

## RAPID COMMUNICATION

## Association of Erythrodermic Cutaneous T-Cell Lymphoma, Superantigen-Positive *Staphylococcus aureus*, and Oligoclonal T-Cell Receptor V $\beta$ Gene Expansion

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Forty-two patients with cutaneous T-cell lymphoma, including 31 with exfoliative erythroderma or Sezary syndrome and 11 with mycosis fungoides, were studied for the occurrence of staphylococcal infection. Thirty-two of 42 (76%) had a positive staphylococcal culture from skin or blood. One half of the patients with positive cultures grew *Staphylococcus aureus*. This group included 11 with Sezary syndrome and 5 with rapidly enlarging mycosis fungoides plaques or tumors. All of the *S aureus* carried enterotoxin genes. Surprisingly, 6 of 16 strains were the same toxic shock toxin-1 (TSST-1)-positive clone, designated electrophoretic type (ET)-41. Analysis of the T-cell receptor V $\beta$  repertoire in 14 CTCL patients found that only 4 had the expected mono-

clonal expansion of a specific V $\beta$  gene, whereas 10 had oligoclonal or polyclonal expansion of several V $\beta$  families. All patients with TSST-1+ *S aureus* had overexpansion of V $\beta$  2 in blood and/or skin lesions. These studies show that *S aureus* containing superantigen enterotoxins are commonly found in patients with CTCL, especially individuals with erythroderma where they could exacerbate and/or perpetuate stimulate chronic T-cell expansion and cutaneous inflammation. Attention to toxigenic *S aureus* in CTCL patients would be expected to improve the quality of care and outcome of this patient population.

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CUTANEOUS T-cell lymphoma (CTCL) is an indolent, epidermotropic CD4<sup>+</sup>CD3<sup>+</sup>CD45Ro<sup>+</sup> T-cell lymphoma characterized by eczematous or psoriasiform skin lesions (mycosis fungoides [MF]) or by diffuse erythroderma and circulating atypical lymphocytes (Sezary syndrome [SS]).<sup>1,2</sup> Alibert first described MF in 1806 and Sezary Bouvain recognized the association between erythroderma and abnormal circulating lymphocytes. This condition has also been called the "Red Man Syndrome" or "L'Homme Rouge" by Besnier and was recognized as a helper T-cell leukemia by Lutzner in 1975.<sup>3</sup> The cause of erythroderma in the SS is unknown. Systemic administration of interleukin-2 (IL-2), a T-cell growth factor, produces erythroderma and scaling.<sup>4</sup> Exfoliative erythroderma also accompanies atopic dermatitis, drug reactions, psoriasis, and toxic shock syndromes caused by *Streptococcus pyogenes* and *Staphylococcus aureus*, including Kawasaki's disease.<sup>5-9</sup> These bacteria contain genes encoding toxins and related molecules that are capable of causing erythroderma and stimulating helper T cells as superantigens.<sup>10-14</sup>

Our clinical experience has taught us that patients with SS who have indwelling catheters virtually always get staphylococcal sepsis accompanied by worsening erythroderma, not uncommonly in the absence of significant fever.<sup>15</sup> In some of our patients, systemic antibiotic therapy may be associated with decreased erythroderma and tumor size, in the absence of other therapy. Tokura et al<sup>16</sup> reported two erythrodermic Sezary patients responding to topical treatment of staphylococcal colonization. In as much as erythroderma can accompany infection or disease progression and is a clinical parameter for determining the outcome of therapy in CTCL patients, we studied the occurrence of staphylococcal infections in our patient population and characterized the strain subtype, superantigen gene presence, and T-cell receptor V $\beta$  gene usage.

### MATERIALS AND METHODS

#### Patient Population and Staging

Patients diagnosed with cutaneous T-cell lymphoma over the course of 2½ years were prospectively studied if they had erythroderma or skin plaques/tumors that were rapidly increasing in size. All patients had a complete physical examination and staging work-up including a blood smear for Sezary cells, skin biopsy for histology and T-cell immunophenotyping, flow cytometry, computed tomography (CT) scan, and bacterial cultures of their skin by swab and/or blood. Sixteen patients had *S aureus* and 14 patients gave consent to have T-cell V $\beta$  analysis performed by reverse transcriptase-polymerase chain reaction (RT-PCR) in skin and blood samples (Table 1). Controls included six healthy donors who consented to give peripheral blood samples or skin biopsy samples.

#### *Staphylococcus* Subtyping and Identification of Superantigen Genes

**Cultures.** Swabs from skin lesions were immediately inoculated into transport media and seeded onto nutrient agar and mannitol salt agar. Blood was drawn from the antecubital fossa using sterile technique into broth containing vacuum tubes.

**Multilocus enzyme electrophoretic analysis of bacterial strains.** Each isolate was grown in 150 mL of tryptic soy broth (Life Technologies, GIBCO-BRL, Grand Island, NY) overnight at 37°C on an orbital shaker and the cells were obtained by centrifugation. The

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Table 1. CTCL Patients With *S aureus* Cultures of T-Cell V $\beta$  Analysis Performed

Patient	Age	Race	Sex	Type CTCL	Stage	Erythrodermic	Staph Infection	Staph Toxin Analysis n = 16	V $\beta$ Analysis n = 14
1	42	White	F	SS	II b	No	Yes	SED ET59	a
2	58	Black	M	SS	IV b	Yes	Yes	SEC, SED ET 108	a
3	56	White	M	SS	IV b	Yes	Yes	Not available	a
4	62	White	M	SS	III	Yes	Yes	SEA, TSST-1ET41	a
5	81	Hispanic	F	SS	III	Yes	Yes	SEB, SEC ET23	a
6	76	White	F	SS	IV a	Yes	Yes	TSST-1, SEA	a
7	40	White	F	SS	III	Yes	Yes	SEA, TSST-1 ET41	a
8	50	Black	M	SS	III	Yes	Yes	Not available	a
9	79	White	M	SS	III	Yes	Yes	TSST-1 ET41	a
10	56	White	F	SS	III	Yes	No	Toxin neg	a
11	53	White	F	SS	III	Yes	No	Toxin neg	a
12	64	White	M	MF	IIb tumor	No	No	Toxin neg	a
13	51	Hispanic	F	MF	IVa	Yes	Yes	SEB ET22	a
14	61	Hispanic	F	MF	tumor	No	Yes	TSST-1 ET41	a
15	62	White	M	SS	IIb tumor	Yes	Yes	SEC ET16	na
16	68	White	F	SS	IV	Yes	Yes	SEA, TSST-1 ET41	na
17	81	White	M	SS	IV	Yes	Yes	SEB, SED ET22a	na
18	68	White	M	MF	IV	No	Yes	SEB, SEC ET23	na
19	65	White	M	SS	II	Yes	Yes	SEB ET24	na
20	56	Hispanic	M	MF	IV	No	Yes	SEC ET14	na
21	43	White	F	SS	II IV	Yes	Yes	SEC, SED ET47	na

