Biochemical and Biological Properties of Staphylococcal Enterotoxin K

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Staphylococcus aureus is an important human pathogen which is implicated in a wide variety of diseases. Major determinants of the virulence of this organism include extracellular virulence factors. Staphylococcal enterotoxins (SEs) are important causative agents in staphylococcal toxic shock syndrome and food poisoning. Our study identified a novel enterotoxin, SEK, and examined its biochemical and biological properties. SEK had a molecular weight of 26,000 and an experimentally determined pI of between 7.0 and 7.5. SEK was secreted by clinical isolates of S. aureus. We demonstrated that SEK had many of the biological activities associated with the SEs, including superantigenicity, pyrogenicity, the ability to enhance the lethal effect of endotoxin, and lethality in a rabbit model when administered by subcutaneous miniosmotic pump. Recombinant SEK was shown to stimulate human CD4+ and CD8+ T cells in a Vβ-specific manner; T-cells bearing Vβ 5.1, 5.2, and 6.7 were significantly stimulated to proliferate.

Staphylococcus aureus is an important human pathogen due to a combination of toxin-mediated virulence, invasiveness, and antibiotic resistance (5, 7, 9, 12, 14, 31, 35). The organism is a significant cause of nosocomial infections, as well as community-acquired disease. The spectrum of staphylococcal infection ranges from pimples and furuncles to toxic shock syndrome (TSS) and sepsis (24, 26, 35).

The virulence factors secreted by S. aureus are major determinants of both disease causation and severity during the course of infection. These factors include several hemolysins (α, β, γ, and δ), leukocidin, exfoliative toxins A and B, and the large family of pyrogenic toxin superantigens (PTSAgs) (reviewed in references 5, 11, and 20). The latter toxins include toxic shock syndrome toxin 1 (TSST-1) and the staphylococcal enterotoxins (SEs) A to J, excluding F (11). All of the staphylococcal PTSAgs are encoded on variable genetic elements, with TSST-1 and enterotoxins B and C, among others, being present on pathogenicity islands (SaPIs) (29).

Numerous studies have shown that PTSAgs are important determinants for TSS (11) and food poisoning (reviewed in reference 2). Todd and coworkers were the first to recognize S. aureus as the etiologic agent of TSS (56). Subsequent work by Schliefert et al. (51) and Bergdoll and colleagues (3) identified TSST-1 as the major toxin associated with this illness, whether menstrual or nonmenstrual associated; TSST-1 accounts for 75% of all TSS cases. Later work by Schliefert and others established that recombinant SEA and -C, were important causes of nonmenstrual-associated TSS (3, 48). The SEs, particularly SEA and -D, and, to a lesser extent, SEB and -C, are also common causes of staphylococcal food poisoning (11, 54).

Crystallographic studies of the PTSAgs have shown that these molecules share the same basic three-dimensional structure (11, 50). The toxins begin with a short N-terminal α helix that leads into a β barrel structure, also known as the B domain or oligonucleotide binding (OB). The OB fold is connected to a C-terminal wall of β strands by a central diagonal α helix, forming domain A. All PTSAgs have these features in common, but some differ in that they have a small number of additional loops. The most notable of these is a cystine loop structure present in the SEs (15). This cystine loop is thought to be important for emetic activity, based on studies of mutants (11, 15). Recently, however, SEI has been identified, which lacks the cystine loop structure; this toxin is both superantigenic and emetic, although the emetic activity is significantly reduced (34).

Overall, the PTSAgs share numerous biological activities, including superantigenicity, pyrogenicity, the capacity to enhance endotoxin shock, and lethality when administered in subcutaneous miniosmotic pumps (11, 50). For superantigenicity (18, 30, 31), PTSAgs bind to the variable region of the β chain (Vβ) of certain T-cell receptors (TCRs). This mode of binding to the TCR is much less specific than the typical TCR-peptide-major histocompatibility complex (MHC) II trimolecular complex that is required for T-cell activation. Depending on the Vβ specificity of the PTSAg, up to 50% of the host T cells may be activated, resulting in massive cytokine release, with concomitant induction of capillary leak (hypotension) (8, 11, 30, 33). The other biological activities, pyrogenicity, endotoxin enhancement, and lethality when given in miniosmotic pumps, are also dependent on cytokine release (11). Among PTSAgs, only SEs have emetic activity, and this activity has been separated from superantigenicity (11, 15).

