

## Selective Expansion of T Cells Expressing V $\beta$ 2 in Toxic Shock Syndrome

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### Summary

Infection with *Staphylococcus aureus* and the production of toxic shock syndrome toxin-1 (TSST-1) have been implicated in the pathogenesis of toxic shock syndrome. Previous in vitro studies have demonstrated that TSST-1 is a powerful but selective stimulator of human T cells, and that the majority of activated cells express the TCR V $\beta$ 2 gene segment. We therefore studied patients with toxic shock syndrome using a modification of the PCR to determine if expansion of V $\beta$ 2<sup>+</sup> T cells is a marker of the in vivo disease process. Five of eight patients studied demonstrated markedly elevated levels of circulating V $\beta$ 2<sup>+</sup> T cells, whereas none showed significantly elevated levels of T cells expressing other V $\beta$  gene segments. The results suggest that toxin-mediated T cell activation, which involves a large fraction of the human T cell repertoire, may be critical in the pathogenesis of this disease.

Toxic shock syndrome remains a serious disease characterized by rapid onset of fever, rash, shock, and multi-organ involvement (1–5). Although the majority of cases are associated with menstruation and tampon use, similar manifestations have been increasingly recognized in other settings associated with focal *Staphylococcus aureus* infections (1–6). Exoproteins purified from *S. aureus*, particularly toxic shock syndrome toxin-1 (TSST-1), have been implicated in the pathogenesis (7, 8), but the mechanism by which TSST-1 results in disease remains unclear.

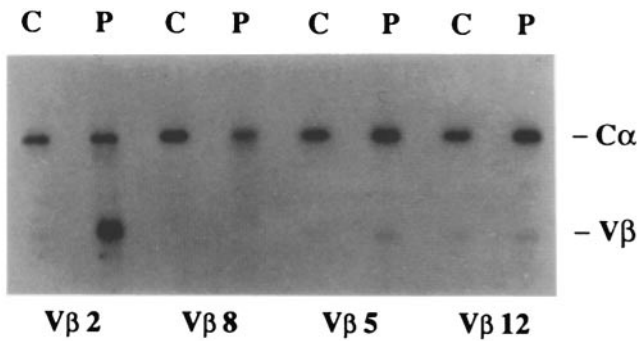
Previous studies have demonstrated that *S. aureus* enterotoxins, including TSST-1, are powerful stimulators of T cell proliferation in culture, in the presence of APCs bearing class II major histocompatibility antigens (reviewed in reference 9). Recent work from our laboratories showed that stimulation of human T cells by individual bacterial toxins is selective for cells expressing particular TCR  $\beta$  chain variable (V $\beta$ ) gene segments (10, 11). Other variable elements (D $\beta$ , J $\beta$ , V $\alpha$ , J $\alpha$ ) of the TCR contribute little to the recognition of these V $\beta$ -specific superantigens, as they do for conventional antigens (9–11). Studies using a modified PCR to quantitate V $\beta$  expression in a population of T cell blasts demonstrated that the majority of T cells stimulated by TSST-1 in culture specifically expressed V $\beta$ 2. Based on these in vitro findings, the present studies were initiated in patients with toxic shock syndrome.

### Materials and Methods

**Patients.** Eight patients were referred for study by their private physicians or through the Colorado Department of Public Health (Pam Shillam, Epidemiology group), and all met the definition for severe toxic shock syndrome (3, 4). Major criteria (all required) for the diagnosis include fever ( $\geq 38.8^\circ\text{C}$ ), rash (diffuse erythematous rash evolving to desquamation), and hypotension (systolic blood pressure  $< 90$  mmHg for adults and/or orthostatic syncope or dizziness). Minor criteria (three required) for the definition include diarrhea and/or vomiting, muscular involvement, mucous membrane hyperemia, decreased renal function or pyuria, elevated liver enzymes, platelet count  $< 100,000/\text{mm}^3$ , and disorientation or altered state of consciousness. Disease in all patients appeared to be related to a focus of *S. aureus* infection.

