

Staphylococcus aureus Superantigens Elicit Redundant and Extensive Human V β Patterns[∇]

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Received 13 November 2008/Returned for modification 17 November 2008/Accepted 23 February 2009

Staphylococcus aureus can produce a wide variety of exotoxins, including toxic shock syndrome toxin 1 (TSST-1), staphylococcal enterotoxins, and staphylococcal enterotoxin-like toxins. These toxins share superantigenic activity. To investigate the β chain (V β) specificities of each of these toxins, TSST-1 and all known *S. aureus* enterotoxins and enterotoxin-like toxins were produced as recombinant proteins and tested for their ability to induce the selective in vitro expansion of human T cells bearing particular V β T-cell receptors (TCR). Although redundancies were observed between the toxins and the V β populations, each toxin induced the expansion of distinct V β subsets, including enterotoxin H and enterotoxin-like toxin J. Surprisingly, the V β signatures were not associated with a specific phylogenetic group of toxins. Interestingly, each human V β analyzed in this study was stimulated by at least one staphylococcal superantigen, suggesting that the bacterium derives a selective advantage from targeting the entire human TCR V β panel.

Staphylococcus aureus produces a broad range of exoprotoxins, including staphylococcal enterotoxins (SEs) and toxic shock syndrome toxin 1 (TSST-1) (9). These toxins were initially implicated in staphylococcal food poisoning (SEs) and TSS (TSST-1) (39). Since the first characterization of SEA and SEB in 1959 to 1960 by Casman and Bergdoll, 18 different SEs have been described; they are designated SEA to SEV, in the chronological order of their discovery (2, 5, 41). Some were renamed SE-like toxins (SEI), because either no emetic properties were detected or because they were not tested in primate models (21, 41).

SEs, SEIs, and TSST-1 share certain structural and biological properties. They have similar sizes (23 to 29 kDa), and their crystal structures, established for SEA, SEB, SEC, SED, SEH, SEI, SEIK, and TSST-1, reveal significant homology in their secondary and tertiary conformations (26). However SEs, SEIs, and TSST-1 can be divided into four phylogenetic groups based on their primary amino acid sequences (41).

SEs, SEIs, and TSST-1 share superantigenic activity (24). Superantigens (SAGs), unlike conventional antigens, do not need to be processed by antigen-presenting cells (APC) before being presented to T cells. They can directly stimulate T cells by cross-linking major histocompatibility complex class II molecules on APC with the variable portion of the T-cell antigen receptor β chain (TCR V β) or the T-cell antigen receptor α chain for SEH (TCR V α), thereby inducing polyclonal cell proliferation (19, 36, 37). SAG binding sites lie outside the peptide-binding groove and therefore do not depend on T-cell

antigenic specificity but rather on the V β and/or V α region of the TCR (8, 19, 37). It was assumed that each SAG elicited a specific pattern of V β and/or V α activation (24). As SAGs are active at very low concentrations (less than 1 pg/ml) (44), which are barely detectable in vivo, SAG-related diseases might theoretically be identified by determining TCR V β specificities in vitro. For example, an expansion of V β 2 T cells on the one hand and of V β 3, -14, and -17 T cells on the other hand, which correspond to TSST-1 and SEB superantigenic activities, respectively, has been detected in patients with TSS (6, 10, 25, 29). Such an approach would be particularly useful for investigating suspected SAG-related diseases, including some inflammatory disorders, Kawasaki disease, and atopy (11). However, the list of staphylococcal SAG (SSAg) V β specificities is not exhaustive, and different activation profiles have been obtained with different methods (41).

To determine the V β specificities of *S. aureus* SAGs, we produced all known SSAGs as recombinant proteins and investigated their V β TCR specificities in vitro, using commercial antibodies.

MATERIALS AND METHODS

Strains and plasmids. The *S. aureus* strains listed in Table 1 were used to produce recombinant toxins. *Escherichia coli* M15 and *S. aureus* RN6390 and RN4220 were used for plasmid amplification and genetic manipulations.

Toxin production and purification. Primers were designed following the identification of suitable hybridization sites in the toxin genes (Table 1). DNA was extracted and used as a template for PCR amplification as previously described (18). The 5' primers were chosen within the coding sequence of each gene, omitting the region predicted to encode the signal peptide, as determined by using the SignalP 3.0 World Wide Web prediction server (<http://www.cbs.dtu.dk/services/SignalP/>). The 3' primers were chosen to overlap the stop codon of SSAG genes (Table 1). The PCR products were codigested with the appropriate restriction enzymes (Promega, Madison, WI), purified with the High Pure PCR product purification kit (Roche Applied Science, Meylan, France), and ligated using T4 DNA ligase (Roche Applied Science, Meylan, France) in either the pQE-30 expression vector (Qiagen, Courtaboeuf, France) or pLUG345 (4) digested with the same restriction enzymes (BamHI and PstI for pQE30 and BglII

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[∇] Published ahead of print on 2 March 2009.