This study was undertaken to purify and characterize a new SE, designated SEK, and to determine whether a functional PTSAg is encoded by its gene. In this report, we demonstrate that recombinant SEK (rSEK) functions as a superantigen and is lethal in rabbit models of TSS, and the toxin is expressed by clinical isolates.
Cloning and sequencing. The gene sek was cloned from S. aureus TSS isolate MNJ. This isolate produces SEB as well as SEK. PCR primers were chosen based on the sek sequence of SaPi1 (29), as well as comparison with the unfinished genome sequence of Staphylococcus aureus strain COL, available online at the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov). PCR primers including several hundred nucleotides at either end of the gene were included in the original clone. The sequences of the sek primers were 5′ AGAAGATGCTGTCGGCGGAGTA and 3′ GAGGTAGCCCGGC. The PCR product was electromorphosed in 1% agarose, and the 1.4-kb band containing the entire sek gene was cut out of the gel. The DNA was purified from the agarose gel by using the GeneClean II kit (Bio101, La Jolla, Calif.) and cloned into the TA vector pGEM T-easy (Promega, Madison, Wis.), resulting in the plasmid pPMO001. The vector plasmid contains TT overhangs that allow for cloning of PCR products directly without restriction digestion. Subsequently, the gene was excised from this vector with EcoRI, using restriction sites on either side of the insertion site in the vector, and ligated with EcoRI-digested plasmid vector pCE104 (44). The resultant plasmid was transformed into Escherichia coli XL-1 Blue (Stratagene, La Jolla, Calif.), and a DNA insert with a restriction fragment of the proper length was verified by plasmid purification with the QiaGen Spin Kit (QIAGEN, Chatsworth, Calif.), followed by electrophoresis in a 0.8% agarose gel. The DNA was purified from the agarose gel by using the GeneClean II kit (Bio101, La Jolla, Calif.) and cloned into the TA vector pGEM T-easy (Promega, Madison, Wis.), resulting in the plasmid pPMO002. The vector plasmid contains TT overhangs that allow for cloning of PCR products directly without restriction digestion. Subsequently, the gene was excised from this vector with EcoRI, using restriction sites on either side of the insertion site in the vector, and ligated with EcoRI-digested plasmid vector pCE104 (44). The resultant plasmid was transformed into E.coli XL-1 Blue (Stratagene, La Jolla, Calif.), and a DNA insert with a restriction fragment of the proper length was verified by plasmid purification with the QiaGen Spin Kit (QIAGEN, Chatsworth, Calif.), followed by electrophoresis in a 0.8% agarose gel. The DNA was purified from the agarose gel by using the GeneClean II kit (Bio101, La Jolla, Calif.) and cloned into the TA vector pGEM T-easy (Promega, Madison, Wis.), resulting in the plasmid pPMO002. The vector plasmid contains TT overhangs that allow for cloning of PCR products directly without restriction digestion. Subsequently, the gene was excised from this vector with EcoRI, using restriction sites on either side of the insertion site in the vector, and ligated with EcoRI-digested plasmid vector pCE104 (44). The resultant plasmid was transformed into E.coli BL21 DE3 for expression with the pRSET system. The deleted signal peptide of SEK was replaced with an N-terminal methionine. In addition, for the purposes of expression in pET28b, the N-terminal glutamine was replaced with glycine. The N-terminal sequence of rSEK was MGGDIGIDNLR. Expression and purification. pET28b clone containing sek (pPM0003) in BL-21 DE3 was grown to early log phase in Luria-Bertani medium supplemented with kanamycin (50 μg/ml) at 37°C with shaking (approximately 100 rpm; OTR Shaker, New Brunswick Scientific, New Brunswick, N.J.) and induced with 200 mM isopropyl-b-D-thiogalactopyranoside (IPTG). At the same time, 1-ml amounts of early-log-phase culture in 15% glycerol were stored at −80°C. The cultures were treated with 4 volumes of absolute ethanol for 48 h. After 4 h, each culture was harvested the next day, and cell pellets were resuspended at a concentration 20 times that of the original culture volume and examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions (21). Proteins were stained with Coomassie blue R250. Induction of the expression system was ascertained by detection of the proper length was verified by plasmid purification with the QiaGen Spin Kit (QIAGEN, Chatsworth, Calif.), followed by electrophoresis in a 0.8% agarose gel. The DNA was purified from the agarose gel by using the GeneClean II kit (Bio101, La Jolla, Calif.) and cloned into the TA vector pGEM T-easy (Promega, Madison, Wis.), resulting in the plasmid pPMO002. The vector plasmid contains TT overhangs that allow for cloning of PCR products directly without restriction digestion. 