Abbreviations: a, analyzed; na, not analyzed.

bacterial pellet was resuspended in 2 mL of lysis buffer (50 mmol/L Tris-HCL, pH 7.5; 5 mmol/L EDTA) containing 100  $\mu$ g/mL lyso-staphin (Sigma, St Louis, MO) and incubated at 37°C for 45 minutes. The suspension was then sonicated for 30 seconds at 50% output with a Branson model 200-sonicator (VWR Scientific, Sugarland, TX) with ice-bath cooling, and centrifuged at 20,000g for 20 minutes. The enzyme-containing supernatants were either immediately analyzed by multilocus enzyme electrophoresis or stored at -80°C. After electrophoresis on starch gels, selective histochemical staining for 20 metabolic enzymes was conducted.<sup>17</sup> Distinctive mobility variants of each enzyme were numbered in order of decreasing anodal migration, and equated with alleles at the corresponding chromosomal structural gene locus. Each isolate was characterized by its combination of alleles at 20 enzyme loci, and unique combinations of electromorphs were designated as electrophoretic types (ETs).<sup>18</sup>

**Detection of genes encoding enterotoxins and toxic shock syndrome toxin-1 (TSST-1).** *S aureus* strains isolated from 16 CTCL patients were analyzed for the presence of genes encoding SEA, SEB, SED and SEE, and TSST-1 (Table 1). Briefly, polymerase chain amplification was performed using oligonucleotide primers and reaction conditions as described.<sup>12,18,19</sup> Bacterial cell lysates generated by the lyso-staphin procedure were treated with RNase and genomic DNA was purified using the method of Vannufel et al.<sup>19</sup> Distinctive PCR products for each toxin were detected by agarose gel electrophoresis.

#### T-Cell Receptor V $\beta$ Gene Analysis

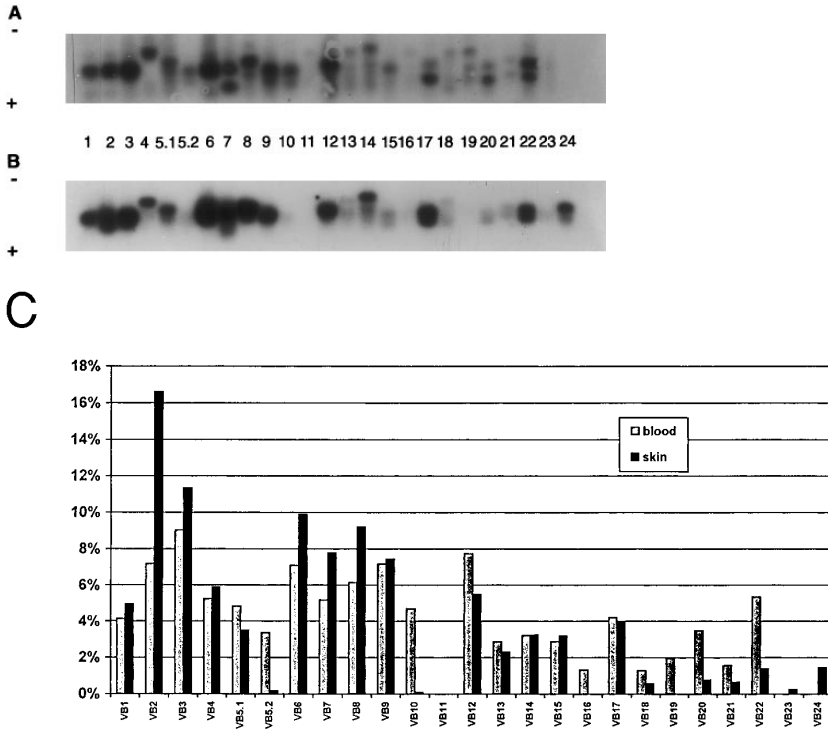
**RNA isolation.** RNA was isolated from peripheral blood mononuclear cells and simultaneously from skin biopsy specimens when possible. Mononuclear cells were separated by gradient centrifugation of the peripheral blood with Ficoll-Paque (Pharmacia, Piscataway, NJ). Four-millimeter skin specimens were snap frozen, and 30 sections of 20- $\mu$ m thickness were cut with a cryostat and homogenized by several passages through 16- to 23-gauge needles. Total RNA was extracted from cells and tissue using TRIzol reagent (Life

Technologies, GIBCO-BRL) according to the manufacturer's protocol. After RNA isolation, the dried pellets were resuspended in RNase-free treated water (diethylpyrocarbonate) and the RNA concentration was measured using a spectrophotometer (Gene Quant, RNA/DNA calculator; Pharmacia).<sup>20</sup> To avoid secondary structure and optimize the synthesis of the cDNA, the RNA was incubated at 70°C for 10 minutes.

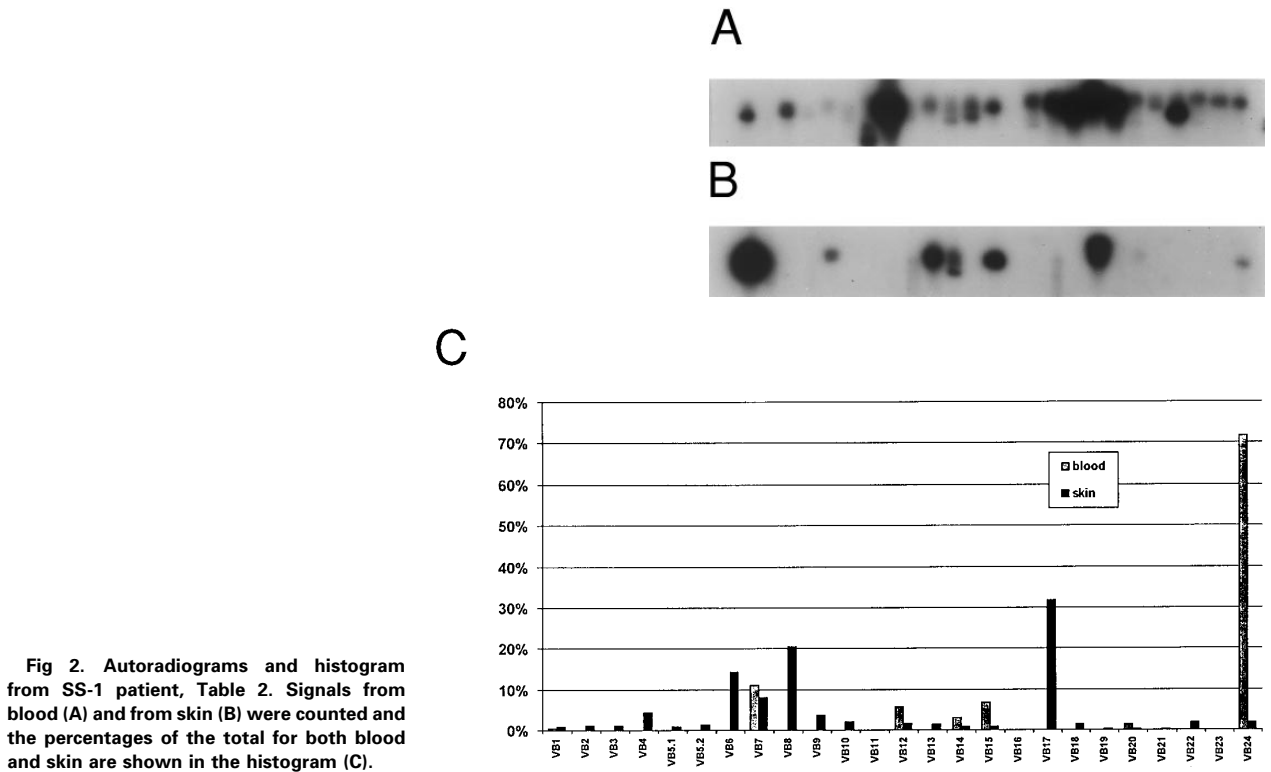
**cDNA synthesis.** Three micrograms of total RNA was used as a template for cDNA synthesis at 42°C for 1 hour in a 120- $\mu$ L reaction using the GeneAmp RNA PCR kit (Cetus-Perkin-Elmer, Emeryville, CA) and protocol with M-MLV reverse transcriptase and random hexamers.