TABLE 1. Sequences of primers and bacterial strains used in this study

Toxin	Strain	Reference or source	Strain and plasmid use for production	Primers ^a
SEA	A87 0502	This study	<i>S. aureus</i> RN6390 pLUG 345	GAAGGCCTAA AAAACAGCA TTTACATTAC TTTTATTCAT TG CGCGGATCCG TTATTAATGA TGATGATGAT GATGAGAACC CCCAC TTGTA TATAAATATA TATCAATATG CATGTTTTC
SEB	COL	12	<i>S. aureus</i> RN6390 pLUG 345	GAAGGCCTTA TAAGAGATTA TTTATTTTAC ATGTAATTTT G CGCGGATCCG TTATTAATGA TGATGATGAT GATGAGAACC CCCCT TTTTC TTTGTCGTAA GATAAAC
SEC	FRI 137	This study	<i>E. coli</i> M15 pQE30	GTAGGATCCG AGAGCCAACC AGACC GGACTGCAGT TATCCATTCT TTGTTGTAAG GTGG
SED	FRI 137	This study	<i>E. coli</i> M15 pQE30	GTTGGATCCA ATGAAAACAT TGATTCAGTA AAAGAG TTGCTGCAGC TACTTTTCAT ATAAATAGAT GTCAATATG
SEE	FRI918	7	<i>E. coli</i> M15 pQE30	CGCGGATCCA AAAAACAGC ATTTATACTA CTTTTATTCA TTGC CCCAAGCTTA GTTGTGTATA AATACAAATC AATATGGAGG
SEG	A99 0372	This study	<i>S. aureus</i> RN6390 pLUG 345	GAAGGCCTAA GAAATTATCT ACTGTAATTA TTATTTTGGAT TCTAGA AATA G GGAAAGATCT TTTATTAATG ATGATGATGA TGATGAGAACC CCCCG TGAGT ATTAAGAAAT ACTTCC
SEH	MW2	1	<i>E. coli</i> M15 pQE30	TCAGGATCCAAAGCAGAAG ATTTACACGA TAAAAG TTTCTGCAGT TATACTTTTT TCTTAGTATA TAGATTTACA TC
SEI	A900322	18	<i>S. aureus</i> RN6390 pLUG 345	GAAGGCCTAA AAAATTTAAA TATAGTTTTA TATTAGTTTT TATATT ACTT TTTAACATTA AAG CGCGGATCCG TTATTAATGA TGATGATGATGAT GAGAACCCCC GT TACTATCT ACATATGATA TTTTCGAC
SEJ	Fukuoka 5	45	<i>E. coli</i> M15 pQE30	TACGGATCCA GCAAAAATGA AACAATTTAA GAAAAGAATT TGCAC TTACTGCAGC TACAGAACCA AAGGTAGACT TATTAATAC
SEIK	HT2003 0702	This study	<i>E. coli</i> M15 pQE30	CGCGGATCCA AAAAATTAAT AAGCATCTTA TTAATAAATA TAATA ATTTT AGGTG CCCAAGCTTT ATCGTTTCTT TATAAGAAAT ATCGACATC
SEIL	HT2003 0702	This study	<i>S. aureus</i> RN6390 pLUG 345	GAAGGCCTAA AAAAAGATTA TTATTTGTAA TTGTTATTAC TTTATT TATTTTTTCTTC GGAAAGATCT TTTATTAATG ATGATGATGA TGATGAGAACC CCCCT CTTTTTGAAATTTTCG ACATCTAGAT GAAATT
SEIM	A900322	18	<i>S. aureus</i> RN6390 pLUG 345	GAAGGCCTAA AAGAATCTT ATCATTTGTT TTTTATTGTT TTG CGCGGATCCG TTATTAATGA TGATGATGAT GATGAGAACC CCCAC TTTCG TCCTTATAAGA TATTTCTGA
SEIN	A900322	18	<i>E. coli</i> M15 pQE30	GAAGGCCTAG ATTGTTCTAC ATAGCTGCAA TTATAATAAC CGCGGATCCG TTATTAATGA TGATGATGAT GATGAGAACC CCCAT CTTTA TATAAAAATA CATCAATATG ATAATTAG
SEIO	A900322	18	<i>S. aureus</i> RN6390 pLUG 345	GAAGGCCTAT TAAAAATAGT AAAGTAATGT TAAATGTATT ATTA TTTTA AATTTAATTG CGCGGATCCG TTATTAATGA TGATGATGAT GATGAGAACC CCCCTG TAAAT AAATAAACAT CAATATGATA GTCTG
SEIP	HT2003 0702	This study	<i>S. aureus</i> RN6390 pLUG 345	GAAGGCCTAG TAAAAATAAAA AAAACAACAT TTATACTACT TTCATT TATT G GGAAAGATCT TTTATTAATG ATGATGATGA TGATGAGAACC CCCCA GTTGT ATATAAATAT ATATCAATAT GCATATTTTT AGAC
SEIQ	COL	12	<i>E. coli</i> M15 pQE30	GCAGGATCCGATGTAGGGG TAATCAATCT TA AAACTGCAGT TATTCAGTTT TCTCATATGA AATCTC
SER	Fukuoka5	31	<i>E. coli</i> M15 pQE30	GTAGGATCCAAACCAGATC CAAGGCCTG CCGCTGCAGT CACATTGTAG TCAGGTGAAC TTC
SEIU	A940624	42	<i>E. coli</i> M15 pQE30	CA GGATCC ATG TTA AAT GGC AAT CCT AAA C CA GC CTGCAG TTA TTT TTT GGT TAA ATG AAC TTC TAC ATT AAT AGA TTT A
SEIV	A940624	42	<i>E. coli</i> M15 pQE30	GCA GGATCC GAT GTC GGA GTT TTG AAT CTT AGG TAA CTGCAG TTA GTT ACT ATC TAC ATA TGA TAT TTC GAC ATC
TSST-1	N315	Kuroda, 2001	<i>S. aureus</i> RN6390 pLUG 345	GAAGGCCTAATAAAAAATTAATGATGATGATGATGATGATGAGAACC GGAAAGATCTTTTATTAATGATGATGATGATGATGATGAGAACC AATTTCTGCTTCTATAGTTTTTATTTTCATC