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The cultures were treated with 4 volumes of absolute ethanol for 48 h to precipitate toxins. The precipitated extracellular and released periplasmic proteins were removed by centrifugation at a concentration of 20 ml intravenously. Three rabbits were injected with each dose. Each rabbit’s temperature was measured with rectal thermometers at 0 and 4 h. After 4 h, each rabbit was injected intravenously with 10 μg of lipopolysaccharide (LPS) from Salmonella enterica (50% lethal dose of endotoxin alone). The lethality of this regimen over a 48-h period was assessed (45, 52). Miniosmotic pump lethality studies. Six American Dutch Belted rabbits had miniosmotic pumps, containing 200 μl (200 μg) of either TSST-1 or SEK, implanted subcutaneously on the left flank (41). Lethality of the toxins was assessed over a period of 15 days. Expression of SEK by clinical isolates. Clinical TSS isolates (36) that tested positive for TSST-1, SEB, or SEC were also evaluated for production of SEK. Crude supernatants (10 ml) of these isolates were collected by centrifugation, and the supernatants were heat-inactivated for 5 min at 65°C. The supernatants were harvested the next day, and cell pellets were resuspended at a concentration of 20 ml intravenously. Three rabbits were injected with each dose. Each rabbit’s temperature was measured with rectal thermometers at 0 and 4 h. After 4 h, each rabbit was injected intravenously with 10 μg of lipopolysaccharide (LPS) from Salmonella enterica (50% lethal dose of endotoxin alone). The lethality of this regimen over a 48-h period was assessed (45, 52). Flow cytometric analysis of T-cell repertoire. Peripheral blood mononuclear cells (PBMCs) obtained from three normal human donors were isolated from heparinized venous blood by density gradient sedimentation over Ficoll-Hypaque (Becton-Dickinson, Cockeysville, Md.). Cells were then washed three times in Hanks balanced salt solution (HBSS; Mediatech Cellogel, Herndon, Va.) and resuspended in medium for cell culture. PBMCs (at 106 cells/ml) were cultured in RPMI 1640 (Mediatech Cellogel) supplemented with 10% heat-inactivated fetal calf serum (Gemini Bioproducts, Woodland, Calif.), 20 mM HEPES buffer (Mediatech Cellogel), 100 U of penicillin per ml (Mediatech Cellogel), 100 μg of streptomycin per ml (Mediatech Cellogel), and 2 mM L-glutamine (Mediatech Cellogel). Cells were cultured in the presence of either anti-CD3 (20 ng/ml) or SEK (100 ng/ml) for 3 days, washed, and allowed to grow for an additional day in the presence of interleukin 2 (50 U/ml) before being washed and stained for immunofluorescence analysis of the T-cell repertoire as previously described (23, 25, 53). For flow cytometry studies, PBMCs were washed in HBSS and resuspended at 10 × 106 cells/ml in a staining solution (PBS with 5% fetal bovine serum [FBS; Gemini Bioproducts, 1% immunoglobulin [Alpha Therapeutic Corp., Los Angeles, Calif.], 0.02% sodium azide [Sigma]). Cells were stained in 96-well, round-bottomed plates with a panel of biotinylated monoclonal antibodies against human Vβ 2, 3, 5, 1, 5, 2, 7, 8, 11, 12, 13, 12, 14, 17, 20, 23, and 22 (Immunotech, Westbrook, Maine); Vβ 9 and 23 (Pharmingen, San Diego, Calif.); and Vβ 6.7-fluorescein isothiocyanate (FITC) (Endogen, Woburn, Mass.) and then incubated for 30 min at 37°C in the dark. After the incubation period, cells were washed twice with washing buffer (PBS, 2% FCS [Gemini Bioproducts], 0.02% sodium azide [Sigma]) by centrifugation at 300 × g for 5 min at 4°C. Cell pellets were resuspended in staining solution and incubated with anti-CD3 allophycocyanin, anti-CD4 phycoerythrin (Becton Dickinson, San
Jose, Calif.), anti-CD8 (FITC) (Becton Dickinson), and a streptavidin-peridinin-chlorophyll protein (PerCP) conjugate (Becton Dickinson) for 30 min at 4°C. Stained cells were again washed twice in washing buffer and once in 0.02% sodium azide (Sigma) in PBS, by centrifugation at 300 g for 5 min at 4°C. Finally, the cells were fixed in 200 ml of 1% (vol/vol) formaldehyde (Polysciences, Warrington, Pa.) in PBS. Analysis was performed by four-color flow cytometry (FACSCalibur; Becton Dickinson) as described previously (53). Methods of cytometer setup and data acquisition have also been described previously (53).

