapy (30).

In support of these clinical observations, Cole and Wuepper (31) first reported that alcohol precipitates of cell-free culture filtrates from *Streptococcus pyogenes* (strain NY-5) induced increased keratinocyte proliferation after intradermal injection into rabbit skin. These culture filtrates also had marked mitogenic activity upon human cultured lymphoid cells and are now known to have contained high levels of the streptococcal superantigens, SPEA, B, and C. Subsequently, it was reported that

Table I. TCR Vβ2 Expression on CD4$^+$ versus CD8$^+$ T Cell Subsets in Guttate Psoriasis

Patient	Percent Vβ2$^+$ in the CD4$^+$ subset			Percent Vβ2$^+$ in the CD8$^+$ subset		
	Dermis	Epidermis	PBMC	Dermis	Epidermis	PBMC
1	30	43	5	21	30	3
2	26	41	7	27	30	4
3	12	40	8	14	20	6
4	27	36	8	24	ND	7
Mean±SD	24±4*	40±1*	7±1	22±3*	27±3*	5±1

* $P < 0.05$ as compared with percent Vβ2+ T cells in the corresponding peripheral blood T cell subset. Patient 4 had a predominance of CD4+ T cells in her epidermal infiltrate and therefore too few CD8+ cells to count.

Table II. Analysis of TCR β Chain Junctional Sequences Containing Vβ2 Gene Segments from Three Patients with Guttate Psoriasis*

Psoriasis patient 1				Psoriasis patient 2				Psoriasis patient 3			
Vβ	NDβN	Jβ		Vβ	NDβN	Jβ		Vβ	NDβN	Jγ	
CSAR	RD	TEAF	(1.1)	CSAR	AIPA	TEAF	(1.1)	CSA	VK	TEAF	(1.1)
CSAR	VGI	YGYT	(1.2)	CSA	ATRQ	TEAF	(1.1)	CSA	VAM	TEAF	(1.1)
CSA	FY	YGYT	(1.2)	CSA	RS	TEAF	(1.1)	CSAR	L	TEAF	(1.1)
CSA	RSL	EKL	(1.4)	CSA	HWA	YGYT	(1.2)	CSA	KREG	YGYT	(1.2)
CSA	ATRK	SPLH	(1.6)	CSA	DT	YGYT	(1.2)	CSA	LNRAD	NTIY	(1.3)
CSA	SRDQS	NEQF	(2.1)	CSA	NNAE	YGYT	(1.2)	CSA	LGTM	NEKL	(1.4)
CSA	PGPC	NEQF	(2.1)	CSAR	QLSEGG	NTIY	(1.3)	CSA	H	NEKL	(1.4)
CSAR	TQEC	NEQF	(2.1)	CSA	RSGT	NEKL	(1.4)	CSAR	EGRP	NQPQH	(1.5)
CSA	GISW	NEQF	(2.1)	CSAR	PTAR	SYNEQF	(2.1)	CSAR	IGGA	NEQF	(2.1)
CSAR	ERAY	NEQF	(2.1)	CSA	TNA	SYNEQF	(2.1)	CSA	APSLQ	NEQF	(2.1)
CSA	VSTR	NEQF	(2.1)	CSA	KRPSG	SYNEQF	(2.1)	CSA	VTRG	NEQF	(2.1)
CSAR	LRFY	NEQF	(2.1)	CSA	GTNRLG	SYNEQF	(2.1)	CSAR	WYGA	NEQF	(2.1)
CSA	PSGGA	GELF	(2.2)	CSA	G	SYNEQF	(2.1)	CSAR	KG	TGELF	(2.2)
CSAR	GGVG	ETQY	(2.5)	CSA	MHS	SYNEQF	(2.1)	CSA	IL	NIQY	(2.4)
CSAR	VSASN	ETQY	(2.5)	CSA	EETQSL	TGELF	(2.2)	CSA	AES	ETQY	(2.5)
CSAR	HAT	ETQY	(2.5)	CSA	VVTQLRG	GELF	(2.2)	CSA	PQN	ETQY	(2.5)
CSAR	IVGLG	ETQY	(2.5)	CSAR	QRAV	TDTQ	(2.3)	CSAR	ARYY	GANVL	(2.6)
CSA	PGLAK	ETQY	(2.5)	CSA	MVALG	NIQY	(2.4)	CSA	VK	GANVL	(2.6)
CSA	GDRGE	SYEQY	(2.7)	CSAR	DERYGG	QETQY	(2.5)	CSA	TQEG	SYEQY	(2.7)
CSAR	VQEV	SYEQY	(2.7)	CSA	LWHT	QETQY	(2.5)				