^a Restriction sites are underlined (BamHI and PstI for pQE30 and BglIII and StuI for pLUG345).

and StuI for pLUG345). The resulting pQE plasmids were transformed into *E. coli* strain M15. Open reading frame integrity was verified by sequencing the junctions between the plasmid and the insert. For toxin expression in *S. aureus*, plasmids were transferred by electroporation into RN4220, a nitrosoguanidine-induced mutant capable of accepting *E. coli* DNA, before transfer to RN6390. His-tagged recombinant toxins were purified by affinity chromatography on a nickel affinity column according to the supplier's in-

structions (New England Biolabs, Ipswich, MA). The protein purity was verified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Lipopolysaccharide was removed from the toxin solutions by affinity chromatography with Detoxi-GEL endotoxin gel (Pierce, Rockford, IL). The QCL-1000 *Limulus* amoebocyte lysate assay (Cambrex-BioWhittaker, Walkersville, MD) showed that the endotoxin content of the recombinant SSAg solutions was less than 0.005 units/ml.

Flow cytometry and CD69 assay. Toxin activities were assessed by measuring CD69 surface expression by T cells upon toxin challenge (22). Briefly, 50 μ l of whole blood was incubated with 1.0 and 0.1 μ g/ml (final concentrations) of recombinant TSST-1, SEA, SEB, SEC, SED, SEE, SEG, SEH, SEI, SEIJ, SEIK, SEIL, SEIM, SEIN, SEIO, SEIP, SEIQ, SER, SEIU, and SEIV in RPMI 1640 culture medium containing 5% heat-inactivated fetal calf serum (Gibco Invitrogen, Paisley, United Kingdom) for 24 h at 37°C in humidified air with 5% CO₂. Culture medium and phytohemagglutinin (PHA; 10 μ g/ml) were used as negative and positive controls, respectively. After ammonium chloride erythrocyte lysis, leukocytes were incubated with a mixture of anti-CD3 conjugated with cyanin-5-phycoerythrin (Dako, Glostrup, Denmark) and anti-CD69 conjugated with fluorescein isothiocyanate (Beckman Coulter, Miami, FL). The cells were then analyzed with a FACScan flow cytometer (Becton Dickinson Biosciences, San Jose, CA), and the results were expressed as the percentage of CD3⁺ lymphocytes expressing CD69. The experiments were done in triplicate with cells from three different blood donors.