List mode multiparameter data files (each file with forward scatter, side scatter, and 4 fluorescent parameters) were analyzed with the Cellquest program (Becton Dickinson). Analysis of activated populations was performed with the light scatter gate set on the T-cell blast population. Negative control reagents were used to verify the staining specificity of experimental antibodies.

RESULTS

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Jose, Calif.), anti-CD8 (FITC) (Becton Dickinson), and a streptavidin-peridinin-chlorophyll protein (PerCP) conjugate (Becton Dickinson) for 30 min at 4°C. Stained cells were again washed twice in washing buffer and once in 0.02% sodium azide (Sigma) in PBS, by centrifugation at 300 g for 5 min at 4°C. Finally, the cells were fixed in 200 ml of 1% (vol/vol) formaldehyde (Polysciences, Warrington, Pa.) in PBS. Analysis was performed by four-color flow cytometry (FACSCalibur; Becton Dickinson) as described previously (53). Methods of cytometer setup and data acquisition have also been described previously (53).

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RESULTS

Sequence of sek. sek was cloned and sequenced from the clinical isolate MN NJ. The open reading frame encoded a polypeptide 242 amino acids in length (Fig. 1). The putative RBS is in boldface, and putative −35 and −10 promoter sequences are underlined. An asterisk marks the predicted amino terminus of the mature protein after removal of the signal peptide.

FIG. 1. Nucleotide and inferred amino acid sequences of sek and SEK, respectively, cloned from staphylococcal TSS isolate MN NJ. The putative RBS is in boldface, and putative −35 and −10 promoter sequences are underlined. An asterisk marks the predicted amino terminus of the mature protein after removal of the signal peptide.

Biochemical properties of recombinant SEK. In order to work with the gene product, a recombinant construct was made with the predicted N-terminal sequence, with a methionine added to the amino terminus for expression in the pET system. This recombinant protein, rSEK, was observed to have a pI of between 7.0 and 7.5. The pI predicted from computer primary sequence analysis was 6.5. The predicted molecular weight of this polypeptide was 25,539. Both of these values were produced with the online Compute pI/MW tool (http://expasy.cbr.nrc.ca/tools/pi_tool.html) with the predicted mature SEK sequence. When purified and evaluated by SDS-PAGE, the recombinant protein had an apparent molecular weight of about 30,000 (Fig. 4). It is not uncommon for PTSAgs to have a higher apparent molecular weight by SDS-PAGE than the predicted amino acid sequence would suggest.

Detection of SEK in clinical isolates. A set of 36 clinical isolates was examined for the ability to produce detectable

FIG. 2. Phylogenetic tree diagram of the family of SEs of serotypes A to L. Three distinct subfamilies can be observed. Groupings are an indication of relatedness, but distances are not quantitatively related to evolutionary distance.

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Detection of SEK in clinical isolates. A set of 36 clinical isolates was examined for the ability to produce detectable
levels of SEK. We were able to detect toxin production by Ouchterlony double immunodiffusion in one isolate (MN JA) and were able to see a reaction with a polyclonal antiserum on a Western blot (Fig. 5). Fourteen of the 36 isolates, including MN NJ, contained bands of the correct size which were reactive with the polyclonal antiserum to rSEK (data not shown).