**Stimulation and Analysis of Peripheral Blood T Cells.** PBMC were isolated from heparinized blood, and to increase the production of functional TCR mRNA, T cells were stimulated with anti-CD3 antibody and IL-2 (10, 11). The cells were then used for either immunofluorescence staining or quickly frozen in liquid nitrogen for subsequent RNA extraction. Indirect immunofluorescence was performed as described using mAbs directed to CD4, CD8, and to epitopes on TCRs bearing V $\beta$ 5, V $\beta$ 6, V $\beta$ 8, and V $\beta$ 12 (10).

**PCR and Quantitation of Amplified Products.** Total RNA prepared from stimulated cells was used for the synthesis of first strand cDNA as described (10, 11). For each PCR, oligonucleotide primers included a V $\beta$ -specific oligomer and an oligomer from the downstream  $\beta$  chain constant region (C $\beta$  primer) as one pair, and two oligomers from the C $\alpha$  region as the other pair. The sequences



**Figure 1.** Autoradiograms of TCR transcripts amplified by PCR. T cells from Patient 1 (P) and control individual (C) were stimulated with anti-CD3 antibody and IL-2 before extraction of RNA and generation of cDNA. Each reaction contained specific oligonucleotide primers to expand the particular V $\beta$  gene segment indicated (170–220 bp), as well as a C $\alpha$  gene segment ( $\sim$ 600 bp).

of the specific primers used and details of the PCR have been published (11). Amplification was performed with  $\sim$ 25 cycles, chosen to ensure that the amount of product synthesized was proportional to the amount of V $\beta$  mRNA in the original preparation. For quantitation of amplified products,  $^{32}$ P end-labeled 3' primers ( $\sim$ 5  $\times$  10<sup>5</sup> cpm each) were added to the reactions. The amplified products were separated on 2% agarose gels, dried, and analyzed with an Ambis Radioanalytic Imaging System (Ambis Systems, San Diego, CA).

### Results and Discussion

T cells from patients and control individuals were stimulated in culture with anti-CD3 antibody and IL-2. Using cDNA generated from T cell blast RNA and a quantitative PCR, TCR gene segments encoding V $\beta$ 2, V $\beta$ 5 (5.2 and 5.3),

V $\beta$ 8 (8.1 and 8.2), and V $\beta$ 12 were amplified and quantitated. To control for the amount of TCR cDNA in the reaction mixture, a C $\alpha$  gene segment was also amplified in each reaction. Fig. 1 shows results with T cells from one patient and a concomitantly studied normal individual. A striking increase in amplified V $\beta$ 2 DNA is apparent in the patient compared with control, whereas little difference is observed in C $\alpha$  or other V $\beta$  products. The data in Table 1 are expressed as a ratio of V $\beta$  DNA to C $\alpha$  DNA amplified in the same reaction. Although all of the controls had V $\beta$ 2/C $\alpha$  ratios less than or equal to 0.10, initial samples from 5 of the 8 patients had ratios greater than 0.17 ( $p = 0.03$  by Fisher's exact test) with one, for Patient 1, as high as 0.78. In contrast to V $\beta$ 2, none of the other V $\beta$ /C $\alpha$  ratios were increased more than 2 SD above control values, indicating the selective nature of V $\beta$ 2 expansion in toxic shock syndrome.