Analysis of T-cell V β repertoires. Peripheral blood mononuclear cells (PBMC) were isolated from the heparinized venous blood of healthy donors by Ficoll density gradient sedimentation (Pancoll; PAN Biotech GmbH, Aidenbach, Germany). The cells were washed three times in Hank's balanced salt solution (Sigma-Aldrich, St. Louis, MO) and suspended in RPMI 1640 medium supplemented with 5% heat-inactivated fetal calf serum, 20 mM HEPES buffer, 2 mM L-glutamine (Sigma-Aldrich), 100 IU/ml penicillin G, and 100 μ g/ml streptomycin (Sigma-Aldrich) at a density of 2×10^6 to 5×10^6 cells per ml. The cells were stimulated with 500 ng/ml of recombinant TSST-1, SEA, SEB, SEC, SED, SEE, SEG, SEH, SEI, SEIJ, SEIK, SEIL, SEIM, SEIN, SEIO, SEIP, SEIQ, SER, SEIU, SEIV, RPMI 1640 (negative control), or 10 μ g/ml PHA (positive control) for three days at 37°C in humidified air with 5% CO₂. In a previous kinetic study of PBMC stimulation with SAGs, we have observed that some V β expansion could be only detected later after stimulation, from day 6 to at least day 10 (42). Thus, to optimize the detection of V β expansion, we have decided to increase the length of the incubation by an additional 9 days of incubation with fresh culture medium. Thus, cells were then washed in Hank's balanced salt solution and incubated in culture medium with 20 to 100 U/ml hu-IL-2 (Eurobio, Courtaboeuf, France) for 9 days at 37°C in humidified air with 5% CO₂. The V β profile was then determined by flow cytometry (FACScan; Becton Dickinson Biosciences, San Jose, CA) using the IOTest Beta Mark kit (Beckman Coulter, Miami, FL), according to the supplier's instructions. This kit is a multiparametric tool designed for the quantitative determination of the TCR V β repertoire of human T lymphocytes by flow cytometry (V β 1, V β 2, V β 3, V β 4, V β 5.1, V β 5.2, V β 5.3, V β 7.1, V β 7.2, V β 8, V β 9, V β 11, V β 12, V β 13.1, V β 13.2, V β 13.6, V β 14, V β 16, V β 17, V β 18, V β 20, V β 21.3, V β 22, and V β 23). To complete the V β panel of IOTest Beta Mark, we performed additional staining and analysis with V β 6.7 antibody (Pierce, Rockford, IL) as previously described (42). The multiparameter data files were analyzed with the Cellquest program (BD Biosciences, Le-Pont-de-Claix, France). The experiments were done in triplicate with cells from three different blood donors. Since the percentage of V β subsets varies between blood donors and after PBMC stimulation by PHA, we expressed the results as ratios of the percentage of TCR V β expansion induced by each toxin relative to that by PHA. The V β subsets that were more abundant with the toxin than with PHA among cells with the three donors were considered to be significantly expanded (Mann-Whitney test, $P = 0.037$).

Phylogenetic analysis. The amino acid sequences of the mature toxins were deduced from the sequences obtained from GenBank and SignalP. The alignment was performed with ClustalX software (43). Evolutionary distances were determined by the Kimura method, and the values were used to construct a dendrogram by means of the neighbor-joining method using SplitsTree4 software (16). At least 1,000 bootstrap trees were generated to investigate the stability of the phylogenetic relationships.

RESULTS

Toxin activities. (i) Induction of CD69 expression. Challenge with 1.0 and 0.1 μ g/ml purified recombinant TSST-1, SEA, SEB, SEC, SED, SEE, SEG, SEH, SEI, SEIJ, SEIK, SEIL, SEIM, SEIN, SEIO, SEIP, SEIQ, SER, SEIU, and SEIV rapidly induced strong CD69 expression on CD3⁺ cells (Fig. 1). Depending on the toxin, CD69 was expressed by 8 to 32% of the cells and by 14 to 35% of the cells at concentrations of 0.1 and 1.0 μ g/ml, respectively. By comparison, CD69 expres-