**Biological activity of rSEK.** The ability of rSEK to stimulate rabbit splenocytes was assessed in a standard superantigenicity assay (Fig. 6). We observed that rSEK was able to stimulate splenocyte proliferation comparable to that of TSST-1. The pyrogenic activity of rSEK and its ability to enhance host susceptibility to endotoxin shock were also examined (Fig. 7). It was observed that rSEK caused fever in rabbits at doses of 4.5 and 0.45 mg/kg, but not at 0.045 mg/kg. The minimum pyrogenic dose of rSEK at 4 h (defined as the dose required to give a 0.5°C average rise in body temperature) was 0.2 µg/kg. This was consistent with minimum doses of other PTSAgs to cause fever. The same doses that were able to cause fever enhanced the lethality of endotoxin, while the nonpyrogenic dose was not active. Finally, the lethality of rSEK when infused into rabbits from subcutaneous miniosmotic pumps was assessed. All three rabbits infused with 200 µg of rSEK died within a 15-day period. The same dose of TSST-1 was also lethal in this model.

The TCR Vβ stimulation profile of rSEK was assessed for human T cells from three volunteers by use of flow cytometry. Antibodies were directed against the different TCR Vβ subsets in human PBMCs. It was observed that TCR Vβs 5.1, 5.2, and 6.7 were preferentially activated by the toxin (Fig. 8). The most prominent subset from all three subjects used was Vβ 5.1, reaching a highest level of 44% of the T-cell population in one case. Vβ 5.2 and 6.7 expansions were not as strong, but were still significant (P = 0.055 and 0.045, respectively). Consistent
with a superantigenic effect, we observed an expansion of Vβs in CD4+ and CD8+ T-cell subsets. In one of the three patients tested, TCR Vβ22 was also expanded, but the significance of this result was not clear. Some T cells bearing certain TCR Vβ subsets were reduced in relative numbers. Examples of this effect include Vβ2, 3, 8, 9, and 13.1. This reduction in relative population size was not the result of apoptosis of T cells expressing those Vβs. Rather, the reduction resulted from those T-cell populations being present in smaller numbers through lack of stimulation relative to the T cells that were preferentially expanded by the toxin.

**DISCUSSION**

The data presented in this work indicate that a novel enterotoxin, designated SEK, is encoded by a gene (sek) contained on a SaPI, designated SaPI3, that also contains the gene (seb) for SEB. sek is also present on SaPI1 (29). SEK shares similar biochemical and biological properties to those described previously for enterotoxins and PTSAgs in general (5, 11). Because the presence of multiple virulence factor genes is a defining feature of pathogenicity islands (29), the presence of both seb and sek on SaPI3 validates the use of the term “pathogenicity island” in this case.

The presence of multiple expressed toxins also makes it less clear whether any single toxin is responsible for all clinical features of TSS induced by such clinical isolates of *S. aureus*. Rather, the data suggest that these strains make multiple toxins with similar activity and that all could contribute to human disease in the absence of protective antibodies; this does not preclude some toxins being more important than others due to differences in amounts made. There is a precedent for expression of multiple toxins by TSS *S. aureus* in the literature. As many as 15% of TSST-1-positive strains also produce SEC, and 75% of TSST-1-positive strains also make SEA (36). It now appears that certain strains may contain even more toxin genes.

Analysis of the sequence of sek and SEK, respectively, shows that SEK fits into a new subfamily of enterotoxins, along with the recently described SEI (34) and SEL (unpublished data). SEK is more closely related to the SEA, -D, and -E subfamily than to SEB and -C (Fig. 3), although sek can be genetically linked to seb, as on SaPI3. Analysis of the sequence 5′ of the putative sek translational start site reveals a Shine-Dalgarno sequence typical of *S. aureus* RBSs previously described (38). sek also contains −10 and −35 putative promoter sequences similar to those previously observed (38).

rSEK was superantigenic, capable of stimulating proliferation of both CD4+ and CD8+ T-cells, and pyrogenic and enhanced the lethal effects of endotoxin in a rabbit model. It was also lethal in a model of TSS in which miniosmotic pumps that deliver a constant amount of toxin over a 7-day period were implanted subcutaneously in rabbits. Although we have not evaluated rSEK for emetic activity, its homology to the other SEs suggests that SEK does belong to the SE subfamily of PTSAgs. It should be noted that the protein used in these studies.
studies was cloned without its putative N-terminal signal sequence, and in order to express the protein in the pET system, a methionine and glycine were added to replace the N-terminal glutamine of the recombinant protein. Thus, the N-terminal sequence of rSEK was MGGDIGIDNLR, while the putative N-terminal sequence of SEK was QGDIGIDNLR. This alteration did not appear to have a significant effect on the biological activity of the toxin, however, since rSEK exhibited comparable immunobiological activity to those of TSST-1 and other PTSAgs (11, 51, 52). A putative degradation product of SEK was present in the supernatant of the clinical isolates we tested, as seen in the Western blot presented (Fig. 5). This is also seen for other SEs, where it has been suggested that staphylococcal proteases cleave the toxins, yielding a fairly stable, lower-molecular-weight, antibody-reactive product (5, 6).

