Analysis of superantigenic toxin Vβ T-cell signatures produced during cases of staphylococcal toxic shock syndrome and septic shock

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ABSTRACT

Most clinical isolates of Staphylococcus aureus harbour genes encoding superantigenic toxins that bind the Vβ domain of T-cells, but little information is available concerning superantigenic toxin production during staphylococcal toxic shock syndrome (TSS) and septic shock. This prospective study investigated 14 patients with staphylococcal TSS or septic shock; the toxin gene profile of each isolate was determined and flow-cytometry was used to identify the discriminant Vβ signature (DVβS) of each superantigenic toxin in vitro. Attempts were also made to identify in-vivo production of superantigenic toxin DVβS in patients’ blood. The DVβS identified in vitro were: toxic shock syndrome toxin (TSST)-1, Vβ 2; staphylococcal enterotoxin (SE), Vβ 9, Vβ 22; SEB, Vβ 3, Vβ 14, Vβ 17; SED, Vβ 1, Vβ 8; egc, Vβ 5.3, Vβ 7.1, Vβ 9, Vβ 23; and SE/K, Vβ 5.1. The DVβS of TSST-1 and SEB were detected in patients with menstrual and non-menstrual TSS, respectively, whereas no Vβ signature was detected during septic shock. All patients with septic shock (but only one patient with TSS) had lymphopenia and/or impaired cellular immunity. Detection of a superantigenic toxin DVβS may help to show which toxin is produced during staphylococcal TSS, thus confirming the diagnosis and hastening the administration of anti-toxin therapy. In contrast, this approach failed to demonstrate superantigenic toxin involvement in cases of septic shock. In this latter condition, a superantigenic toxin may not be produced by S. aureus, or its production may occur without expansion of targeted T-cells because of T-cell apoptosis and/or anergy.

Keywords Diagnosis, septic shock, Staphylococcus aureus, superantigens, toxic shock syndrome, Vβ signatures

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INTRODUCTION

Staphylococcus aureus produces a wide range of toxins that interact with the immune system. Superantigenic toxins, also called pyrogenic toxin superantigens, which include toxic shock syndrome toxin-1 (TSST-1) and staphylococcal enterotoxins (SEA, SEB, etc.), mediate a potent activation of immune cells [1,2]. These molecules bypass normal antigen presentation pathways by cross-linking major histocompatibility complex class II molecules on antigen-presenting cells to the Vβ domain of the T-cell receptor [1,2]. In this way, each superantigenic toxin selectively stimulates and expands one or several Vβ subsets, thereby producing a Vβ signature for each superantigenic toxin [2–4]. Except for Vβ 2, which is specific to TSST-1, a given Vβ subset may be expanded by different superantigenic toxins, and the discriminant Vβ signature (DVβS) of each superantigenic toxin (i.e., the combination of Vβ subsets specific to a given superantigenic toxin) is unknown [2–4]. The massive T-cell activation mediated by superantigenic toxins leads
to uncontrolled release of pro-inflammatory cytokines, which are responsible for clinical manifestations of toxin-mediated diseases, e.g., toxic shock syndrome (TSS) [2,5].

TSST-1 was first identified in the 1980s as the toxin responsible for menstrual TSS, while both TSST-1 and enterotoxins are involved in non-menstrual TSS [6,7]. For many years, the identification of superantigenic toxins involved in TSS was based mainly on the in-vitro toxin production or toxin gene content of an isolate [6,8–10]. However, superantigenic toxins are also produced by *S. aureus* isolates from patients without TSS [11]. This includes patients with septic shock, thereby adding to the controversy surrounding the involvement of superantigenic toxins in the pathogenesis of *S. aureus* septic shock [12].