* Results are expressed as derived amino acid sequences.

psoriatic lesions could be induced by intradermal injection of small amounts of killed streptococcal material (28). Furthermore, in vitro, both guttate and chronic plaque psoriasis patients have been shown to have enhanced T lymphocyte responses to group A streptococcal antigens (32). These observations suggested the hypothesis that bacterial toxins derived from *streptococci*, acting as superantigens, could mediate the activation of infiltrating T cells and monocytes in at least a subset of patients with psoriasis.

In this report, we have demonstrated that there is selective accumulation of Vβ2+ T cells infiltrating into the perilesional and lesional skin of patients with guttate psoriasis. This bias for the use of Vβ2-encoded gene products was not seen in perilesional or lesional skin from patients with atopic dermatitis, or inflammatory skin lesions induced by sodium lauryl sulfate in normal subjects. No increases in Vβ2+ T cells have been

Table III. Characteristics of Group A Streptococci Isolates from Throat Cultures of Patients with Guttate Psoriasis

	Typing		Pyrogenic exotoxins		
Patient	M	T	SPEA	SPEB	SPEC
1	4	4	Negative	Negative*	Positive
2	1	1	Positive	Negative*	Positive
3	Nontypeable	12	Positive	Positive	Positive
4	18	27	Negative	Negative	Positive
5	Unknown	Unknown	Negative	Negative	Positive
6	Unknown	Unknown	Negative	Negative	Positive
7	4	4	Negative	Negative	Positive

* Variable expression of SPEB.

observed in normal skin although Vβ1, -7, -14, and -16+ T cells have been reported to be frequently highly expressed in normal skin relative to peripheral blood (33). Thus, the observed Vβ2+ T cells in guttate psoriasis skin lesions do not appear to be due to nonspecific skin inflammation.

We used immunocytochemistry and mAbs directed against specific TCR Vβ families to avoid the criticism of selection bias that has been associated with use of semiquantitative PCR techniques to analyze Vβ repertoire. The 10 mAbs used in this study detect ~ 50% of the T cell repertoire. Therefore, a potential criticism of our study is that we could have missed the increase of a Vβ family that was not stained for with our panel of mAbs. This would appear unlikely because Vβ2+ T cells accounted for over 50% of the T cell infiltrate in some lesions. Furthermore, in selected skin lesions, we have used a previously published semiquantitative PCR technique to analyze TCR repertoire in guttate psoriasis lesions and found primarily overexpression of the Vβ2 gene product (Trumble, A. E., and D. Y. M. Leung, unpublished observations).

Our data are consistent with a report by Lewis et al. (11) who also described a increased expression of Vβ2+ T cells in the acute skin lesions of patients with guttate psoriasis. In our study, however, we provide further support for the concept that the Vβ2+ T cell expansion in guttate psoriasis is the result of superantigen activation. In this regard, we have found that there was selective expansion of Vβ2+ cells in both the CD4 and the CD8+ infiltrating T cells in the dermis and epidermis of guttate psoriasis skin (Table I). More importantly, sequence analysis of TCR β chain genes of Vβ2-expressing T cells in the skin from patients with guttate psoriasis showed extensive junctional region diversity (Table II).

In view of the marked selective expansion of Vβ2+ T cells in acute guttate psoriasis, the extensive β chain junctional diver-

sity suggests that these Vβs play a dominant role in recognition. This type of recognition is not characteristic of responses to most peptide antigens (10). A complex response to multiple antigens or epitopes seems unlikely since multiple Vβ regions would be expected to be involved. Taken together, this high frequency of response and the prominent role for Vβ2 are most consistent with the hypothesis that T cell activation during the acute phase of guttate psoriasis is mediated by a superantigen which induces Vβ2 expansion.

