Involvement of Enterotoxins G and I in Staphylococcal Toxic Shock Syndrome and Staphylococcal Scarlet Fever

SOPHIE JARRAUD,1 GREGOIRE COZON,2 FRANCOIS VANDENESCH,1 MICHELÈ BES,1 JEROME ETIENNE,1 AND GERARD LINA1*

Centre National de Référence des Toxémies Staphylococciques, Faculté de Médecine, 69372 Lyon Cedex 08, and Unité d’Immunologie, Hôpital de la Croix-Rousse, 69317 Lyon Cedex 04, France.

Received 12 February 1999/Returned for modification 19 March 1999/Accepted 27 April 1999

We investigated the involvement of the recently described staphylococcal enterotoxins G and I in toxic shock syndrome. We reexamined Staphylococcus aureus strains isolated from patients with menstrual and nonmenstrual toxic shock syndrome (nine cases) or staphylococcal scarlet fever (three cases). These strains were selected because they produced none of the toxins known to be involved in these syndromes (toxic shock syndrome toxin 1 and enterotoxins A, B, C, and D), enterotoxin E or H, or exfoliative toxin A or B, despite the fact that superantigenic toxins were detected in a CD69-specific flow cytometry assay measuring T-cell activation. Sets of primers specific to the enterotoxin G and I genes (seg and sei, respectively) were designed and used for PCR amplification. All of the strains were positive for seg and sei. Sequence analysis confirmed that the PCR products, corresponded to the target genes. We suggest that staphylococcal enterotoxins G and I may be capable of causing human staphylococcal toxic shock syndrome and staphylococcal scarlet fever.

Toxic shock syndrome (TSS) is a life-threatening multisystem disorder caused by strains of Staphylococcus aureus. It is characterized by rapid onset of fever, arterial hypotension, scarlatiniform rash, and multiorgan failure (4). Originally described for children by Todd and Fishaut in 1978 (32), TSS has been extensively studied over the past 18 years, since the observation of superantigenic toxins associated with menstruation and the use of a newly introduced superabsorbent brand of tampon (3, 4). These tampons were withdrawn from the market, and the use of a newly introduced superabsorbant brand of tampon has not been associated with TSS (7). All of these staphylococcal enterotoxin G and I (SEG and SEI) have not been linked to TSS (24). In this study we used PCR to detect the SEG and SEI genes (seg and sei, respectively) in CD69-positive, SEA- to SEE-, SEH-, and TSST-1-negative strains isolated from patients with a diagnosis of TSS or SSF. seg and sei were detected in all of these strains, suggesting a clinical importance for these new toxins.

MATERIALS AND METHODS

Patients. The 12 patients included in this study corresponded to nine cases of TSS (including two menstrual case) and three cases of SSF. They were all colonized or infected by S. aureus strains that did not produce the usual superantigenic toxins associated with TSS. They were selected from among 170 cases of TSS, 5 cases of REDD, and 105 cases of SSF reported to the Centre National de Référence des Toxémies à Staphylococques (Lyon, France) between January 1985 and 31 January 1999 from hospitals throughout France. Cases were first identified by chart review if the patient was from the Lyon area or otherwise from accompanying notes sent to the staphylococcal reference center. Cases met the definition of TSS, REDD, or SSF (4, 6, 16). The patients were epidemiologically unrelated.

Strains. S. aureus strains from patients with TSS or SSF were cultured from sites including the genital tract, blood, skin, throat, and soft tissues. Strains were identified as S. aureus by their ability to coagulate citrated rabbit plasma (bioMérieux, Marcy-l’Etoile, France) and to produce a clumping factor (Staphylococcal enterotoxin A, B, C, D, and E; bioMérieux). Isolates were typed by using phage and serotyping techniques (33).