It has been shown previously that the in-vivo Vβ signature of superantigenic toxins can be used to confirm a diagnosis of TSS [13,14]; indeed, expansion of Vβ 2 T-cells, which corresponds to TSST-1 superantigenic activity, has been detected in patients with TSS [13–15]. Selective expansion of Vβ T-cells has not yet been investigated in patients with enterotoxin-associated non-menstrual TSS or in patients with *S. aureus* septic shock. The purpose of the present study was to detect in-vivo production of superantigenic toxins early after onset of staphylococcal TSS and septic shock. An attempt was made to identify superantigenic toxin DVβS in peripheral blood mononuclear cells (PBMCs) of patients who had been enrolled prospectively after determination of (i) the toxin gene profile of each isolate and (ii) the DVβS obtained in vitro by flow-cytometry with the culture supernatant of the corresponding *S. aureus* isolate.

**MATERIALS AND METHODS**

**Patients and clinical data**

Patients with *S. aureus* TSS or septic shock were enrolled prospectively between 1 January 2006 and 31 December 2006. The study was approved by the local ethics committee. Septic shock was defined according to the criteria of the American College of Chest Physicians/Society of Critical Care Medicine Conference [16]. *S. aureus* TSS was defined according to CDC criteria [17].

The following data were recorded for all patients: demographical characteristics (age, gender), underlying disorders associated with impaired cellular immunity (haematological immunosuppressive disease, ongoing malignancy, infection with human immunodeficiency virus, cirrhosis, etc.) and current corticosteroid therapy (>1 month) or other immunosuppressive therapy. Therapy with antimicrobial agents and intravenous immunoglobulin was also recorded, and the 28-day mortality rate was determined.

**Microbiological investigations**

*S. aureus* isolates from vaginal swabs and other sites of infection were identified by standard microbiological methods, including Gram’s stain and use of the Phoenix Automated Microbiology System (Becton Dickinson Biosciences, Franklin Lakes, NJ, USA). Each *S. aureus* isolate was also tested for production of coagulase (bioMérieux, Marcy-l’Étoile, France), catalase and clumping factor (Pastorex Staph-plus; Bio-Rad, Hercules, CA, USA).

Genomic DNA was extracted from all isolates and analysed by PCR for genes encoding various superantigenic toxins, i.e., TSST-1, SEA, SEB, SEC, SED (isolates positive for the gene encoding SED were also considered to be positive for the gene encoding SEJ, as these two genes are located on the same plasmid), SEH, SEJ, SEK, SEI, SEI, SEIO (isolates positive for genes encoding SEIM and SEIO were also considered to be positive for SEG, SEI and SEN, as they form part of a single operon known as the enterotoxin gene cluster, egc), SEIP, SEIQ and SEJR [4,18]. The mecA gene coding for methicillin resistance was also detected by PCR [10].