To identify a superantigen which might be involved in the activation of Vβ2+ T cell activation in guttate psoriasis, we analyzed streptococcal isolates from these patients by M typing and secretion of pyrogenic exotoxins A, B, and C. Similar to previous studies, streptococci from our patients did not express any consistent M protein type (34). Some of the isolates secreted SPEA or SPEB, which could explain the expansion of Vβ8 seen in some patients. Interestingly, all streptococci secreted SPEC, a superantigen known to stimulate the marked expansion of Vβ2+ T cells (23). We did not test isolates in this study for two recently described superantigens, streptococcal superantigen (SSA) and SPEF (35, 36). It is highly unlikely that SSA was made by all of the isolates in this study since it has been shown that only selected M types of streptococci, not including M1 and M18, for example, make SSA. In contrast, all of the isolates in our study likely also made SPEF since in addition to SPEC all isolates tested in a previous study (36) from a variety of streptococcal diseases made SPEF (35). SPEF stimulates T cells bearing Vβ2 as well as Vβ4, 8, 15, and 19. However, the consistent expression of SPEC by streptococci from guttate psoriasis patients suggests that it may have an important role in the pathogenesis of this disease.

Although a remarkable accumulation of Vβ2+ T cells has now been reproducibly found in independent studies to occur in the epidermis and dermis of patients with guttate psoriasis, analyses of T cell repertoire in the skin lesions of patients with chronic plaque psoriasis have yielded inconsistent results. In one study, Chang et al. (37) found that CD8+, but not CD4+, T cells in the epidermis of psoriatic plaques from five patients preferentially used Vβ3 and/or Vβ13.1 genes. Sequence analyses of these two Vβ genes demonstrated monoclonality or marked oligoclonality. In two patients in which Vβ3 and/or Vβ13.1 was not increased, an increase in Vβ17 gene use and clonality was found. In a separate study, by Boehncke et al. (38), only three of nine patients with chronic plaque psoriasis showed evidence of TCR Vβ skewing of their skin-infiltrating T cells. In these cases, there was a marked accumulation of intraepidermal Vβ19 and/or Vβ22+ T cells. In contrast, the study by Lewis et al. (11) reported a predominance of Vβ2+ T cells in the skin infiltrate of the majority of patients with chronic plaque psoriasis. These data suggest that the host factors and immunologic triggers likely differ in guttate versus chronic plaque psoriasis.

In the case of acute guttate psoriasis, induction of psoriatic lesions occurs after pharyngeal infection with superantigen-secreting streptococci. To date, we have been unable to recover streptococci or staphylococci from the local skin lesions of these patients (Leung, D. Y. M., J. B. Travers, and D. A. Norris, unpublished observations). Since these patients do not develop toxic shock and there is no evidence for Vβ expansion of circulating T cells, we postulate that after streptococcal pharyngitis, skin-seeking T cells are induced in lymph nodes draining the pharynx, i.e., at a site distant from the skin. After activation, these T cells then home to the skin via (CLA)/E-selectin inter-

actions and may be locally activated further in psoriatic patients by a skin-specific antigen that is recognized by Vβ2+ T cells. This hypothesis is consistent with our observation that the percentage of Vβ2+ T cells in the perilesional skin was higher than that found in the actual guttate psoriasis lesion. In further support of this concept, we have also found recently that SPEC and other bacterial superantigens potently induce the expression of the skin homing receptor (CLA) on T lymphocytes, in an IL-12–dependent manner (18).

Nearly 70% of patients with guttate psoriasis develop chronic plaque psoriasis. In such patients, superantigens may activate skin-infiltrating autoreactive T cells which remain persistently activated due to the abnormal recognition of specific skin antigens (39). The additional factors which determine whether patients have self-limited guttate psoriasis or develop chronic plaque psoriasis are unknown but presumably depend in large part on host genetic factors which are involved in antigen-binding, e.g., HLA molecules, as well as the actual antigen-triggering T cell activation. Indeed, chronic psoriasis has been associated with the HLA-Cw6 allele and a raised incidence of DR7 (4). Recent oligonucleotide typing studies have also revealed a strong association with the extended HLA-DRB1*0701/2,DQA1*0201,DQB1*0303 haplotype (40).