Toxins. Superantigen activity in culture supernatants was detected by measuring T-cell activation in a CD3- and CD69-specific flow cytometric assay (15). Since fewer than 1% of unstimulated CD3+ lymphocytes spontaneously expressed CD69, T cells were considered to be activated when more than 2% expressed CD69 (15). Sequences specific for sea to see, seg to sei, eta, eth, and rat, encoding SEA to SEE, SEG to SEI, exfoliative toxin A (ETA), ETB, and TSST-1, respectively, were detected by PCR. Genomic DNA was extracted from staphylococcal cultures and used as a template for amplification with the primers described in Table 1 (Eurogentec, Seraing, Belgium). The thermal conditions were as follows: denaturation for 1 min at 94°C, annealing for 1 min at 55°C, and extension for 1 min at 72°C. Amplification of gyrB (11) was used as a control to confirm the quality of each DNA extract and the absence of PCR inhibitors. All PCR products were analyzed by electrophoresis through 1% agarose gels (Sigma, Saint Quentin Fallavier, France). The following S. aureus strains were used to

* Corresponding author. Mailing address: Faculté de Médecine, Laboratoire de Bactériologie, Rue Guillaume Paradin, 69372 Lyon Cedex 08, France. Phone: 33 (0) 478 77 86 57. Fax: 33 (0) 478 77 86 58. E-mail: geralina@univ-lyon1.fr.
control the specificity of PCR amplification: RN-450 (negative control), A970237 (negative control), A99024 (negative control), CA-15580 (ATCC 13566) (sea" seb"), FRI-137 (ATCC 10905) (sec"), FRI-1511m (sed"), FRI-326 (ATCC 27764) (sec"), FRI-569 (ATCC 51811) (etb"), FRI-1169 (eta"), TC-7 (eta"), and TC-146 (etb") (1, 7, 11, 13, 15, 20, 26, 27, 31). Since no control strains for SEG and SEI were available, the specificity of seg and sei amplification was assessed by DNA sequencing of selected PCR products (Genome Express, Grenoble, France). To rule out the possibility of false-negative PCR results due to minor variations in the DNA sequences, Southern blotting of selected strains was performed as follows: total DNA was digested with HindIII (Boehringer Mannheim, Meylan, France), separated on a 1% agarose gel, vacuum transferred to positively charged nylon membranes (Boehringer Mannheim), and cross-linked by exposure to UV light. The seg and sei PCR products were labelled with digoxigenin (DIG) by using a DIG DNA Labelling and Detection Kit (Boehringer Mannheim) for use as probes. Hybridization and washing steps were carried out at 68°C in standard buffer solutions (Boehringer Mannheim). Hybridizing bands were detected with anti-DIG–alkaline phosphatase conjugate and the chemiluminescent substrate CSPD, using the DIG Luminescent Detection kit in accordance with the instructions of the supplier (Boehringer Mannheim). Lumi-Film (Boehringer Mannheim) was subsequently exposed to the membranes for 1 h. The sizes of the hybridizing bands were estimated by using a 1-kb DNA ladder (Gibco BRL, Cergy Pontoise, France).

RESULTS

The 12 S. aureus strains induced CD69 expression by over 2% of CD3+ lymphocytes (Table 2), in a manner similar to that observed with supernatants from control strains producing known superantigenic toxins (Table 5). In contrast, the toxin-negative control strain (RN450) induced CD69 expression in only 0.4% ± 0.2% of CD3+ lymphocytes, as observed with strains that do not produce superantigenic toxins (15).

The 12 strains were examined for the presence of sea to sei, seg to sei, eta, and etb by PCR amplification, and were all positive for seg and sei only (Table 2), while RN-450 was negative for all of these genes. Several of the control strains harboring sea to sei, eta, and etb by PCR amplification, and were all positive for both seg and sei (Table 3). seg and sei amplicons from 3 of the 12 clinical strains were sequenced, and the sequences were 100% identical to the published sequences (GenBank accession no. AF064773 and AF064774, respectively). Since a change in only a few bases could cause false-negative results in seg or sei PCR products, DNAs from three PCR-positive strains and five PCR-negative strains were analyzed by Southern blotting with seg and sei probes. Only strains which were positive for seg and sei by PCR hybridized to both DNA probes (Fig. 1), thus confirming the PCR results. The sizes of the hybridizing fragments were identical for both probes but differed between strains, from 2.9 kb (strains A900322 and TC7) to >7.1 kb (strain A980483) (Fig. 1).