**Flow-cytometry**

*In-vitro determination of the DVβS of individual superantigenic toxins.* Culture supernatants of each clinical isolate, grown for 24 h at 37°C with vigorous shaking in RPMI-1640 medium (Invitrogen, Paisley, UK) supplemented with fetal bovine serum (Invitrogen) 5% v/v, were filtered (Sartorius 0.22-μm Minisart; VivaSciences, Hannover, Germany) and used to stimulate PBMCs (2 × 10⁶/mL) purified from two healthy donors by Ficoll density gradient sedimentation (PANCOLL; PAN Biotech GmbH, Aidenbach, Germany). After incubation for 2 days, the cells were washed twice in phosphate-buffered saline (Invitrogen) and resuspended in fresh RPMI-1640 medium supplemented with heat-inactivated fetal bovine serum 10% v/v, 20 mM HEPES buffer, 2 mM L-glutamine (Sigma-Aldrich, Poole, UK), streptomycin and penicillin (Invitrogen) 100 mg/L in 24-well Falcon plates (Becton Dickinson) for 9 days. The medium was changed every 3 days. After culture for 11 days, PBMCs were labelled with anti-CD3 and anti-Vβ antibodies (24 Vβ antibodies are quantified in the Immunotech IO Test β Mark; Beckman Coulter, High Wycombe, UK) according to the supplier’s instructions. The Vβ repertoire was determined by flow-cytometry (FACScan; Becton Dickinson) and the multiparameter data files were analysed with the CellQuest program (Becton Dickinson). To assign the DVβS of a given superantigenic toxin, the Kruskal–Wallis non-parametric test was used to compare the percentage of each Vβ subset, obtained for all isolates, in the presence and absence of each superantigenic toxin gene, with p <0.05 considered to be statistically significant. Finally, by combining all the results, a single Vβ subset, or a combination of Vβ subsets, was considered to be the DVβS of the corresponding toxin. The results obtained were compared with those published previously [3,4,15, 19–29].
Determination of the V\textsubscript{b} repertoire in patients with staphylococcal toxic or septic shock and detection of a superantigenic toxin D\textsubscript{V}S in blood. The V\textsubscript{b} repertoire of each patient was determined as early as possible during the first 10 days after the onset of symptoms. PBMCs from the patients were purified by Ficoll density gradient sedimentation and immediately labelled with anti-CD3 and anti-V\textsubscript{b} antibodies to determine the V\textsubscript{b} repertoire (see above). When possible, the V\textsubscript{b} repertoire of each patient was also determined after 1 week in order to detect changes in the V\textsubscript{b} pattern with time. Control values for each V\textsubscript{b} subset were obtained using samples from 39 healthy donors. For each V\textsubscript{b} subset, a positive cut-off value for V\textsubscript{b} expansion in vivo was taken as the mean plus two standard deviations of the values obtained for the same V\textsubscript{b} subset in the controls.

**RESULTS**

**Clinical characteristics**

Fourteen patients were enrolled in this study, five with S. aureus TSS (three menstrual and two non-menstrual cases) and nine with S. aureus septic shock. The demographical characteristics and medical histories of these patients are summarised in Table 1. Patients with TSS had a mean age of 30 years (range 18–63 years) and no history of immunosuppression; only one patient had transient lymphopenia (patient 5), and all survived at day 28. Patients with septic shock were older (mean age 61 years, range 35–85 years) and lymphopenia was present in all eight patients tested, with four (44\% of patients) having a medical history of cellular immunosuppression. Five (56\% of these patients) died before day 28. Except for patients 1 and 2 (who had only postural hypotension), all patients required admission to an intensive care unit and vaso-active drug therapy (e.g., norepinephrine) after fluid resuscitation.

**Table 1. Characteristics associated with 14 patients with Staphylococcus aureus toxic shock syndrome (TSS) or septic shock**

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (years), gender</th>
<th>Clinical diagnosis</th>
<th>Cellular immunosuppression</th>
<th>Lymphocyte count (\times 10^9/L)</th>
<th>Site of isolation</th>
<th>Type of infection</th>
<th>ICU admission</th>
<th>Surgery for drainage</th>
<th>Antibiotics</th>
<th>IVIG</th>
<th>28-day mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20, F, mTSS</td>
<td>No</td>
<td>4.53</td>
<td>Vagina</td>
<td>No</td>
<td>Clindamycin, oxacillin</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>24, F, Recurrent mTSS</td>
<td>No</td>
<td>3.26</td>
<td>Vagina</td>
<td>No</td>
<td>Clindamycin, oxacillin</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>18, F, mTSS</td>
<td>No</td>
<td>2.36</td>
<td>Vagina</td>
<td>No</td>
<td>Clindamycin, oxacillin</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>26, M, nmTSS</td>
<td>No</td>
<td>2.08</td>
<td>Surgical site infection</td>
<td>Surgical site</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>63, M, nmTSS</td>
<td>No</td>
<td>0.46</td>
<td>Surgical site infection</td>
<td>Surgical site</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>6</td>
<td>48, F, Septic shock</td>
<td>Chemotherapy</td>
<td>0.2</td>
<td>Noocomial pneumonia</td>
<td>Blood</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>53, M, Septic shock</td>
<td>No</td>
<td>0.85</td>
<td>Endocarditis</td>
<td>Blood</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>35, M, Septic shock</td>
<td>No</td>
<td>1.41</td>
<td>Endocarditis</td>
<td>Blood</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>9</td>
<td>62, F, Septic shock</td>
<td>Corticosteroids</td>
<td>0.2</td>
<td>Catheter infection</td>
<td>Blood</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>10</td>
<td>70, M, Septic shock</td>
<td>Alcoholic cirrhosis</td>
<td>12</td>
<td>Catheter infection</td>
<td>Blood</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>11</td>
<td>61, M, Septic shock</td>
<td>No</td>
<td>0.64</td>
<td>Intra-abdominal infection</td>
<td>Blood</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>12</td>
<td>78, M, Septic shock</td>
<td>No</td>
<td>NA</td>
<td>Endocarditis</td>
<td>Blood</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>13</td>
<td>85, F, Septic shock</td>
<td>No</td>
<td>0.83</td>
<td>Osteoarthritis</td>
<td>Blood</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>14</td>
<td>60, M, Septic shock</td>
<td>No</td>
<td>1.12</td>
<td>Pneumonia</td>
<td>Blood</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