The previous reports documenting the involvement of CD8+ T cells bearing different TCR Vβs, that are clonally expanded in chronic plaque psoriasis, suggest that these T cells have been expanded in situ in response to an antigen(s) in the skin. Potential candidate skin autoantigens include keratins (41) or carbohydrates (42), which have crossreactive determinants with bacterial antigens. Alternatively, some bacterial-derived superantigen may be accumulated by either cutaneous professional (43) or nonprofessional antigen (44) presenting cells and thereby may locally activate the T cells to induce keratinocyte proliferation. Of interest, there have been reports of patients with plaque psoriasis who had exacerbation of their skin disease after cutaneous infection with superantigen-secreting S. aureus or Candida albicans (20). Since the perilesional skin, which has increased Vβ2+ T cells, does not necessarily become a lesion, there is probably a second event in the skin which is needed for complete evolution of the clinical psoriatic skin lesion.

In conclusion, our study provides strong evidence that guttate psoriasis is triggered by streptococcal superantigen(s). The extent to which other types of psoriasis are also induced by superantigens requires further study. It is highly likely, however, that psoriasis will be associated with a number of microbial and nonmicrobial triggers. The inability to find microbial triggers for other forms of chronic psoriasis may stem from the lack of a clear starting point to their disease. Acute guttate psoriasis may therefore be an important model to examine early events in the triggering of psoriasis. Identification of the T cells and triggers involved in psoriasis will be critical for the examination of early upstream events involved in its pathogenesis and allow the development of more effective and specific therapeutic targets in this important skin disorder.

Acknowledgments

References

1. Nickoloff, B. J. 1991. The cytokine network in psoriasis. *Arch. Dermatol.* 127:871–884.

2. Krueger, J. G., J. F. Krane, D. M. Carter, and A. B. Gottlieb. 1990. Role of growth factors, cytokines, and their receptors in the pathogenesis of psoriasis. *J. Invest. Dermatol.* 94:135s–140s.

3. Strange, P., K. D. Cooper, E. R. Hansen, G. Fisher, J. K. Larsen, D. Fox, C. Krag, J. J. Voorhees, and O. Baadsgaard. 1993. T-lymphocyte clones initiated from lesional psoriatic skin release growth factors that induce keratinocyte proliferation. *J. Invest. Dermatol.* 101:695–700.

4. Baker, B. S., A. F. Swain, L. Fry, and H. Valdimarsson. 1984. Epidermal T lymphocytes and HLA-DR expression in psoriasis. *Br. J. Dermatol.* 110:555–564.

5. Ellis, C. N., D. C. Gorsulowsky, T. A. Hamilton, J. Billings, M. Brown, J. Headington, K. Cooper, O. Baadsgaard, E. Duell, and T. Annesley. 1986. Cyclosporine improves psoriasis in a double-blind study. *JAMA (J. Am. Med. Assoc.)* 256:3110–3116.

6. Henderson, C. A., and A. S. Highet. 1988. Acute psoriasis associated with Lancefield group C and group G cutaneous streptococcal infections. *Br. J. Dermatol.* 118:559–562.

7. Whyte, J. H., and R. D. Baughman. 1964. Acute guttate psoriasis and streptococcal infection. *Arch. Dermatol.* 89:350–356.

8. Tomai, M., M. Kotb, G. Majumdijar, and E. H. Beachey. 1990. Superantigenicity of streptococcal M protein. *J. Exp. Med.* 172:359–362.

9. Abe, J., J. Forrester, T. Nakahara, J. A. Lafferty, B. L. Kotzin, and D. Y. M. Leung. 1991. Selective stimulation of human T cells with streptococcal erythrogenic toxins A and B. *J. Immunol.* 146:3747–3750.

10. Kotzin, B. L., D. Y. M. Leung, J. Kappler, and P. Marrack. 1993. Superantigens and human disease. *Adv. Immunol.* 54:99–166.

11. Lewis, H. M., B. S. Baker, S. Bokth, A. V. Powles, J. J. Garioch, H. Valdimarsson, and L. Fry. 1993. Restricted T-cell receptor Vβ gene usage in the skin of patients with guttate and chronic plaque psoriasis. *Br. J. Dermatol.* 129:514–520.

12. Hanifin, J. M., and G. Rajka. 1980. Diagnostic features of atopic dermatitis. *Acta Dermatol-Venereol. (Stockh.).* 92:44–47.