Since all 12 strains were positive for both genes by PCR and the sizes of the hybridizing fragments were identical for both probes, the possibility that the seg and sei loci were adjacent to each other was investigated by attempting to coamplify the two genes with all combinations of seg- and sei-specific primers. Only the combination of primer SE1-1 with primer SEG-2 produced an amplicon, of about 3.2 kb, while the other primer combinations gave negative results. Partial sequence analysis showed that the 3.2-kb amplicon contained portions of both seg and sei, in tandem orientation with a 1.9-kb intergenic segment.

Eight of the clinical strains harboring seg and sei were analyzed by phage typing and serotyping. They were not clonal, as they had distinct phage types. Four strains belonged to phage group III, one belonged to group V, and three were untypeable (Table 2). Serotyping confirmed the absence of clonality.

DISCUSSION

Among the staphylococcal superantigenic toxins, only TSST-1, SEA, SEB, SEC, and SED have been linked to TSS or SSF (8, 18, 21). This study shows that two additional staphylococcal enterotoxins, SEG and SEI, are likely associated with TSS or SSF (8, 18, 21). This study shows that two additional staphylococcal enterotoxins, SEG and SEI, are likely associated with TSS or SSF (8, 18, 21). This study shows that two additional staphylococcal enterotoxins, SEG and SEI, are likely associated with TSS or SSF (8, 18, 21).
TABLE 2. Toxin production by *S. aureus* isolates from patients with TSS or SSF that do not produce TSST-1, SEA to SEE, SEH, ETA, or ETB

<table>
<thead>
<tr>
<th>Case</th>
<th>S. aureus strain</th>
<th>Bacterial characteristics</th>
<th>Patient characteristics</th>
<th>Clinical manifestation(s)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A890375</td>
<td>CD69 expression (%)</td>
<td>4.4 ± 1.6</td>
<td>Blood</td>
<td>Alive</td>
</tr>
<tr>
<td>2</td>
<td>A890376</td>
<td>CD69 expression (%)</td>
<td>6 ± 0.6</td>
<td>Abscess</td>
<td>Alive</td>
</tr>
<tr>
<td>3</td>
<td>A890326</td>
<td>CD69 expression (%)</td>
<td>6.1 ± 0.3</td>
<td>Vagina, placenta, and TSS</td>
<td>Alive</td>
</tr>
<tr>
<td>4</td>
<td>A900322</td>
<td>CD69 expression (%)</td>
<td>7.4 ± 2.9</td>
<td>Tampon, vagina</td>
<td>Alive</td>
</tr>
<tr>
<td>5</td>
<td>A900422</td>
<td>CD69 expression (%)</td>
<td>5.2 ± 0.0</td>
<td>Pharynx</td>
<td>Alive</td>
</tr>
<tr>
<td>6</td>
<td>A900472</td>
<td>CD69 expression (%)</td>
<td>5.9 ± 1.0</td>
<td>Blood, bronchoalveolar fluid</td>
<td>Alive</td>
</tr>
<tr>
<td>7</td>
<td>A900432</td>
<td>CD69 expression (%)</td>
<td>3.1 ± 0.8</td>
<td>Urine</td>
<td>Alive</td>
</tr>
<tr>
<td>8</td>
<td>A900422</td>
<td>CD69 expression (%)</td>
<td>2.9 ± 0.1</td>
<td>Sinus, blood</td>
<td>Alive</td>
</tr>
<tr>
<td>9</td>
<td>A900444</td>
<td>CD69 expression (%)</td>
<td>1 ± 1.0</td>
<td>Wound</td>
<td>Alive</td>
</tr>
<tr>
<td>10</td>
<td>A900438</td>
<td>CD69 expression (%)</td>
<td>4.2 ± 1.2</td>
<td>Tampon, vagina, blood</td>
<td>Alive</td>
</tr>
<tr>
<td>11</td>
<td>A900422</td>
<td>CD69 expression (%)</td>
<td>4.3 ± 1.1</td>
<td>Wound</td>
<td>Alive</td>
</tr>
</tbody>
</table>