NA, not available; ICU, intensive care unit; IVIG, intravenous immunoglobulin; mTSS, menstrual toxic shock syndrome; nmTSS, non-menstrual toxic shock syndrome; M, male; F, female.

\(^a\)No S. aureus was isolated from vaginal swab or blood.

\(^b\)Introduced after V\textsubscript{b} analysis revealed in-vivo production of superantigenic toxin (patient 4 had persistent fever and remained in shock despite treatment with clindamycin and teicoplanin, and patient 5 had persistent fever despite treatment with oxacillin and gentamicin).
the nine isolates responsible for septic shock, five harboured the sea gene (plus sed, selr, selk or selq in four cases), while two were positive only for the egc locus. One isolate was negative for all the superantigenic toxin genes tested. Among the total of 12 S. aureus isolates, four were positive for the mecA gene (three of which corresponded to the predominant hospital-acquired methicillin-resistant S. aureus clone in France), and these four isolates were responsible for septic shock.

In-vitro determination of the Vβ signature of superantigenic toxins

To determine which Vβ subset was expanded significantly in the presence of each superantigenic toxin gene, the Vβ subset obtained with an isolate positive for the gene encoding a given superantigenic toxin (e.g., the sea gene) was compared with the results obtained for the isolates negative for this gene. Using this approach, the DVS was Vβ 2 for TSST-1, Vβ 9 and Vβ 22 for SEA, Vβ 3, Vβ 14 and Vβ 17 for SEB, and Vβ 1 and Vβ 8 for SED (Table 2). The DVS of superantigenic toxins encoded by the egc locus was Vβ 5.3, Vβ 7.1, Vβ 9 and Vβ 23. The selk and selq genes, which were always jointly present in the isolates investigated, were associated significantly with Vβ subset 5.1. In contrast to the other toxins, no specific Vβ T-cell expansion was revealed in response to isolates positive for SEIR.

Detection of a superantigenic toxin DVβS in peripheral blood

The Vβ repertoire in PBMCs from each patient with S. aureus TSS or septic shock is shown in Table 3 and Fig. 1. A Vβ 2 T-cell expansion was observed in the three patients with menstrual TSS, even when the corresponding isolates showed in-vitro expansion not only of Vβ 2, but also of other Vβ subsets corresponding to the production of SEA and egc-encoded superantigenic toxins. In patient 2, the percentage of the Vβ 2 subset was initially less than two standard deviations above that of the controls, but rose two-fold within 9 days (Fig. 1). As this case fully corresponded to the case definition, it was considered that the two-fold expansion of a Vβ subset, albeit less than two standard deviations above that of the controls, could result from superantigenic toxin activation.