13. Stevens, D. L., M. H. Tanner, J. Winship, R. Swarts, K. M. Ries, P. M. Schlievert, and E. Kaplan. 1989. Severe group A streptococcal infections associated with a toxic shock-like syndrome and scarlet fever toxin A. *N. Engl. J. Med.* 321:1–7.

14. Tyler, S. D., W. M. Johnson, and J. C. Huang. 1992. Streptococcal erythrogenic toxin genes: detection by polymerase chain reaction and association with disease in strains isolated in Canada from 1940 to 1991. *J. Clin. Microbiol.* 30:3127–3131.

15. Lee, P. K., and P. M. Schlievert. 1989. Quantification and toxicity of group A streptococcal pyrogenic exotoxins in an animal model of toxic shock syndrome-like illness. *J. Clin. Microbiol.* 27:1890–1894.

16. Abe, J., B. L. Kotzin, K. Jujo, M. E. Melish, M. P. Glode, T. Kohsaka, and D. Y. M. Leung. 1992. Selective expansion of T cells expressing T cell receptor variable regions Vβ2 and Vβ8 in Kawasaki disease. *Proc. Natl. Acad. Sci. USA.* 89:4066–4070.

17. Abe, J., B. L. Kotzin, C. Meissner, M. E. Melish, T. Masato, D. Fulton, F. Romagne, B. Malissen, and D. Y. M. Leung. 1993. Characterization of T cell repertoire changes in acute Kawasaki disease. *J. Exp. Med.* 177:791–796.

18. Leung, D. Y. M., M. Gately, A. Trumble, B. Ferguson-Darnell, P. M. Schlievert, and L. J. Picker. 1995. Bacterial superantigens induce T cell expression of the skin-selective homing receptor, the cutaneous lymphocyte-associated antigen (CLA). *J. Exp. Med.* 181:747–753.

19. Giorno, R. 1984. Technical considerations in immunohistology of lymphoid cell membrane antigens. *Surv. Synth. Pathol. Res.* 3:165–188.

20. Leung, D. Y. M., P. Walsh, R. Giorno, and D. A. Norris. 1993. A potential role for superantigens in the pathogenesis of psoriasis. *J. Invest. Dermatol.* 100:225–228.

21. Wagner, L., and C. P. Worman. 1988. Color-contrast staining of two different lymphocyte subpopulations: a two-color modification of alkaline phosphatase monoclonal anti-alkaline phosphatase complex technique. *Stain Technol.* 63:129–136.

22. Toneguzzo, F., S. Glynn, E. Levi, S. Mjolsness, and A. Hayday. 1988. Use of a chemically modified T7 DNA polymerase for manual and automated sequencing of supercoiled DNA. *Biotechniques.* 6:460–469.

23. Tomai, M. A., P. M. Schlievert, and M. Kotb. 1992. Distinct T-cell receptor V beta gene usage by human T lymphocytes stimulated with the streptococcal pyrogenic exotoxins and pep M5 protein. *Infect. Immun.* 60:701–705.

24. Hauser, A. R., D. L. Stevens, E. L. Kaplan, and P. M. Schlievert. 1991. Molecular analysis of pyrogenic exotoxins from *Streptococcus pyrogenes* isolates associated with toxic shock-like syndrome. *J. Clin. Microbiol.* 29:1562–1567.

25. Schlievert, P. M., K. M. Bettin, and D. W. Watson. 1979. Production of pyrogenic exotoxin by groups of streptococci: association with group A. *J. Infect. Dis.* 140:676–680.

26. Kapur, V., K. Nelson, P. M. Schlievert, R. K. Selander, and J. M. Musser. 1992. Molecular population genetic evidence of horizontal spread of two alleles of the pyrogenic exotoxin C gene (*speC*) among pathogenic clones of *Streptococcus pyogenes*. *Infect. Immun.* 60:3513–3517.

27. Morel, P., J. P. Revillard, J. F. Nicolas, J. Wijdenes, H. Rizova, and J. Thivolet. 1992. Anti-CD4 monoclonal antibody therapy in severe psoriasis. *J. Autoimmun.* 5:465–477.

28. Rosenberg, E. W., and P. W. Noel. 1988. The Koebner phenomenon and the microbial basis of psoriasis. *J. Am. Acad. Dermatol.* 18:151–158.