T cells isolated from patients and controls were also analyzed for subset alterations by indirect immunofluorescence and cytofluorographic analysis. No consistent alterations in the percentage of CD4<sup>+</sup> and CD8<sup>+</sup> T cells were noted even in patients with markedly elevated V $\beta$ 2 levels. For example, the percentages of CD4<sup>+</sup> and CD8<sup>+</sup> cells, respectively, were 57 and 41 for Patient 1, 31 and 62 for Patient 2, and 78 and 22 for Patient 6. These data are consistent with previous studies demonstrating that *S. aureus* enterotoxins stimulate both CD4 and CD8 T cell populations in vitro (10). Cells from 7 of the 8 patients were also stained for the expression of V $\beta$ 5, V $\beta$ 6, V $\beta$ 8, and V $\beta$ 12. Values outside the normal range were not observed for these non-V $\beta$ 2 T cell subsets. Mean percent ( $\pm$  SE) values for patients vs. controls ( $n = 12$ ) were: V $\beta$ 5, 2.4  $\pm$  0.25 vs. 3.1  $\pm$  0.30; V $\beta$ 8, 4.3  $\pm$  0.63 vs. 3.7  $\pm$  0.50; V $\beta$ 6, 3.0  $\pm$  0.46 vs. 3.4  $\pm$  0.41; and V $\beta$ 12, 1.6  $\pm$  0.14 vs. 1.5  $\pm$  0.09.

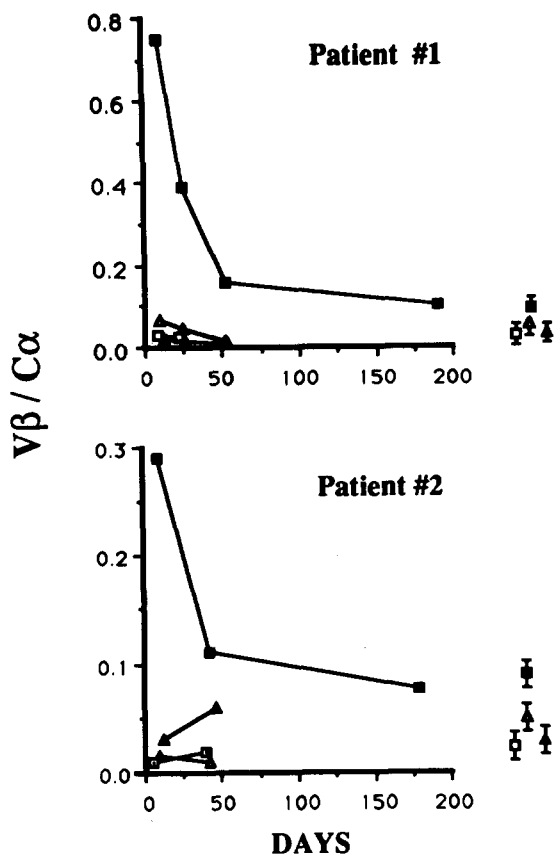
**Table 1.** V $\beta$  Expression in Peripheral Blood T Cells from Patients with Toxic Shock Syndrome

Patients*	Source of <i>S. aureus</i> infection	V $\beta$ /C $\alpha$ ratio <sup>†</sup>			
		V $\beta$ 2	V $\beta$ 5 <sup>‡</sup>	V $\beta$ 8 <sup>‡</sup>	V $\beta$ 12
1	Vagina, menstruation-related	0.78	0.07	0.03	0.02
2	Vagina, menstruation-related	0.25	0.02	0.01	0.03
3	Sinus	0.18	0.03	0.03	0.03
4	Vagina, menstruation-related	0.18	0.04	0.03	0.01
5	Vagina, post-surgical	0.11	0.04	0.04	0.01
6	Buttocks abscess	0.46	0.04	0.03	0.01
7	Peritonsillar cellulitis	0.08	0.04	0.04	0.01
8	Vagina, menstruation-related	0.05	0.04	0.04	0.01
Controls ( $n = 7$ ) (mean $\pm$ SE)		0.09 $\pm$ 0.01	0.05 $\pm$ 0.01	0.025 $\pm$ 0.01	0.03 $\pm$ 0.01

\* Peripheral blood was drawn from patients 3–14 d after the onset of symptoms.

<sup>†</sup> Quantitation of the amount of labeled primer incorporated into the V $\beta$  and C $\alpha$  bands (see Fig. 1) allowed for calculation of the ratios presented (11).

<sup>‡</sup> Primers specific for a sequence common to V $\beta$ 5.2 and 5.3 or V $\beta$ 8.1 and 8.2 were used (11).