In the two non-menstrual TSS cases, the SEB DVS was detected. Vβ 3, Vβ 14 and Vβ 17 were

Table 2: In-vitro production in culture supernatants of 12 Staphylococcus aureus isolates of discriminant Vβ signatures (p values shown in bold) for each superantigenic toxin investigated

<table>
<thead>
<tr>
<th>Toxins</th>
<th>Vβ signatures described previously</th>
<th>Ref.</th>
<th>Genes</th>
<th>Vβ 1</th>
<th>Vβ 2</th>
<th>Vβ 3</th>
<th>Vβ 5.1</th>
<th>Vβ 5.2</th>
<th>Vβ 5.3</th>
<th>Vβ 7.1</th>
<th>Vβ 7.2</th>
<th>Vβ 8</th>
<th>Vβ 9</th>
<th>Vβ 14</th>
<th>Vβ 17</th>
<th>Vβ 22</th>
<th>Vβ 23</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSST-1 2</td>
<td>-</td>
<td>[3,15]</td>
<td>tst</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.004</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SEA 1, 5, 5.2, 5.3, 6.4, 7.2, 8, 9, 16, 18, 21, 22, 23</td>
<td>[3,22,24]</td>
<td>sea</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
</tbody>
</table>
Table 3. In-vivo production of superantigenic toxins according to the Vβ signature defined in vitro

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Clinical data</th>
<th>Microbiological data</th>
<th>Flow-cytometry investigation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CDC criteria for TSS</td>
<td>meca</td>
<td>Toxin gene profile</td>
</tr>
<tr>
<td>1</td>
<td>mTSS</td>
<td>Yes (probable)</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>Recurrent mTSS</td>
<td>Yes (confirmed)</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>mTSS</td>
<td>Yes (probable)</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>nmTSS</td>
<td>Yes (confirmed)</td>
<td>No</td>
</tr>
<tr>
<td>5</td>
<td>nmTSS</td>
<td>Yes (confirmed)</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>Septic shock</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>7</td>
<td>Septic shock</td>
<td>No</td>
<td>No</td>
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<tr>
<td>8</td>
<td>Septic shock</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>9</td>
<td>Septic shock</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>10</td>
<td>Septic shock</td>
<td>No</td>
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<tr>
<td>11</td>
<td>Septic shock</td>
<td>No</td>
<td>Yes</td>
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<tr>
<td>12</td>
<td>Septic shock</td>
<td>No</td>
<td>No</td>
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<td>13</td>
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<td>No</td>
<td>No</td>
</tr>
<tr>
<td>14</td>
<td>Septic shock</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

ND, not done; mTSS, menstrual toxic shock syndrome; nmTSS, non-menstrual toxic shock syndrome; TSST, toxic shock syndrome toxin; SEB, staphylococcal enterotoxin B.

Vβ subsets expanded by more than two standard deviations above that of the controls are shown in bold; Vβ subsets expanded by less than two standard deviations above the controls, but with >1.5-fold variation between two time-points, are not shown in bold; Vβ subsets comprising a detected D/VβS are underlined.

*The diagnosis of TSS could not be confirmed, as the patient was lost to follow-up after discharge (2 weeks after admission) and no information was available concerning desquamation.

**Corresponding to the predominant clone of methillin-resistant Staphylococcus aureus detected in France.

The T-cell Vβ repertoire was determined once, at the onset of symptoms.

Minor expansion of Vβ 2-positive T-cells (17.7%, without tis gene detection) and minor expansion of Vβ 3-positive T-cells (slightly above two standard deviations of that of the controls, without expansion of Vβ 14 and Vβ 17 and without detection of the selr gene) were detected. The expansion of Vβ 5.1 was less than two standard deviations above that of the controls, but fell 1.6-fold between the two time-points (without detection of the selr gene).

The value of Vβ 14 increased by more than two standard deviations (8.5%) above that of the controls between day 7 (7.10%) and day 14 (10.5%) and remained mildly elevated (11.5%) on day 28 (without expansion of Vβ 3 or Vβ 17 and without detection of the selr gene).

The value of Vβ 8 increased by more than two standard deviations (5.3%) above that of the controls between day 1 (5.5%) and day 11 (6.73%) (without expansion of Vβ 1 and without detection of the selr gene).