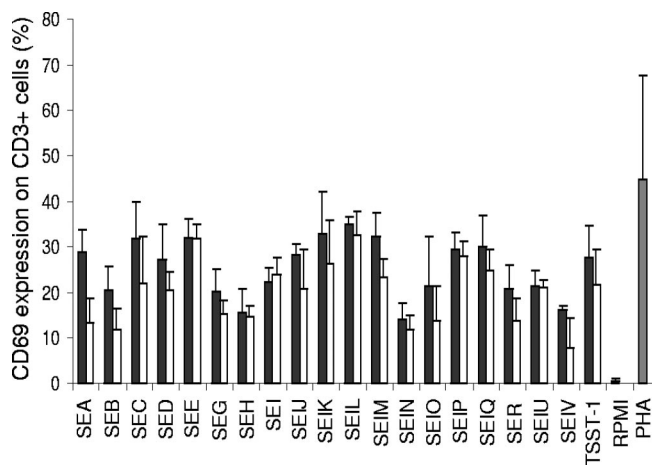
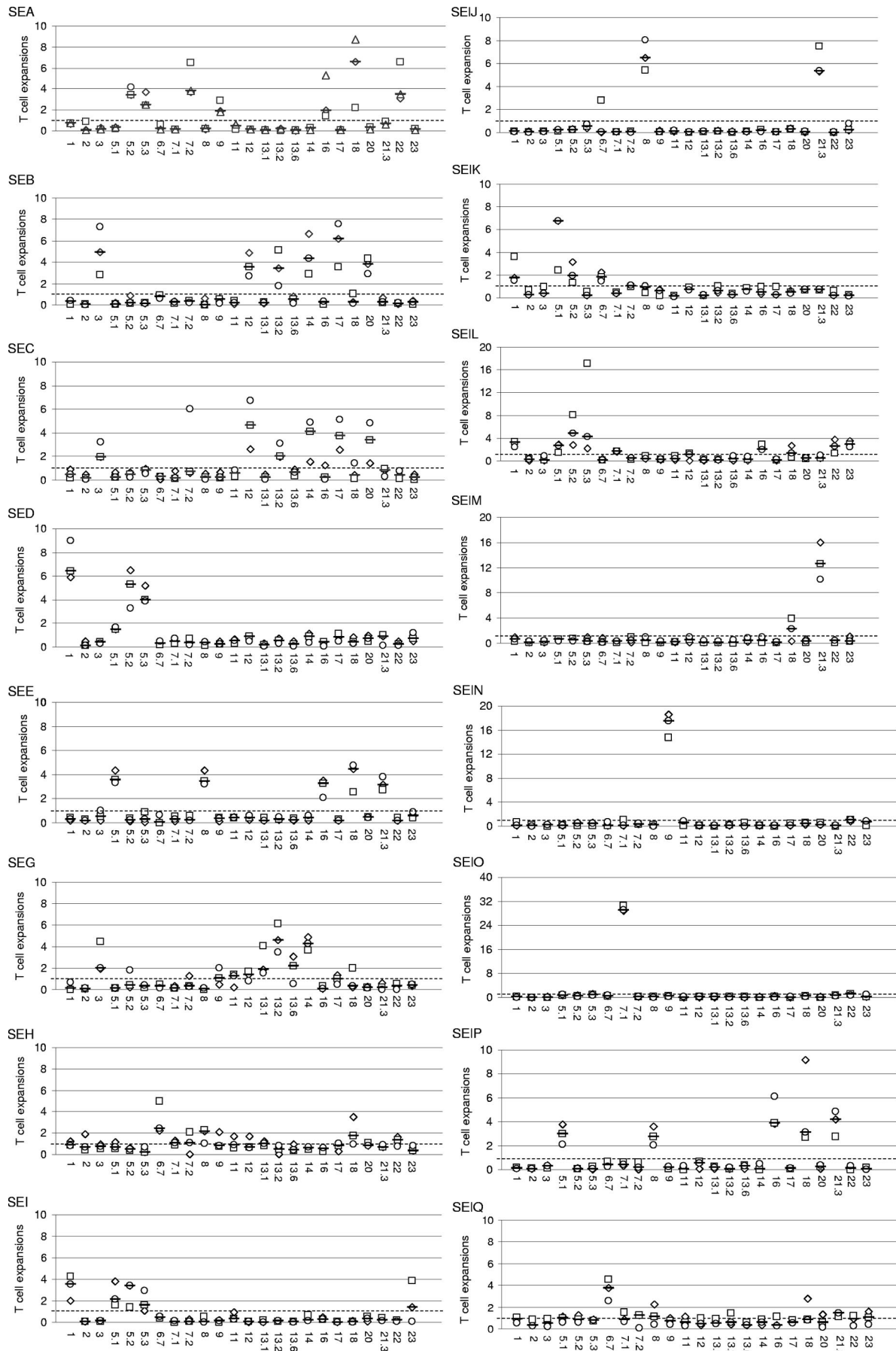


FIG. 1. CD69 expression by T lymphocytes upon *S. aureus* SAG challenge. CD69 expression was measured on T lymphocytes (CD3⁺) after 24 h of incubation with 1.0 μ g/ml of SSAGs (black bars) and 0.1 μ g/ml of SSAGs (white bars) of whole blood, Eagle's minimal essential medium (negative control; RPMI [gray bar]), or 10 μ g/ml PHA (positive control; gray bar). Results are means \pm standard deviations ($n = 3$).

sion was below 1% when the cells were incubated with RPMI medium and 44% when they were incubated with PHA. These experiments showed that our recombinant TSST-1, SEA, SEB, SEC, SED, SEE, SEG, SEH, SEI, SEIJ, SEIK, SEIL, SEIM, SEIN, SEIO, SEIP, SEIQ, SER, SEIU, and SEIV could activate T cells.