29. Marples, R. R., C. L. Heaton, and A. M. Kligman. 1973. *Staphylococcus aureus* in psoriasis. *Arch. Dermatol.* 107:568–570.

30. Rosenberg, E. W., P. W. Noah, M. D. Zanolli, R. B. Skinner, M. J. Bond, and N. Crutcher. 1986. Use of rifampin with penicillin and erythromycin in the treatment of psoriasis. *J. Am. Acad. Dermatol.* 14:761–764.

31. Cole, G. W., and K. D. Wuepper. 1978. Isolation and partial characterization of a keratinocyte proliferative factor produced by *Streptococcus pyogenes* (strain NY-5). *J. Invest. Dermatol.* 71:219–223.

32. Baker, B. S., S. Bokth, A. Powles, J. J. Garioch, H. Lewis, H. Valdimarsson, and L. Fry. 1993. Group A streptococcal antigen-specific T lymphocytes in guttate psoriatic lesions. *Br. J. Dermatol.* 128:439–499.

33. Dunn, D. A., A.-S. Gadenne, S. Simha, E. A. Lerner, M. Bigby, and P. A. Bleicher. 1993. T-cell receptor Vβ expression in normal human skin. *Proc. Natl. Acad. Sci. USA.* 90:1267–1271.

34. Telfer, N., R. Chalmers, K. Whale, and G. Colman. 1992. The role of streptococcal infection in the initiation of guttate psoriasis. *Arch. Dermatol.* 128:39–42.

35. Reda, K. B., V. Kapur, J. A. Mollick, J. G. Lamphear, J. M. Musser, and R. R. Rich. 1994. Molecular characterization and phylogenetic distribution of the streptococcal superantigen gene (*ssa*) from *streptococcus pyogenes*. *Infect. Immun.* 62:1867–1874.

36. Norrby-Teglund, A., D. Newton, M. Kotb, S. E. Holm, and M. Norgren. 1994. Superantigenic properties of the group A streptococcal exotoxin SpeF (MF). *Infect. Immun.* 62:5227–5233.

37. Chang, J. C. C., L. R. Smith, K. J. Froning, B. J. Schwabe, J. A. Laxer, L. L. Caralli, H. H. Kurland, M. A. Karasek, D. I. Wilkinson, D. J. Carlo, and S. W. Brostoff. 1994. CD8+ TR cells in psoriatic lesions preferentially use T-cell receptor Vβ3 and/or Vβ13.1 genes. *Proc. Natl. Acad. Sci. USA.* 91:9282–9286.

38. Boehncke, W.-H., C. Küenzlen, T. M. Zollner, V. Mielke, and W. Sterry. 1994. Predominant usage of distinct T-cell receptor Vb regions by epidermotropic T cells in psoriasis. *Exp. Dermatol.* 3:161–163.

39. Valdimarsson, H., B. Baker, I. Jónsdóttir, and L. Fry. 1986. Psoriasis: a disease of abnormal keratinocyte proliferation induced by T lymphocytes. *Immunol. Today.* 7:256–259.

40. Schmitt-Egenolf, M., W.-H. Boehncke, M. Ständer, T. H. Eiermann, and W. Sterry. 1993. Oligonucleotide typing reveals association of type I psoriasis with the HLA-DRB1 *0701/2, -DQA1 *0201, -DQB1 *0303 extended haplotype. *J. Invest. Dermatol.* 100:749–752.

41. McFadden, J., H. Valdimarsson, and L. Fry. 1991. Cross-reactivity between streptococcal M surface antigen and human skin. *Br. J. Dermatol.* 125:443–447.

42. Shikhman, A. R., and M. W. Cunningham. 1994. Immunological mimicry between *N*-acetyl-β-D-glucosamine and cytokeratin peptides. *J. Immunol.* 152:4375–4387.

43. Nestle, F. O., L. A. Turka, and B. J. Nickoloff. 1994. Characterization of dermal dendritic cells in psoriasis. *J. Clin. Invest.* 94:202–209.

44. Nickoloff, B. J., R. S. Mitra, J. Green, X.-G. Zheng, Y. Shimizu, C. Thompson, and L. A. Turka. 1993. Accessory cell function of keratinocytes for superantigens: dependence on lymphocyte function-associated antigen-1/intercellular adhesion molecule-1 interaction. *J. Immunol.* 150:2148–2159.