DISCUSSION

This study revealed that nearly all cases of TSS were associated with specific Vβ expansion, whereas no specific T-cell modifications were found in patients with septic shock, despite the presence of superantigenic toxin genes in the corresponding isolates and their expression in vitro. As most clinical isolates of S. aureus responsible for TSS and septic shock harbour two or more superantigen-encoding genes [9,12], it was first necessary to determine the precise D/VβS corresponding to each toxin. TSST-1 is known to activate only the Vβ 2 subset in vitro [3,15]. A Vβ signature has also been documented for most enterotoxins in vitro, but the situation is not straightforward as: (i) a given Vβ subset may be part of the Vβ signature of different enterotoxins; (ii) most enterotoxins are associated with the expansion of multiple Vβ subsets; (iii) some Vβ subsets are considered to be major or minor components of the Vβ signature of a given enterotoxin; and (iv) discriminant combinations of Vβ subsets (i.e., the D/VβS) are not known for each enterotoxin [2,3]. To our knowledge, the present investigation is the first study to determine the discriminant Vβ signatures of superantigenic toxins, especially enterotoxins, produced by clinical isolates of S. aureus. For example, the study revealed that only Vβ 9 and Vβ 22 formed the D/VβS of SEA (despite previous reports of the possible expansion of 13 different Vβ subsets; Table 2), and that only Vβ 3, Vβ 14 and Vβ 17 comprised the D/VβS of SEB (despite previous reports of the possible expansion of eight different Vβ subsets; Table 2) [3,22,24]. Determining the
DVβS by analysing the T-cell Vβ repertoire in a patient’s blood may therefore be a rapid (a few hours) and reliable method of detecting production of superantigenic toxins in vivo, thereby guiding the use of anti-toxin agents (e.g., antibiotics to block toxin production, or intravenous immunoglobulin to block toxin activity in vitro) [30]. At present, identification of superantigenic toxins is based mainly on detection of the corresponding gene [9]. However, most clinical S. aureus isolates responsible for TSS and septic shock harbour at least one gene encoding a superantigenic toxin if newly described enterotoxins, e.g., those encoded by the egc locus, are included [9,11,12,31].

Biological criteria are needed for early diagnosis of TSS, as the full CDC case definition of ‘definite’ TSS includes desquamation, which may
occur only several weeks after the onset of symptoms [2,5]. It has been proposed that other biological criteria, e.g., the absence of acute-phase antibodies against TSST-1, should be included to facilitate early diagnosis of TSS [32]. The present study revealed specific Vβ 2 expansion in the three patients with menstrual TSS, which confirmed the findings of previous case reports. This specific T-cell expansion reflected a pathogenic role of TSST-1, since: (i) only TSST-1 is responsible for menstrual TSS; (ii) the isolates carried the gene encoding TSST-1; (iii) Vβ 2 expansion was observed both in the patient’s blood and with the culture supernatant in vitro; and (iv) TSST-1 is the only superantigenic toxin known to target the Vβ 2 subset. In patient 2, who had recurrent TSS, Vβ 2 expansion was only minor, perhaps because of Vβ 2-positive T-cell anergy. Indeed, immunological tolerance and anergy towards a Vβ targeted by a superantigenic toxin have been described in animal models challenged repeatedly with the same superantigenic toxin, and also in humans after a first episode of neonatal TSS-like exanthematous disease, which is another TSST-1-associated disease [33,34]. Vβ 3, Vβ 14 and Vβ 17 expansion was observed in the two cases of non-menstrual TSS. In at least one case (patient 4), specific T-cell expansion revealed SEB intoxication, as: (i) few enterotoxins (only SEB and SEC) can induce concomitant expansion of Vβ 3, Vβ 14 and Vβ 17 [3]; (ii) the isolate carried the gene encoding SEB; and (iii) Vβ 3, Vβ 14 and Vβ 17 expansion was observed in the patient’s blood, and it was demonstrated that these subsets corresponded to the SEB DVβS in vitro. In the second non-menstrual case (patient 5), it was not possible to clearly demonstrate the involvement of SEB, as the isolate was lost before analysis of toxin genes and culture supernatant assays could be performed. However, the patient fulfilled all the criteria for TSS (including desquamation) and, 15 days after the onset of symptom, Vβ 14 expansion associated with Vβ 3 and Vβ 17 expansion was detected, corresponding to the SEB DVβS. It is noteworthy that this patient with TSS developed profound lymphopenia (reported infrequently during TSS) early in the disease, and that normal values returned by the time that the SEB DVβS was detected. In the early stage of the disease, transient depletion has been described before T-cell expansion, possibly following mobilisation from peripheral blood and accumulation in lymph nodes or the spleen [34].