**PCR amplification and detection.** Twenty-five specific primers for the T-cell receptor variable or V $\beta$  5' families, a constant C $\beta$  3' primer, and primers for the constant C $\alpha$  region, as a control, were purchased from Clontech Laboratories (Palo Alto, CA). Two hundred nanograms of cDNA was amplified in a 50- $\mu$ L reaction using AmpliTaq DNA polymerase (Cetus-Perkin-Elmer) by following the manufacturer's recommended buffers and instructions. All the components were added in a master mix and aliquoted to each tube and 0.5  $\mu$ L of each of the 25 V $\beta$  5' primers was incorporated individually. A positive control was prepared with the C $\alpha$  controls primers as well as a negative control. PCR reactions were performed on an MJ thermocycler (MJ Research, Inc, Watertown, MA) at 95°C for 1 minute, 55°C for 1 minute, 72°C for 1 minute  $\times$  35 cycles, followed by a 72°C extension for 7 minutes.

**Southern blot hybridization.** PCR products were detected on a 1.8% agarose/0.5 Tris-Borate-EDTA electrophoresis gel following ethidium bromide staining by UV light visualization. The DNA was transferred to Zetabind nylon membranes (Cuno Inc, Meriden, CT) and hybridized to an end-labeled [ $\gamma$ -<sup>32</sup>P]-ATP 3' C $\beta$  control probe (Figs 1 and 2). The membranes were directly counted on an Instant Imager (Packard, Meriden, CT), exposed overnight on film at -70°C (Hyperfilm; Amersham, Arlington Heights, IL).<sup>20</sup> Each V $\beta$  signal was expressed as a percentage of total signal after background sub-



**Fig 1.** Autoradiograms and histogram for TCR V $\beta$  study from SS patient 6 (SS-6, Table 2). The signals were detected by Southern blot hybridized to a labeled constant primer C $\beta$  and quantitated on a Instant Imager. The blots were then exposed to film overnight and the signals for each receptor gene studied (numbers) are shown for blood (A) and for skin (B). A histogram with the relative amounts of each signal are shown as a percentage of the total counts for blood and for skin (C).



**Fig 2.** Autoradiograms and histogram from SS-1 patient, Table 2. Signals from blood (A) and from skin (B) were counted and the percentages of the total for both blood and skin are shown in the histogram (C).

traction with expression at a value of 6% or more of the total value considered significant overexpression of a TCR V $\beta$  gene (Tables 2 and 3).

## RESULTS

### *Enterotoxin Genes, Especially TSST-1 ET-41, Are Present in CTCL S aureus Isolates*

We next characterized the enterotoxin genes present in the 16 *S aureus* isolates from our CTCL patients (Tables 1 and 2). A total of 10 distinct electrophoretic types (ETs), marking *S aureus* clones, were identified in the sample of 16 isolates. However, 6 patients had the identical ET (ET-41) that is characterized by the presence of the TSST-1 gene. One of these patients (no. 7) developed SS after breast implants<sup>21</sup> and had the same strain of *S aureus* with TSST-1, ET-41, cultured from skin lesions, breast implants at removal, blood, and vagina over the course of 6 months. A complete and durable remission of SS has occurred after treatment with prolonged systemic antibiotics as well as photopheresis,  $\alpha$ -interferon, and gammaglobulin therapy. Five of the *S aureus* isolates contained other toxin genes: SEA, SEB, SEC, or SED. Nine patients' *S aureus* isolates were found to have two toxin genes, including 3 with both TSST-1 ET-41 and SEA. Four patients had SEC in combination with SEB and SED, and 1 patient's strain had SEB with SED.

### *Staphylococci Are Frequently Isolated From CTCL Patients*

Forty-two CTCL patients were enrolled in the study over the course of the 2.5 years including 31 with erythroderma or the SS and 11 with MF. On enrollment, 32 (76%) had a skin or blood culture positive for staphylococcus. Coagulase negative staphylococci or *S epidermidis* were cultured from one half of the 32 patients. The other 16 patients had cultures positive for *S aureus*, including 11 patients with erythroderma or SS and 5 patients with rapidly enlarging MF lesions (Table 1).

### *Oligoclonal Expansion of the T-Cell V $\beta$ Receptor Is Frequently Observed in CTCL*

Because staphylococcal enterotoxins function as superantigens to amplify T cells with specific V $\beta$  receptors, we next determined the T-cell receptor (TCR) V $\beta$  repertoire use in paired skin lesion and peripheral blood samples in 14 consenting CTCL patients. These included 9 patients from whom *S aureus* enterotoxins were determined (Table 2). Only 4 of 14 CTCL patients had a predominant T-cell clone identified. Two had a single TCR V $\beta$  gene that was expressed at a level of more than 25% in skin and blood and 2 had a major TCR clone in their skin. The other 10 patients had three or more TCR V $\beta$  genes expressed at greater than 6% of the total, which represented oligoclonal or polyclonal expansion. The TCR V $\beta$  usage was variable, both within blood and skin specimens from the same patient and among the different individual patients studied.

Of the 25 T-cell V $\beta$  families studied by RT-PCR, we found that TCR genes V $\beta$  6, 7, 8, and 12 were most often

amplified in the skin and blood of CTCL patients and controls. TCR V $\beta$  6 was increased to greater than 6% of the total in 10 of 11 skin samples and 8 of 11 blood lymphocyte samples from CTCL patients compared with 3 of 6 or 4 of 6 control samples, respectively. In contrast, the TCR V $\beta$  5.2 and V $\beta$  11, which are not known to be recognized by staphylococcal toxins,<sup>5,9,10,22</sup> were rarely amplified to greater than 6% predominance.