(ii) TCR V β repertoires. Purified recombinant TSST-1, SEA, SEB, SEC, SED, SEE, SEG, SEH, SEI, SEIJ, SEIK, SEIL, SEIM, SEIN, SEIO, SEIP, SEIQ, SER, SEIU, and SEIV were studied for their ability to induce the selective expansion of T cells bearing particular TCR V β regions in PBMC culture. V β profiles were determined by flow cytometry using the IOTest Beta Mark kit completed with a V β 6.7 antibody. The antibody against TCR V β 4 generated nonspecific labeling, making it impossible to identify the TCR V β 4 cells. Consequently, the TCR V β 4 results were excluded from the study. As shown in Fig. 2, recombinant TSST-1, SEA, SEB, SEC, SED, SEE, SEG, SEH, SEI, SEIJ, SEIK, SEIL, SEIM, SEIN, SEIO, SEIP, SEIQ, SER, SEIU, and SEIV induced the selective expansion of distinct V β subpopulations with various potencies. TSST-1, SEA, SEG, SEH, SEIJ, SEIK, SEIL, SEIM, SEIN, SEIO, SEIQ, SER, SEIU, and SEIV induced unique expansion patterns. As summarized in Table 2, TSST-1 induced the expansion of V β 2; SEA induced V β 5.2, V β 5.3, V β 7.2, V β 9, V β 16, V β 18, and V β 22; SEG induced V β 3, V β 13.1, V β 13.2, and V β 14; SEH induced V β 6.7 and V β 8; SEIJ induced V β 8 and V β 21.3; SEIK induced V β 1, V β 5.1, V β 5.2, and V β 6.7; SEIL induced V β 1, V β 5.1, V β 5.2, V β 5.3, V β 7.1, V β 16, V β 22, and V β 23; SEIM induced V β 21.3; SEIN induced V β 9; SEIO induced V β 7.1; SEIQ induced V β 6.7 and V β 21.3; SER induced V β 3, V β 12, and V β 14; SEIU induced V β 13.2 and V β 14; and SEIV induced V β 6.7, V β 18, and V β 21.3. By contrast, similar patterns of V β expansion were observed with SEB and SEC (V β 3, V β 12, V β 13.2, V β 14, V β 17, and V β 20), SED and SEI (V β 1, V β 5.1, V β 5.2, and V β 5.3), and SEE and SEIP (V β 5.1, V β 8, V β 16, V β 18, and V β 21.3). When similar V β subpopulations were stimulated by more



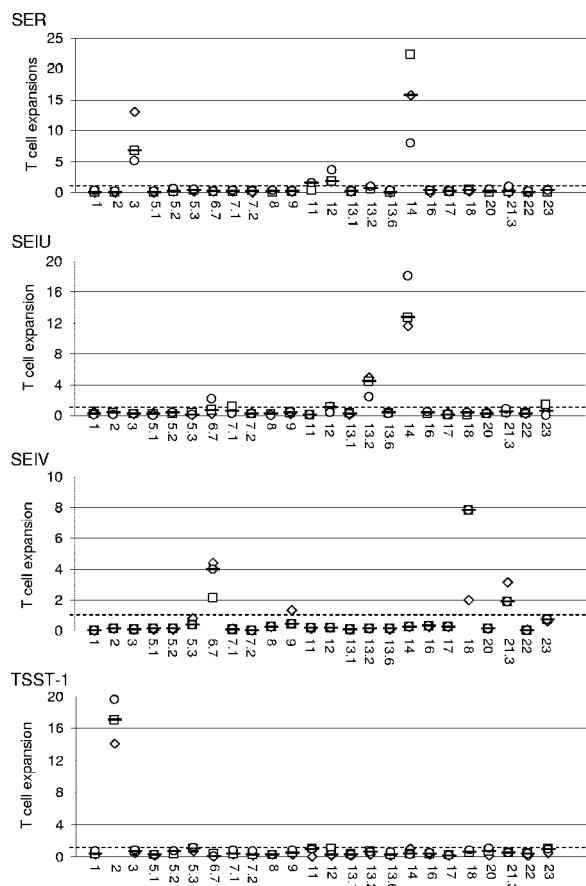


FIG. 2. Human Vβ expansion induced by *S. aureus* SAGs as detected with the IOTest Beta Mark kit. Purified recombinant toxins and PHA were studied for their ability to induce the selective expansion of T cells bearing particular TCR Vβ regions in PBMC culture. Vβ profiles were determined by flow cytometry using the IOTest Beta Mark kit completed with a Vβ6.7 antibody. Results are expressed as ratios of the percentage of TCR Vβ expansion induced by each toxin relative to that of PHA. The x axis label indicates Vβs. The data shown here are the representative ratios observed with each of the three blood donors (□, ◇, ○) plus the medians of these ratios (•), while the horizontal dashed line represents a ratio of 1.

than one toxin, the potencies of the toxins sometimes differed. For example, the expansion of Vβ3 was stronger with SEB than with SEC, while Vβ12 expansion was stronger with SEC than with SEB.

Phylogenic groups. A phylogenetic tree was constructed from the deduced amino acid sequences of the mature TSST-1, the enterotoxins, and the enterotoxin-like toxins by using the neighbor-joining method (Fig. 3). The nodes were well supported (>70% bootstrap values) with four exceptions, namely, the nodes for TSST-1, SEH, the common branch SEIO/SEIN, and SEIK/SEI/SEIM/SEIV. We identified four groups within the tree: SEA, SED, SEE, SEH, SEIJ, SEIN, SEIO, and SEIP; SEB, SEC, SEG, SER, and SEIU; SEI, SEIK, SEIL, SEIM, SEIQ, and SEIV; and TSST-1 alone. The Vβ specificity of each toxin is indicated in the tree (Fig. 3). Surprisingly, we observed no correlation between the phylogenic groups and the Vβ specificities. Similar Vβ subsets (e.g., Vβ1, Vβ5.1 to -5.3, Vβ6.7, Vβ7.1, Vβ16, Vβ18, Vβ21.3, and Vβ22) were elicited

by toxins belonging to different monophyletic groups, such as SEA and SEI. For instance, SED (from the SEA phylum) and SEI both induced Vβ1, -5.1, -5.2, and -5.3 T-cell expansion. By contrast, all the Vβ subsets that were activated by most of the toxins belonging to the SEB group (except for SEB and SEC, which had the same Vβ specificity) and by TSST-1 were specific to these phylogenetic groups.