**Figure 2.** Longitudinal changes in T cell repertoire in two patients studied serially after toxic shock syndrome.  $V\beta 2/C\alpha$  (■),  $V\beta 5/C\alpha$  (△),  $V\beta 8/C\alpha$  (□), and  $V\beta 12/C\alpha$  (▲) ratios are presented for Patient 1 (top graph) and Patient 2 (bottom graph) in relation to the number of days after the acute onset of symptoms. Normal values (mean  $\pm$  SE) are indicated by the symbols in the right of each graph.

There is currently no mAb specific for human T cells expressing  $V\beta 2$ , and therefore no direct method to quantitate the percentage of these cells in peripheral blood. However, based on those  $V\beta$ s against which specific mAbs are available,  $V\beta/C\alpha$  values obtained with the PCR can be converted to the percentage of T cells expressing the respective  $V\beta$  (11). PCR data in this study suggest that  $V\beta 2^+$  T cells in normal individuals are  $\sim 10\%$  of the peripheral blood T cell population. In contrast, peak values for patients 1 and 2 were  $\sim 70\%$  and  $30\%$ , respectively, emphasizing the striking stimulation occurring in some patients.

Serial samples were obtained from two patients to examine longitudinal changes in T cell subsets after the acute disease.

Fig. 2 shows that  $V\beta 2^+$  T cell percentages in these patients returned nearly to normal by 45–60 d after the acute episode. The fact that levels do normalize indicates that the increase in  $V\beta 2^+$  T cells occurs after the onset of toxic shock syndrome and is not a factor influencing susceptibility. These serial studies also emphasize the relative lack of fluctuation over time in T cell subsets expressing other  $V\beta$  segments, an observation also made in studies of normal individuals (10).

Three of the eight patients studied did not demonstrate elevated  $V\beta 2$  levels. Our initial hypothesis was that disease in these patients was caused by a *S. aureus* toxin different than TSST-1, and that other sets of T cells would be expanded in a  $V\beta$ -specific fashion. We therefore measured a much larger proportion of the T cell repertoire in these patients and controls using 22 different oligonucleotide  $V\beta$ -specific primers (11) and quantitative PCR. Despite accounting for nearly 70% of the T cell repertoire, selective expansion of another T cell subset could not be demonstrated (data not shown). It is still possible that an expanded subset was not included in this analysis. However, we believe it is more likely that selective  $V\beta$  expansion was not reflected in the peripheral blood of some patients because of the timing of blood sampling. Studies in rodents after immunization have indicated that antigen-specific T cells are sequestered in lymphoid tissues (the site of antigen activation) where they are activated and expanded in numbers before emigrating into the recirculating pool (12, 13). It is interesting that two of the three patients with normal  $V\beta 2^+$  T cell levels were studied within 3 d of the onset of symptoms, whereas the highest  $V\beta 2^+$  T cell percentages were observed  $\sim 10$ –14 d after onset of disease.

It is not entirely clear as to how a toxin such as TSST-1 interacts with the host to produce the diverse manifestations of toxic shock syndrome. There is little evidence that TSST-1 directly mediates the decreased vasomotor tone and increased capillary leakage that results in shock, and humoral mediators such as IL-1 and especially TNF have been implicated (14, 15). These cytokines can be shown to be released in cultures of mononuclear cells or macrophages after the addition of TSST-1 or other *S. aureus* enterotoxins (reviewed in references 9 and 14). Recent studies in mice have also indicated that the toxicity of *S. aureus* enterotoxin B is dependent on the presence of T cells (16). In support of a critical role for T cells in the human disease, the data presented here indicate that during toxic shock syndrome T cell stimulation occurs on a scale not observed in response to conventional antigens (9–11). These activated T cells are likely to be releasing large quantities of lymphokines such as IL-2, IFN- $\gamma$  and lymphotoxin (TNF- $\beta$ ), all of which could be involved in the induction of shock (15, 17, 18).

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