During septic shock, several lines of evidence suggest that superantigenic toxins and other virulence factors may act in concert, resulting in overlap between toxic shock and septic shock in a type of ‘mixed shock’ [35]. First, it has been shown previously that the presence of the sea gene correlates with the onset of septic shock during S. aureus infection [12]. Second, vaccination with modified SEA, SEC or TSST-1 lacking superantigenic properties confers protection in animal models of sepsis induced by S. aureus strains producing SEA, SEC or TSST-1 in vitro [36–38]. No DVβS were detected in patients with septic shock, supporting the hypothesis that superantigenic toxins are not produced in vivo during septic shock. The slight Vβ expansion observed during septic shock did not correspond to that observed in vitro with the corresponding isolates. These results indicate that: (i) superantigenic toxin is not produced during S. aureus septic shock; or (ii) the Vβ expansion pick time has been missed in patients with septic shock (as described in a few patients with TSS [15]); or (iii) that superantigenic toxin production occurs without expansion of targeted T-cells. The last possibility could be explained by T-cell apoptosis and/or anergy [39]. Apoptosis is an early event during the course of septic shock, leading rapidly to profound lymphopenia [40]. All of the septic shock patients (but only one TSS patient) had lymphopenia, and four of them also had impaired cellular immunity resulting from underlying illness or immunosuppressive therapy. Minor ‘expansion’ of Vβ 2-positive T-cells was detected in a patient infected by a strain that did not produce TSST-1. Minor expansion of Vβ 14- and Vβ 8-positive T-cells was also revealed in two other patients, respectively, but these ‘expansions’ did not correspond to any DVβS. This might be because lymphopenia results from differential apoptosis among the 24 major Vβ subsets. As a consequence, during septic shock, T-cell Vβ expansion in response to superantigenic toxins might be limited by apoptosis and/or by anergy, which perhaps explains why it is difficult to demonstrate superantigen involvement in this setting.

A limitation of this study was the small number of patients studied, especially patients with TSS. However, TSS is rare in France, with only one case per 106 inhabitants per year. Also, markers of
T-cell activation, e.g., CD45RO, used in neonatal TSS-like exanthematous disease or in some TSS cases associated with TSST-1 production [14,34], were not employed. Activation of an expanded Vβ subset may provide indications of superantigen production, and may be useful for distinguishing expansion caused by superantigen toxins from relative expansion caused by age-related changes in the T-cell repertoire or proliferative disorders, e.g., myeloma, myelodysplastic syndromes and T-cell lymphoproliferation [41,42]. However, each patient was sampled on several occasions in order to detect short-term variations in a Vβ subset that may provide information about its putative stimulation and expansion. In a previous study, Kamel et al. [43] failed to demonstrate a clear-cut correlation between results in vivo and in vitro in patients with multiple myeloma and non-menstrual toxic shock or septic shock.

In conclusion, analysis of the Vβ T-cell repertoire appears to be useful for detecting the superantigenic toxin DVβS in vivo. This may help to confirm the diagnosis of TSS and hasten the administration of anti-toxin therapy. Further investigations (e.g., analysis of apoptosis of putative targeted Vβ subsets) are required to demonstrate in-vivo production of superantigenic toxins during S. aureus septic shock.

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