DISCUSSION

To determine the Vβ specificities of SSAGs, we produced all known *S. aureus* SAGs as recombinant proteins and studied their ability to induce the selective expansion of T cells bearing particular TCR Vβ regions in PBMC culture. All the recombinant toxins induced T-cell activation, as shown by enhanced CD69 expression. Specific Vβ expansion was observed with all the toxins, confirming their SAG status. Importantly, SEIJ, which induced strong Vβ8 and -21.3 expansions, and SEH, which induced significant Vβ6.7 and weak Vβ8 expansions, had not previously been shown to induce specific Vβ repertoires. We also found that SEIQ induced the weak expansion of Vβ21.3.

The Vβ profiles observed with our recombinant toxins and with the IOTest Beta Mark kit completed with a Vβ6.7 antibody were similar to those described elsewhere (3). However, several of our results appear to conflict with published data. We did not detect the previously described Vβ1.1 expansion induced by SEA (27), Vβ8.1 and Vβ12.1 by SED (20), Vβ12 and Vβ13.6 by SEG (18), Vβ23 by SEI (18), Vβ18 by SEIM,

TABLE 2. Human Vβ expansion induced by *S. aureus* superantigens, as detected with the IOTest Beta Mark kit

Toxin	TCR Vβ ^a
SEA	5.2, 5.3, 7.2, 9, 16, 18, 22
SEB	3, 12, 13.2, 14, 17, 20
SEC	3, 12, 13.2, 14, 17, 20
SED	1, 5.1, 5.2, 5.3
SEE	5.1, 8, 16, 18, 21.3
SEG	3, 13.1, 13.2, 14
SEH	6.7, 8
SEI	1, 5.1, 5.2, 5.3
SEIJ	8, 21.3
SEIK	1, 5.1, 5.2, 6.7
SEIL	1, 5.1, 5.2, 5.3, 7.1, 16, 22, 23
SEIM	21.3
SEIN	9
SEIO	7.1
SEIP	5.1, 8, 16, 18, 21.3
SEIQ	6.7, 21.3
SER	3, 12, 14
SEIU	13.2, 14
SEIV	6.7, 18, 21.3
TSST-1	2

^a Purified recombinant toxins were tested for their ability to induce the selective expansion of T cells bearing particular TCR Vβ regions in PBMC culture. Vβ profiles were determined by flow cytometry with the IOTest Beta Mark kit completed with a Vβ6.7 antibody. Experiments were done in triplicate with cells from three donors. Since the percentage of Vβ subsets varies between blood donors and after PBMC stimulation by PHA, we expressed the results as ratios of the percentage of TCR Vβ expansion induced by each toxin relative to that by PHA. Only Vβ subsets that were more abundant with the toxin than with PHA among cells with the three donors were considered to be significantly expanded (Mann-Whitney test, P = 0.037) and are indicated.

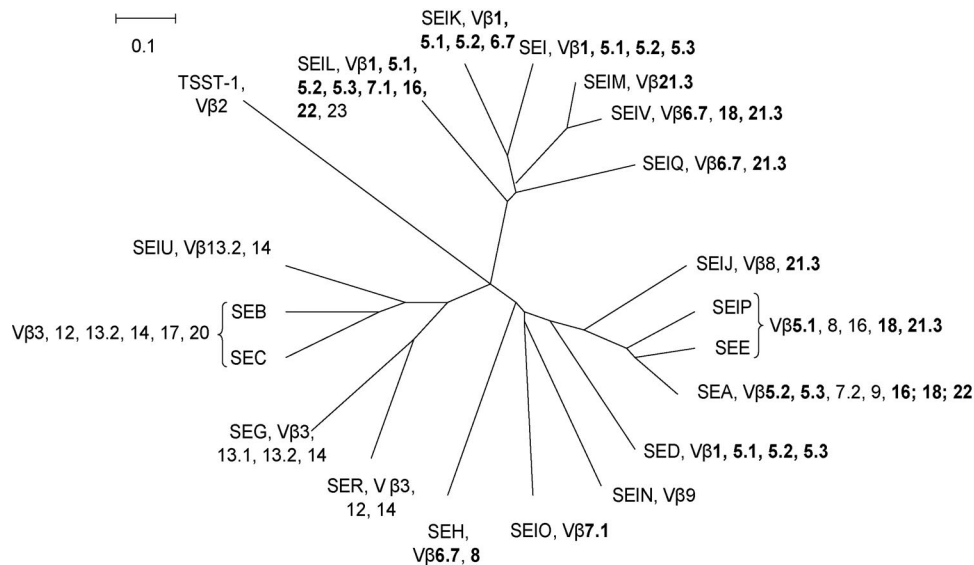


FIG. 3. Reconstitution of the phylogenetic tree of *S. aureus* SAGs. Amino acid sequences of the mature toxins were deduced from sequences obtained from GenBank and SignalP analysis. Alignment was performed with ClustalX software. Evolutionary distances were determined by the Kimura method, and the values were used to construct a dendrogram by means of the neighbor-joining method using SplitsTree4 software. V β expansion induced by each toxin is indicated. V β expansions observed in response to several SSAG phylogenetic groups are boldfaced.