### *T-Cell V $\beta$ 2 Expansion Is Seen in CTCL Patients in Association With TSST-1*

It was previously reported that the staphylococcal toxin TSST-1 is associated with amplification of TCR V $\beta$ 2.<sup>5,10</sup> Therefore, we examined its frequency among 11 CTCL patients who had both the TCR V $\beta$  repertoire and staph toxin typing performed. Figure 1 shows the polyclonal TCR expansion including V $\beta$ 2 in skin and blood of SS patient 6 with TSST-1. Five of 11 patients, including 4 with SS, had TSST-1 present in their staphylococci, and all had TCR V $\beta$  2 expanded in either skin or blood (Table 2 and Fig 1). Four overexpressed TCR V $\beta$  2 in blood (6.2% to 16%), 3 in skin only (8% to 17%), and 2 in both skin and blood. This is compared with 2 of 6 control bloods and 1 of 6 control skin specimens (Table 3).

In the remaining CTCL patients with *S aureus*, only 1 SS patient with SEB toxin had overexpression of V $\beta$  2 in blood. Figure 2 shows the TCR V $\beta$  repertoire of a patient whose *S aureus* contained SED enterotoxin. V $\beta$ 2 was not amplified in blood but was weakly amplified in skin. However, V $\beta$  5 and 12, associated with this toxin, were also amplified weakly in skin. TCR V $\beta$  2 gene was not over-expressed in 3 CTCL patients who were culture-negative for *S aureus*.

## DISCUSSION

Bacterial superantigens, which stimulate clonal expansion of T cells by mechanisms involving specific HLA molecules,<sup>10,13,23-28</sup> have also been hypothesized to cause inflammatory skin diseases.<sup>7-9,22,29-31</sup> The evidence to support this mechanism for cutaneous T-cell lymphoma is limited to in vitro stimulation of T cells by superantigens<sup>32</sup> and to report of two patients treated for staphylococcal colonization.<sup>16</sup>

This is the first report of the occurrence of staphylococcus and its enterotoxins in a large, prospective cohort of CTCL patients. We found that 32 of 42 consecutive CTCL patients had a staphylococcal culture positive from either skin or blood and that coagulase positive *S aureus* was present in 16 patients. In a smaller subset of 14 patients studied for T-cell receptor V $\beta$  expansion, 78.6% had *S aureus*-containing toxins. Unexpectedly, a specific *S aureus* strain (ET-41) was present in 1 of 3 MF patients and in 4 of 11 Sezary patients whose cultures were positive for *S aureus*. This toxic shock toxin-1 strain occurred in association with oligoclonal expansion of several V $\beta$  genes, including the expected TCR V $\beta$  2. Our data support the role of *S aureus* enterotoxins in the pathogenesis of CTCL and raise the question "To what extent do superantigens contribute to the features of this disease?"

In cutaneous T-cell lymphomas there is a "malignant"

Table 2. TCR V $\beta$  Repertoire in Blood Cells and Skin Lesions of CTCL Patients

V $\beta$ %	SS-1 staph <sup>+</sup>		SS-2 staph <sup>+</sup>		SS-3 staph <sup>+</sup>		SS-4 staph <sup>+</sup>		SS-5 staph <sup>+</sup>		SS-6 staph <sup>+</sup>	
	B	S	B	S	B	S	B	S	B	S	B	S
1	<1	<1	+	+	+	+	<u>9.1</u>	+	+	<u>10</u>	+	+
2		+	+	+	+	+	<u>10</u>	<u>8.6</u>	+	+	<u>7.2</u>	<u>16.6</u>
3		+	+	+	+	<u>8.7</u>	<u>8.3</u>	<u>10.4</u>	<u>16.8</u>	+	<u>9</u>	<u>11.3</u>
4		+	+	+	+	+	+	+	+		+	+
5.1		+	+	+	+	+	+	+	+	<1	+	+
5.2	+	+					+	+	+		+	<1
6		<u>14.4</u>	<u>9.4</u>			<u>8.8</u>	<u>12.8</u>	<u>16.6</u>	<u>6</u>	<u>33.9</u>	<u>7.1</u>	<u>9.9</u>
7	<u>11</u>	<u>8.1</u>	+		+	+	<u>10.5</u>	+	+		+	<u>7.8</u>
8		<u>20.4</u>	<u>9.6</u>	+	+	+	<u>19.9</u>	<u>12.4</u>	<u>6.2</u>	<u>7</u>	<u>6.1</u>	<u>9.2</u>
9		+	+	+	+	+	<u>8.1</u>	+	<u>10.5</u>	<u>6.2</u>	<u>7.1</u>	<u>7.4</u>
10		+	+	+	+	+	+	+	+		+	<1
11			+						<1			
12	+	+	<u>12.6</u>	+	<u>15.6</u>	+	+	<u>6.8</u>	+	<u>12.8</u>	<u>7.7</u>	+
13		+	<u>20.3</u>	<u>21.9</u>		+		+	<1	+	+	+
14	+	<1	+	<u>23.3</u>		+		+	+		+	+
15	<u>6.7</u>	+	+	+		+	+	+	+		+	+
16			+	+		+	+	<1	<1		+	+
17		<u>31.9</u>	+	+	+	+	+	<u>7.3</u>	+	<1	+	+
18			+	+	<1	+		<1	+		+	<1
19		+		+	<1		+		+		+	+
20	+	<1	+	<1	+	+		<1	+		+	<1
21		<1	+	+	<u>24</u>	<u>9</u>		<1	+		+	<1
22		+	<u>15.3</u>	<u>9.2</u>	<u>7.5</u>	<u>6.5</u>		+	<u>9.6</u>		+	+
23			+	+	<u>7.8</u>	<u>7.9</u>						<1
24	<u>71.6</u>	+	+	+	<u>6.3</u>	+	+	+	+	<u>15.3</u>		+
	SED		SEC,SED		na		TSST-1		SEB,SEC		SEA, TSST-1	

(Continued on following page)

The estimation of the percentage of T cells bearing each V $\beta$  was obtained by Southern blotting with hybridization to the constant region probe. Total counts per minute for each PCR band was determined by phosphorimaging. Percentages >6% are shown in the table as the numerical values. Values between 1 and 6% are indicated as positive (+). V $\beta$  signals expressed at a level <1% are also indicated. V $\beta$  2 percentages correlated with TSST-1 toxin are underlined. The toxin from staph isolated from each patient are shown on the bottom line.

Abbreviations: na, not available; B, blood mononuclear cells; S, skin lesions.

\* PBL analyzed 3 months later.