*All toxins were detected by PCR assay of the corresponding genes.*

<table>
<thead>
<tr>
<th>CD69 expression (%)</th>
<th>Testing detected*</th>
<th>Toxic gene*</th>
<th>Phage typing pattern</th>
<th>Scenotype</th>
<th>Serotype</th>
<th>Age (yr)/sex</th>
<th>Clinical manifestation(s)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.4 ± 1.6</td>
<td>1</td>
<td>SEG, SEI</td>
<td>Group II: 29/52</td>
<td>19/F</td>
<td>ND</td>
<td>77/M</td>
<td>Arteriovenous shunt abscess, TSS</td>
<td>Dead</td>
</tr>
<tr>
<td>6 ± 0.6</td>
<td>2</td>
<td>SEG, SEI</td>
<td>Group I: 1/1</td>
<td>17/M</td>
<td>ND</td>
<td>Thigh abscess, TSS</td>
<td>Alive</td>
<td></td>
</tr>
<tr>
<td>6.1 ± 0.3</td>
<td>3</td>
<td>SEG, SEI</td>
<td>Group III: 84</td>
<td>27/F</td>
<td>ND</td>
<td>Puerperal SSF, Mother alive, newborn blood, newborn dead</td>
<td>Alive</td>
<td></td>
</tr>
<tr>
<td>7.4 ± 2.9</td>
<td>4</td>
<td>SEG, SEI</td>
<td>Group I/III: 29/52/53/54/83A/84/85/87</td>
<td>18/F</td>
<td>ND</td>
<td>Vagina, placenta, and TSS</td>
<td>Alive</td>
<td></td>
</tr>
<tr>
<td>5.2 ± 0.0</td>
<td>5</td>
<td>SEG, SEI</td>
<td>Group II: 29/52</td>
<td>19/F</td>
<td>ND</td>
<td>Thigh abscess, TSS</td>
<td>Alive</td>
<td></td>
</tr>
<tr>
<td>5.9 ± 1.0</td>
<td>6</td>
<td>SEG, SEI</td>
<td>Group III: 84</td>
<td>23/M</td>
<td>ND</td>
<td>Bacteriemia, pneumonia, meningitis, and TSS</td>
<td>Alive</td>
<td></td>
</tr>
<tr>
<td>3.1 ± 0.8</td>
<td>7</td>
<td>SEG, SEI</td>
<td>Group II: 29/52</td>
<td>19/F</td>
<td>ND</td>
<td>Pharyngitis, SSF</td>
<td>Alive</td>
<td></td>
</tr>
<tr>
<td>2.9 ± 0.1</td>
<td>8</td>
<td>SEG, SEI</td>
<td>Group I: 1/1</td>
<td>15/M</td>
<td>ND</td>
<td>Postendoscopy urinary tract infection, TSS</td>
<td>Alive</td>
<td></td>
</tr>
<tr>
<td>1 ± 1.0</td>
<td>9</td>
<td>SEG, SEI</td>
<td>Group I/III: 29/52/53/54/83A/84/85/87</td>
<td>9/F</td>
<td>ND</td>
<td>Vagina, placenta, and TSS</td>
<td>Alive</td>
<td></td>
</tr>
<tr>
<td>4.2 ± 1.2</td>
<td>10</td>
<td>SEG, SEI</td>
<td>Group II: 29/52</td>
<td>44/F</td>
<td>ND</td>
<td>Menstrual TSS</td>
<td>Dead</td>
<td></td>
</tr>
<tr>
<td>4.3 ± 1.1</td>
<td>11</td>
<td>SEG, SEI</td>
<td>Group II: 29/52</td>
<td>35/M</td>
<td>ND</td>
<td>Postoperative spondylitis, TSS</td>
<td>Alive</td>
<td></td>
</tr>
<tr>
<td>4.3 ± 1.1</td>
<td>12</td>
<td>SEG, SEI</td>
<td>Group II: 29/52</td>
<td>39/F</td>
<td>ND</td>
<td>Sinusitis, TSS</td>
<td>Alive</td>
<td></td>
</tr>
</tbody>
</table>