V β 5.1 and V β 22 by SEIO (18), V β 2.1 and V β 5.1 by SEIQ (35), or V β 11 by SER (31). In contrast, we observed the specific expansion of V β 1 by SEIK (34), V β 6.7 and V β 8 by SEH, and V β 1 and V β 7 by SEL (33). These discrepancies may be explained by differences in the methods used to detect V β expansion (other sets of antibodies or reverse transcriptase PCR), the length of the incubation, and the use of different cutoff values used to define the significant enhancement of V β expansion. In several cases, V β expansion was only observed with two blood donors for V β 13.6 and SEG, V β 18 and SEIM, and V β 11 and SER.

Interestingly, our phylogenetic analysis showed no correlation between the toxin phyla and V β specificity. Toxins from different phyla sometimes induced similar V β subsets, while several V β subsets were sometimes induced by more than one toxin. This suggests that only a few key amino acids in the toxin sequences are responsible for V β specificity. Only TSST-1, SEA, SEG, SEH, SEIJ, SEIK, SEIL, SEIN, SEIM, SEIO, SEIQ, SER, SEIU, and SEIV generated unique V β patterns. Thus, it would be risky to attempt to identify the SSAG involved in a given SSAG-related disease simply by determining the V β repertoire of blood cells. Indeed, the results should be interpreted according to the toxin profile of the corresponding isolate. This would help to show which toxins are expressed in vivo and could provide pathophysiological insights, especially into putative SSAG-related diseases.

Depending on the *S. aureus* SAG, between one and eight (median, >3) V β T-cell subpopulations were activated, and each V β was induced by between one and six SSAGs (median, 3). These results emphasize the redundancy of *S. aureus* SSAGs. V β 5.2 and V β 14 were targeted by five SSAGs and V β 5.1, V β 18, and V β 21.3 by six SSAGs, suggesting that *S. aureus* might derive a selective advantage by activating these particular T-cell subpopulations. It would be hazardous to assume that all SSAGs with similar V β specificities are biologi-

cally equivalent. At the level of APC/T-cell interaction, while SEB and SEC induced the expansion of similar T-cell subsets in our study, they bind preferentially to distinct major histocompatibility complex class II isotypes (14, 38). SEB shows higher affinity for human leukocyte antigen DR-like molecules, and SEC for human leukocyte antigen DQ-like molecules.

A given *S. aureus* strain harbors only a small number of SSAG genes (17). Sometimes, genes encoding toxins that share similar V β specificities can be found on the same genetic background (30). The expression of each of these genes by *S. aureus* is precisely controlled by several regulatory systems but in a different manner (28). As suggested by Grumann et al., we suspect that it is beneficial for *S. aureus* to extend the conditions when the immune system is triggered by SSAGs (13).

It is important to note that all the V β specificities tested were activated by at least one SSAG. Even other V β s, such as V β 15 (not tested with the IOTest Beta Mark kit) and V β 4 are activated by SSAGs (3). It is widely thought that SSAGs benefit *S. aureus* by disrupting the immune system and notably by inducing immune anergy through T-cell suppressor activity, along with B-cell depression and the inhibition of antibody responses (15, 18, 23, 40). Our results are in keeping with this hypothesis, as they show that SSAGs interact with the entire V β repertoire in humans.

In conclusion, by examining the ability of all known SSAGs to induce selective TCR V β expansion, we found a certain redundancy among SSAG V β specificities. This clearly hinders SSAG identification based on elicited V β profiles. Interestingly, each human V β was stimulated by at least one SSAG, suggesting that the bacterium derives a selective advantage from targeting the entire TCR V β panel.

While the experimental work was finished, Ono et al. has reported the discovery of two novel staphylococcal enterotoxins, SES and SET (32). SES induced strong V β 9 and -16

T-cell expansions, but no V β specificity was detected for SET.

ACKNOWLEDGMENTS

We thank Grégoire Cozon for the preliminary characterization of the SSAGs; Martine Rougier, Annie Martra, Christine Courtier, Christine Gardon, Céline Spinelli, Caroline Bouveyron, and Florence Couzon for their technical advice; and David Young for editorial guidance.

The laboratory received research grants from the French Ministry of Health and Education, from the French medical research council (INSERM), and from Pfizer.

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Editor: S. R. Blanke