† V $\beta$ 2 is absent in this patient's blood and was true in a course time of 1 year (data not shown).

proliferation of atypical CD4<sup>+</sup>CD45Ro<sup>+</sup> T cells that is thought to initially expand in skin and later invade lymph nodes and other organs, including blood (SS).<sup>2,33,34</sup> Although Tan et al<sup>35</sup> first suggested that CTCL arises from persistent antigenic stimulation of T cells, the identity of a putative antigen and its ability, if any, to stimulate T cells is unknown and speculative.<sup>21,36-38</sup> If CTCL results from antigen persistence, then colonizing skin flora including staphylococci or streptococci could provide the necessary antigens, and superantigens, for initiation and perpetuation of T-cell proliferation in some, if not all, patients with this disease.

Clonal expansion of TCR V $\beta$  genes has been shown for CTCL, as well as for a number of other autoimmune diseases (Table 4), but no single TCR variable gene has been identified. This has been interpreted to imply lack of a common antigen (or superantigen). Our study was surprising in that it suggested that oligoclonal or polyclonal (rather than monoclonal) expansion of T-cell V $\beta$  genes is more common in CTCL patients' skin and blood, as detected by RT-PCR analysis with the limitations of this technique and the availability of primer sequences. Secondly, CTCL patients with TSST-1<sup>+</sup> *S aureus* had the expected expansion of the V $\beta$ 2

gene. In addition, most patients' TCR V $\beta$  repertoires included expansion of TCR V $\beta$  6 and 8, frequently cited in other studies.<sup>39-44</sup> Although V $\beta$  6 and 8 genes are also expanded in normal cutaneous T cells relative to blood, this is hypothesized to be the result of their reactivity to common skin flora.<sup>45</sup> Epidermotropic T cells of CTCL lymphoma may be similar.

We hypothesize that like other T-cell-mediated skin diseases, CTCL occurs in the setting of a genetically determined host (HLA determinants), a trigger (antigens or superantigens), and an immune response with cytokine and chemokine production. In CTCL, T cells are attracted into the epidermis and they may be unable to limit their proliferation (absent apoptosis). Staphylococcal superantigen, presented either by Langerhans cells or by class II-bearing keratinocytes, results in cytokines that stimulate T cells.<sup>35</sup> It is reasonable that persistent colonization with toxigenic bacteria would expand the population of epidermotropic T cells and elicit production of T-cell-activating cytokine/chemokines, such as interferon-inducible protein 10, capable of perpetuating the reaction and recruiting additional cells.<sup>46</sup>

In T-cell-mediated autoimmune diseases (Table 4), ge-

**Table 2 (Cont'd). TCR Vβ Repertoire in Blood Cells and Skin Lesions of CTCL Patients**

SS-7 staph <sup>+</sup>			SS-8 staph <sup>+</sup>		SS-9 staph <sup>+</sup>		SS-10 staph <sup>-</sup>		SS-11 staph <sup>-</sup>		MF-1 staph <sup>-</sup>		MF-2 staph <sup>+</sup>		MF-3 staph <sup>+</sup>	
B	B*	S	B	S	B	S	B†	S	B	S	B	S	B	S	B	S
9.2	+	+	+	8.5	6.1	+		11		19.	+	+	+	+	+	+
16	14	+	+	8.3	6.1	+				2	+	+	8.6	+		8
18	10	+	+	8	8	6.2		14.		+	+	+	7.8	+	8.8	6
+	+	+	14	+		7.6	15	3	+	+	14	+	+	+	+	+
+	+	+	+	+	+	19				+	+	<1	+	+	+	+
+	+	+	+	+	+	+				+	+	+	+	<1	+	+
13	8	10.1	11	7.8	12	8.2			+	+	+	9.3	7	8	8	9
6.8	17	+	8	11	12	+	19	10.	+	13	+	7.5	9	14	8	+
+		15.8	12	10	+	15		5	+	+	15	6.3	9.3	13		+
+		+	+	6.8	6.5	+	10	54.	+	12	+	+	6.6	7	+	+
	+	+	+	+	+	<1	<1	5	+	14.	+	+	+	+	15	+
		+				<1	<1	9.7		4	<1	+	+	<1		+
+	8	9.8	7.8	+	+	+	+		6.8	+	6	+	+	6.1	+	8
<1		+	+	+	+	<1	+	+	+	+	+	+	+	+		+
<1		+	+	+	<1	+	+	+	+	+	+	27	+	+		+
		+	+	+	+	+			+	<1	+	+	+	+	6.3	+
+		+	+	+	<1	<1			13	<1	+	<1	<1	+	+	+
+	+	+	+	+	+	+			9.2	<1	+	+	<1	+	+	+
+		<1	+	+	<1	+			8	+	+	<1	+	+	+	+
+	+	<1			<1	<1			7.5	+	+	<1	+	+		+
		<1	+	+	+					+	+	+	+	+	<1	+
+		+			<1				6.8	+	+	+	+	+	9.6	+
<1	11	+	+	+	7	+			+	<1	+	+	+	+	+	+
<1		+			9	+			+	<1	+	+	+	+	+	+
	12	12.7	+	+	8	+	41		+	+	+	+	+	+	13	+
SEA, TSST-1			na		TSST-1		staph neg		staph neg		staph neg		SEB		SEA, TSST-1	

**Table 3. TCR Vβ Analysis in Six Controls**

Vβ (%)	Control 1		Control 2		Control 3		Control 4		Control 5		Control 6	
	B	S	B	S	B	S	B	S	B	S	B	S
1	7.2	19.2	+	+	<1	+	+	+	6.3	+	+	+
2	8.8	6.1	+	+	+	+	+	+	7		+	+
3	8.9	10.3	+	13.1	+	9.6	7	6.3	9.8	+	+	6.5
4	+	+	+			+	8.5		6.4		7.3	+
5.1	+	+	+	+	+	+	+	+	+	+	+	+
5.2	+		+				+		+		+	
6	8	18.5	+	11.9	+	13.1	8.1		8.6		6.7	+
7	+	11.7	+	16.3	+	6.2	6.9		11.8	+	7.9	+
8	7.7	7.7	+	17.8	7.1	14	+	+	11	+	+	+
9	8.5	+	+		+	6.8	+	<1	6.8	+	+	+
10	7.7	<1	+	+	+	+	+	<1	+	+	+	11
11	+		+				<1		+	+	+	<1
12	+	+	+	6.7		9.9	7.1	+	6.2	15	+	+
13	+	<1	+	+	+	<1	+	+	+		+	+
14	+	<1	+	+	+	+	+	+	+	6.5	+	+
15	+	+	+	+	+	+	+	+	+	7	+	<1
16	+	+	+	+		<1	+	+	10.5	+	+	<1
17	+	+	+	+	+	+	+	7	+	8.5	+	<1
18			+	+		<1	+	+	15.4	+	+	<1
19	<1	+	+		9.4		+	+	+	+	+	<1
20		+	+		24.6	+	+	+	+	+	+	<1
21		+	+	+		+	+	+	+	+	+	+
22	+	+	8	+	13.4	11	+		+	+	+	+
23	<1	+	+	+		<1	+	+	+	+	+	+
24	<1	+	+	+	+	+	+	+	+	+	+	<1