*All toxins were detected by PCR assay of the corresponding genes.*

FIG. 1. Southern blot hybridization of DNAs of *S. aureus* strains with sei and seg probes. Total DNA was digested with *Hind*III, separated by agarose gel electrophoresis, transferred to positively charged nylon membranes, and probed with the indicated DIG-labelled probes. Lanes contain DNAs of three *S. aureus* strains found to be PCR positive for sei and seg (lanes 1, A980483; lanes 2, TC-7; and lanes 3, A900322) and five *S. aureus* strains found to be PCR negative for seg and sei (lanes 4, FR1-1169; lanes 5, FR1-569; lanes 6, RN-450; lanes 7, A970237; and lanes 8, A990204).

In conclusion, *S. aureus* strains that produce both SEG and SEI may be associated with SSF and TSS (including menstrual cases), and the SEG and SEI determinants are close to each other on the *S. aureus* chromosome. The PCR amplification products and Southern blotting (Fig. 1). SEG and SEI produced by these strains were probably responsible for the T-cell activation detected in a CD69-specific flow cytometry assay. It is also conceivable that the SEG and SEI produced by these strains caused the clinical manifestations of TSS or SSF.

As seg and sei were initially cloned from two different strains (FR1-572 and FR1-445, respectively) (24), we were surprised that both seg and sei were detected in all 12 clinical strains and also in several reference strains (Table 3). The positive PCR amplification obtained with the sei-1 and SEG-2 primers in our study indicates that seg and sei are in tandem orientation and are separated by 1.9 kb of intergenic DNA. Sequencing of this intergenic region is under way to determine whether it contains additional open reading frames, as suggested by the reported observation that strain FR1-445 contains part of an enterotoxin-like gene upstream of sei (24). The link between two staphylococcal superantigenic toxins such as seg and sei is not uncommon; it has been described for plasmid pIB485, which contains both seg and sei (34), and for pathogenicity islands containing both tst and an enterotoxin-like gene (19).

Phage typing and serotyping ruled out a clonal origin of our *S. aureus* clinical strains harboring seg and sei and responsible for TSS, contrasting with the clonal origin of strains isolated from patients with menstrual TSS that produced TSST-1 (25). Three of the strains that produced SEG and SEI were associated with two cases of menstrual TSS and a case of puerperal SSF, respectively. Previous findings suggested that the vast majority of cases of menstrual TSS were due to TSST-1-producing strains (29). However, some vaginal isolates from women with menstrual TSS did not produce TSST-1, suggesting that other staphylococcal toxins might be responsible for the clinical manifestations (4, 9, 10, 21); indeed, cases of menstrual TSS related to strains producing only SEA to SED have been described (8, 23). Our data suggest that *S. aureus* strains producing SEG and SEI but not TSST-1 can also cause menstrual TSS, although this needs to be confirmed by experimental and epidemiological studies.

In conclusion, *S. aureus* strains that produce both SEG and SEI may be associated with SSF and TSS (including menstrual cases), and the SEG and SEI determinants are close to each other on the *S. aureus* chromosome. The PCR amplification...
method used in this study is an efficient way of identifying strains harboring seg and set.

ACKNOWLEDGMENTS

We are grateful to N. Viollant, C. Courtier, C. Gordon, and Y. Benito for technical assistance and to A. C. Wong for the gift of strain FRI-569.

REFERENCES