**Table 4. V $\beta$  TCR Repertoire Reported in CTCL and Autoimmune Diseases**

Disease	Author	Methods	Patients	Site	V $\beta$ Dominant
CTCL	Hunt et al <sup>60</sup>	7 MoAbs	13	Skin	V $\beta$ 5
CTCL	Jack et al <sup>39</sup>	2 MoAbs	16	Skin/tumor	V $\beta$ 8
CTCL	Lessin et al <sup>43</sup>	RNA-PCR assay	1	PBL	V $\beta$ 6,4
MF	Wood et al <sup>40</sup>	RNA-PCR assay	1	Skin	Multiple with V $\beta$ 6 dominant
MF	Bahler et al <sup>41</sup>	RNA-PCR assay	28	Skin	Multiple families with V $\beta$ 6 & 8 dominant
SS	Kono et al <sup>61</sup>	RNA-PCR assay	19	PBL	Multiple with V $\beta$ 8
CTCL	Longley et al <sup>44</sup>	RNA-PCR assay + Abs	6	PBL	Polyclonal random
SS	Gorochov et al <sup>42</sup>	RNA-PCR assay	16	Skin	V $\beta$ 5, 6, 8, 13, 18
RA	Paliard et al <sup>62</sup>	RNA-PCR assay	6	Skin/PBL	Multiple with V $\beta$ 14 dominant
RA	Howell et al <sup>63</sup>	RNA-PCR assay	5	Synovial fluid	Multiple with V $\beta$ 14, 17 dominant
RA	Bucht et al <sup>64</sup>	RNA-PCR assay	9	Synovial fluid	Random
Psoriasis	Chang et al <sup>62</sup>	RNA-PCR assay	8	Skin/PBL	Multiple with V $\beta$ 3, 13.1 dominant
Kawasaki	Abe et al <sup>5</sup>	RNA-PCR assay + Abs	19	PBL	Multiple with V $\beta$ 2, 8 dominant

Abbreviations: MoAbs, monoclonal antibodies; Abs, antibodies; PBL, peripheral blood lymphocytes.

netic susceptibility may be conferred to some degree by major histocompatibility locus antigens.<sup>47</sup> We confirmed that CTCL patients have an association with HLA-DR5 (DR11),<sup>48</sup> and found stronger associations with HLA-DQ\*03 alleles and with HLA-DQ\*0502 in SS (odd's ratio [OR] = 7.75).<sup>49</sup> HLA class II antigens are clearly known to affect binding of bacterial superantigens SEA, SEB, and TSST-1 and to present processed antigen to CD4<sup>+</sup> T cells.<sup>13,23,26-28,47</sup> There may be an HLA-mediated sensitivity to superantigens as suggested by the data that fibroblasts transfected with HLA-DR5 $\beta$ 3 are more highly stimulated with SEA superantigen than those with other HLA chains.<sup>50</sup> HLA type may influence nasal colonization with *S aureus*, specifically HLA-DR3, in combination with HLA-DR5.<sup>51</sup> Thus, HLA-DR5 presence may play a role in susceptibility to colonization and to sensitivity to superantigen stimulation once colonization occurs.

The boundary between normal T-cell proliferation to antigen stimulation and "malignant" clonal expansion of T cells is not always clear, especially with the finding that psoriatic lesions may have clones of T cells with specific V $\beta$  restriction.<sup>52</sup> Early "CTCL/mycosis fungoides and pre-Sezary" are notoriously difficult to diagnose, sharing clinical and histologic features with "benign" dermatitis/eczema and psoriasis including a high incidence of *S aureus* colonization and a similar response profile to therapy.<sup>7,29,53</sup> The design of our study underestimated the prevalence of staphylococcus and toxigenic staphylococci in CTCL patients and it did not address the difficult question of whether *S aureus* is a primary cause or a secondary effect of the "disease." If the propensity for establishing staphylococcal colonization and ability to tolerate enterotoxins without fever or hypotension is the host-related integral part of this disease, then the question of which came first may be unanswerable.

The dogmatic view is that staphylococcal colonization is secondary and the result of profound immunosuppression.<sup>54</sup> In this sense, CTCL patients resemble patients with acquired immunodeficiency syndrome who cannot clear the skin of staphylococcus and have protracted pruritus and erythrodermic psoriasis.<sup>55-57</sup> Regardless of etiology, clinical management of CTCL patients would improve by recognizing that

TSST-1 *S aureus* may be found in CTCL and cause bacteremia or sepsis, sometimes in the absence of fever. Disease evaluation is generally measured by degree of skin erythroderma and scale<sup>58</sup> so that reactive superantigen erythema may be interpreted as "disease progression" and result in change to more aggressive therapy. Rapid clinical improvement within days has occurred with administration of systemic antibiotics in our erythrodermic and MF patients, and administration of intravenous gammaglobulin, containing antibodies inhibitory to superantigen activation,<sup>59</sup> may also benefit the hypogammaglobulinemic patient. Erythroderma skin score has improved with photopheresis as serum Ig levels are restored to normal.<sup>15</sup>

The association between staphylococcal infections and the erythrodermic form of CTCL deserves further attention and study. Genetic susceptibility and disease initiation or persistence may involve the ability to be colonized and/or relative sensitivity to superantigens in common skin flora. Because allelic variation in bacterial superantigens can significantly alter T-cell interactions, it will be critical to more precisely characterize the enterotoxin genes found in the *S aureus* isolates cultured from our patients. Attention to the infectious component of CTCL would be expected to greatly impact the outcome of care, improve prognosis and mortality, and lead to novel forms of therapy and disease prevention.

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#### REFERENCES

1. Abel EA, Wood GS, Hoppe RT: Mycosis fungoides: Clinical and histologic features, staging, evaluation, and approach to therapy. *CA Cancer J Clin* 43:93, 1993
2. Braverman IM: Cutaneous T-cell lymphoma. *Curr Prob Dermatol Nov/Dec*:181, 1991
3. Lutzner M, Edelson R, Schein P, Green I, Kirkpatrick C, Ahmed A: Cutaneous T-cell lymphomas: The Sezary syndrome, mycosis fungoides, and related disorders. *Ann Intern Med* 83:534, 1975

4. Gaspari AA, Lotze MT, Rosenberg SA, Stern JB, Katz SI: Dermatologic changes associated with Interleukin 2 administration. *JAMA* 258:1624, 1987
5. Abe J, Kotzin BL, Kazuhito J, Melish ME, Glode MP, Kohsaka T, Leung DYM: Selective expansion of T cells expressing T-cell receptor variable regions Vb2 and Vb8 in Kawasaki disease. *Proc Natl Acad Sci USA* 89:4066, 1992
6. McFadden JP, Noble WC, Camp RDR: Superantigenic exotoxin-secreting potential of staphylococci isolated from atopic eczematous skin. *Br J Dermatol* 128:631, 1993
7. Leung DYM, Walsh P, Giorno R, Norris DA: A potential role for superantigens in the pathogenesis of psoriasis. *J Invest Dermatol* 100:225, 1993
8. Leung DYM, Meissner HC, Fulton DR, Murray DL, Kotzin BL, Schlievert PM: Toxic shock syndrome toxin-secreting *Staphylococcus aureus* in Kawasaki syndrome. *Lancet* 342:1385, 1993
9. Drake CG, Kotzin BL: Superantigens: Biology, immunology, and potential role in disease. *J Clin Immunol* 12:149, 1992
10. Choi Y, Kotzin B, Herron L, Callahan J, Marrack P, Kappler J: Interaction of *Staphylococcus aureus* toxin "superantigens" with human T cells. *Proc Natl Acad Sci USA* 86:8941, 1989
11. Marrack P, Kappler J: The staphylococcal enterotoxins and their relatives. *Science* 248:705, 1990
12. Johnson WM, Tyler SD, Ewan EP, Ashton FE, Pollard DR, Rozee KR: Detection of genes for enterotoxins, exfoliative toxins, and toxic shock syndrome toxin 1 in *Staphylococcus aureus* by the polymerase chain reaction. *J Clin Microbiol* 29:426, 1991
13. Mollick JA, Chintagumpala M, Cook RG, Rich RR: Staphylococcal exotoxin activation of T cells. *J Immunol* 146:463, 1991
14. Mollick JA, Miller GG, Musser JM, Cook RG, Grossman D, Rich RR: A novel superantigen isolated from pathogenic strains of *Streptococcus pyogenes* with aminoterminal homology to Staphylococcal enterotoxins B and C. *J Clin Invest* 92:710, 1993
15. Duvic M, Hester JP, Lemak NA: Photopheresis therapy for cutaneous T cell lymphoma. *J Am Acad Dermatol* 35:573, 1996
16. Tokura Y, Yagi H, Ohshima A, Kurokawa S, Wakita H, Yokote R, Shriahama S, Furukawa F, Takigawa M: Cutaneous colonization with staphylococci influences the disease of Sezary syndrome: A potential role for bacterial superantigens. *Br J Dermatol* 133:6, 1995
17. Selander RK, Caugant DA, Ochman H, Musser JM, Gilmour MN, Whittam TS: Methods of multilocus enzyme electrophoresis for bacterial population genetics and systems. *Appl Environ Microbiol* 51:873, 1986
18. Musser JM, Kapur V: Clonal analysis of methicillin-resistant *Staphylococcus aureus* strains from intercontinental sources: Association of the *mec* gene with divergent phylogenetic lineages implies dissemination by horizontal transfer and recombination. *J Clin Microbiol* 30:2058, 1992
19. Vannufel, Gigi PJ, Ezzedine H, Vandercam B, Delmee M, Waunters G, Gala JL: Specific detection of methicillin-resistant staphylococcus species by multiplex PCR. *J Clin Microbiol* 33:2864, 1995
20. Duvic M, Chema D, Annarella M, Cho M, Esgleyes-Ribot T, Remenyik E, Ulmer R, Rapini R, Sacks PG, Clayman GL, Davies PJA, Thacher S: Keratinocyte transglutaminase expression varies in squamous cell carcinomas. *J Invest Dermatol* 102:462, 1994
21. Duvic M, Moore D, Menter A, Vonderheid EC: Cutaneous T-cell lymphoma in association with silicone breast implants. *J Am Acad Dermatol* 32:939, 1995
22. Skov L, Baadsgaard O: Superantigens: Do they have a role in skin diseases? *Arch Dermatol* 131:829, 1995
23. Jardetzky TS, Brown JH, Gorga JC, Stern L, Urban RG, Chi YI, Stauffacher C, Strominger JL, Wiley DC: Three-dimensional structures of a human class II histocompatibility molecule complexed with superantigen. *Nature* 368:711, 1994
24. Janeway CA: Are there cellular superantigens? *Immunol Rev* 131:189, 1993
25. Uchiyama T, Araake M, Yan XJ, Miyanaga Y, Igarashi H: Involvement of HLA class II molecules in acquisition of staphylococcal enterotoxin A-binding activity and accessory cell activity in activation of human T cells by related toxins in vascular endothelial cells. *Clin Exp Immunol* 87:322, 1992
26. Von Bonin A, Erhlich S, Malcherek G, Fleischer B: Major histocompatibility complex class II associated peptides determine the binding of the superantigen toxic shock syndrome toxin-1. *Eur J Immunol* 25:2894, 1995
27. Fleischer B, Schrezenmeier H, Conrad P: T lymphocyte activation by staphylococcal enterotoxins: Role of class II molecules and T cell surface structures. *Cell Immunol* 120:92, 1989
28. Labrecque N, Thibodeau J, Mourad W, Sekaly RP: T cell receptor-major histocompatibility complex class II interaction is required for the T cell response to bacterial superantigens. *J Exp Med* 180:1921, 1994
29. Leung DYM, Harbeck R, Bina P, Reiser RF, Yang E, Norris DA, Hanifrin JM, Sampson HA: Presence of IgE antibodies to staphylococcal exotoxins of the skin of patients with atopic dermatitis. *J Clin Invest* 92:1374, 1993
30. Boehncke WH, Zollner TM, Dressel D, Kaufmann R: Induction of psoriasisiform inflammation by a bacterial superantigen in human skin grafts transplanted onto SCID mice. *J Invest Dermatol* 106:833, 1996
31. Yokote R, Tokura Y, Furukawa F, Takigawa M: Susceptible responsiveness to bacterial superantigens in peripheral blood mononuclear cells from patients with psoriasis. *Arch Dermatol Res* 287:443, 1995
32. Tokura Y, Heald PW, Yan SL, Edelson RL: Stimulation of cutaneous T-cell lymphoma cells with superantigenic staphylococcal toxins. *J Invest Dermatol* 98:33, 1992
33. Hermans LH, Noordijk EM, Meijer CJLM, Scheffer E, Van Vloten WA: Cutaneous T-cell lymphoma: Clinicopathological relationships, therapy and survival in ninety-two patients. *Br J Dermatol* 107:145, 1982
34. Wieselthier JS, Koh HK: Sezary syndrome: Diagnosis, and critical review of treatment options. *J Am Acad Dermatol* 22:381, 1990
35. Tan RSH, Butterworth CM, McLaughlin H, Malka S, Samman PD: Mycosis fungoides—A disease of antigen persistence. *Br J Dermatol* 91:607, 1974
36. Grossman D, Duvic M: Cutaneous T cell lymphoma occurring after blood transfusion. *Lancet* 342:1483, 1993
37. Grossman D, Rapini RP, Osborne B, Duvic M: Emergence of leprosy in a patient with mycosis fungoides. *J Am Acad Dermatol* 30:313, 1994
38. Pancake BA, Zucker-Franklin D, Coutavas EE: The cutaneous T cell lymphoma, mycosis fungoides, is a human T cell lymphotropic virus-associated disease. *J Clin Invest* 95:547, 1995
39. Jack AS, Boylston ASJ, Carrel S, Grigor I: Cutaneous T cell lymphoma cells employ a restricted range of t-cell antigen receptor variable region genes. *Am J Pathol* 136:17, 1990
40. Wood GS, Bahler DW, Hoppe RT, Warnke RA, Sklar JL, Levy R: Transformation of mycosis fungoides: T-cell receptor beta gene analysis demonstrates a common clonal origin for plaque-type mycosis fungoides and CD30+ large-cell lymphoma. *J Invest Dermatol* 101:296, 1993
41. Bahler DW, Berry G, Warnke RA, Levy R: Diversity of T-cell antigen receptor variable genes used by mycosis fungoides. *Am J Pathol* 140:1, 1992
42. Gorochoff G, Bachelez H, Cayuela JM, Legac E, Laroche L,



Dubertret L, Sigaux F: Expression of V beta segments by Sezary cells. *J Invest Dermatol* 105:56, 1995

43. Lessin SR, Rook AH, Rovera G: Molecular diagnosis of cutaneous T-cell lymphoma: Polymerase chain reaction amplification of T-cell antigen receptor beta-chain gene rearrangements. *J Invest Dermatol* 96:299, 1991

44. Longley J, Tyrrell L, Lu SZ, Farrel JA, Ding TG, Yan S, Sallee D, Heald P, Berger C, Tigelaar R, Edelson R: Malignant and normal T cells show random use of t-cell receptor alpha chain variable regions in patients with cutaneous T cell lymphoma. *J Invest Dermatol* 105:62, 1995

45. Dunn DA, Gadenne AS, Simha S, Lerner EA, Bigby M, Bleicher PA: T-cell receptor Vbeta expression in normal human skin. *Proc Natl Acad Sci USA* 90:1267, 1993

46. Sarris AH, Esgleyes-Ribot T, Crow M, Broxmeyer H, Karasavvas N, Pugh W, Grossman D, Deisseroth A, Duvic M: Cytokine Loops involving Interferon-gamma and IP-10, a cytokine chemotactic for CD4-positive lymphocytes: An explanation for the epidermotropism of cutaneous T cell lymphoma. *Blood* 86:651, 1995

47. Sinha AA, Lopez MT, McDevitt HO: Autoimmune diseases: The failure of self tolerance. *Science* 248:1380, 1990

48. Safai B, Myskowski PL, Dupont B, Pollack MS: Association of HLA-DR5 with mycosis fungoides. *J Invest Dermatol* 80:395, 1983

49. Jackow CM, McHam JB, Friss A, Alvear J, Reveille JR, Duvic M: HLA-DR5 and DQB\*03 class II alleles are associated with CTCL. *J Invest Dermatol* 107:373, 1996

50. Mollick JA, Cook RG, Rich RR: Class II molecules are specific receptors for staphylococcus enterotoxin A. *Science* 244:817, 1989

51. Kinsman OS, McKenna R, Noble WC: Association between histocompatibility antigens (HLA) and nasal carriage of staphylococcus aureus. *J Med Microbiol* 16:215, 1983

52. Chang JCC, Smith LR, Froning KJ, Schwabe BJ, Laxer JA, Caralli LL, Kurland HH, Karasek MA, Wilkinson DI, Carlo DJ, Brostoff SW: CD8+ T cells in psoriatic lesions preferentially use T-cell receptor Vb3 and/or Vb13.1 genes. *Proc Natl Acad Sci USA* 91:9282, 1994

53. Shapiro PE, Pinto FJ: The histologic spectrum of mycosis fungoides/Sezary syndrome (cutaneous T cell lymphoma). *Am J Surg Pathol* 18:645, 1994

54. Heald P, Yan S-L, Edelson R: Profound deficiency in normal circulating T cells in erythrodermic cutaneous T-cell lymphoma. *Arch Dermatol* 130:198, 1994

55. Duvic M: Staphylococcal infections and the pruritus of AIDS-related complex (Letter to the Editor). *Arch Dermatol* 123:1599, 1987

56. Smith KJ, Wagner KF, Yeager J, Skelton HG, Ledsy R: Staphylococcus aureus carriage and HIV-1 disease: Association with increased mucocutaneous infections as well as deep soft-tissue infections and sepsis. *Arch Dermatol* 130:521, 1994

57. Duvic M, Johnson T, Rapini RP, Freese T, Rios A: AIDS associated psoriasis and Reiter's syndrome. *Arch Dermatol* 123:1622, 1987

58. Edelson RL, Berger CL, Gasparro FP, Jegasothy BV, Heald PW, Wintroub B, Vonderheid E, Knobler R, Wolff K, Plewig G, McKiernan G, Christensen I, Oster M, Honigsmann H, Wilfore H, Kokoschka E, Rehle T, Perez M, Stingl G, Laroche L: Treatment of cutaneous T cell lymphoma by extracorporeal photchemotherapy. *N Engl J Med* 316:287, 1987

59. Takei S, Arora YK, Walker SM: Intravenous immunoglobulin contains specific antibodies inhibitory to activation of T cells by Staphylococcal toxin superantigens. *J Clin Invest* 91:602, 1993

60. Hunt SJ, Charley MR, Jegasothy BV: Cutaneous T-cell lymphoma: Utility of antibodies to the variable regions of the human T-cell antigen receptor. *J Am Acad Dermatol* 26:552, 1992

61. Kono DH, Baccala R, Balderas RS, Kovac SJ, Heald PW, Edelson RL, Theofilopoulos AN: Application of a multiprobe RNase protection assay and junctional sequences to define Vb gene diversity in Sezary syndrome. *Am J Pathol* 140:823, 1992

62. Paliard X, West SG, Lafferty JA, Clements JR, Kappler JW, Marrack P, Kotzin BL: Evidence for the effects of a superantigen in rheumatoid arthritis. *Science* 253:325, 1991

63. Howell MD, Diveley JP, Lundeen KA, Esty A, Winters ST, Carlo DJ, Brostoff SW: Limited T-cell receptor B-chain heterogeneity among interleukin 2 receptor-positive synovial T cells suggests a role for superantigen in rheumatoid arthritis. *Proc Natl Acad Sci USA* 88:10921, 1991

64. Bucht A, Oksenberg JR, Lindblad S, Gronberg A, Steinman L, Klareskog L: characterization of T-cell receptor alpha-beta repertoire in synovial tissue from different temporal phases of rheumatoid arthritis. *Scand J Immunol* 35:159, 1992