The study of SEK is likely to increase our understanding of the structure-function relationships within the PTSAgs. The structures of TSST-1 (1, 42), SEA to -D (46, 55), and several streptococcal pyrogenic exotoxins (40, 45, 49) have been solved, as well as those of a variety of mutant staphylococcal toxins. In addition, SEB and -C have been crystallized in complex with the TCR (13, 28), as well as TSST-1 and SEB in complex with MHC class II (16, 17, 19). These studies have allowed detailed models of PTSAg activation of T cells to be developed. However, there are many facets of PTSAg activities which have not been explained in terms of structural differences. A prime example of this is emetic activity. The enterotoxins are uniquely characterized by their abilities to cause emetic responses when administered orally to monkeys (11), whereas other PTSAgs are not emetic (11). However, we still do not completely understand what parts of the SE molecules are required for this emetic function. It has been proposed that the cystine loop, located in the OB fold of the toxins, is important for emesis (15, 50), but this has only been incompletely studied. Because SEK has only one cysteine, the molecule does not contain a cystine loop at the usual position. One other enterotoxin (SEI) has been identified that lacks the cystine loop, and that protein was shown to be only weakly emetic compared to SEA, -B, and -C (34). Based on primary structure, it is likely that SEK will act more like SEI than the other SEs and thus be less emetic than other toxins. Structural studies of SEs, such as SEK and -I, and comparison to structures of highly emetic SEs may help elucidate what SE structural components are necessary for this activity.

Structural studies of the SEK, -L, and -I subfamily of PTSAgs may also increase our understanding of how superantigens interact with immune cells. For example, SEK generally functions similar to other PTSAgs in stimulation of T cells dependent on the composition of the TCR Vβ, but it has a TCR Vβ profile distinct from those previously observed. Only one other characterized toxin stimulates TCR Vβ 5.1 (SEE), and no other toxins have been observed to stimulate Vβ 5.2 or 6.7. The structure of the PTSAg must dictate the TCR Vβ profile of the stimulated T cells (10, 16, 18). It has been seen with other toxins that a large region at the top front (in the standard view of SEB and -C) or top back of TSST-1 is important for TCR binding (11, 13, 22, 28). Where SEK, SEI, and SEL interact with either TCR or MHC II remains to be determined.

It is noteworthy that SEK has the strongest homology with other SEs in the C-terminal β grasp domain, whereas some PTSAgs (SEA, for example) bind to MHC class II, opposite the usual MHC II site in the OB fold domain (46, 55). In SEA, zinc is coordinated by His187, His225, and Asp227, which are important residues in this MHC II binding site (46, 55). These residues are present in the same position relative to one another in SEK (His169, His208, and Asp210), suggesting SEK may interact with zinc and may have an MHC II binding site in this position. The zinc binding site in SEA also requires Ser1. Homologous residues are not present in either SEK or rSEK, implying that some other residue would be necessary to make up the final piece of the tetrahedral coordination site in this toxin. We do not know if zinc is present in rSEK, but clearly the N-terminal residue normally present on SEK (Gln1) is not required for superantigenicity, as Ser1 is for SEA (46); rSEK, which lacks Gln1, retains superantigenic activity.

We have shown that human PBMCs containing TCR Vβ 5.1, 5.2, and 6.7 were significantly stimulated by rSEK in vitro. TCR Vβ 5.1 in particular has been observed to be overrepresented in several diseases of unknown etiology, in particular Crohn’s disease, a severe small bowel inflammatory disorder (43). T cells from the Vβ 5 family have also been implicated in juvenile rheumatoid arthritis and periodontitis (32, 37). In the latter case, TCR Vβ 6.7 has also been observed to be overrepresented (37). PTSAgs from both S. aureus and group A streptococci have also been implicated in forms of psoriasis and atopic dermatitis (23–27, 53). Previous studies have isolated S. aureus from psoriatic lesions, and some of the organisms were categorized as non-enterotoxin producing based on antibody testing against toxins identified at the time (26). It is possible that new SEs, such as SEK, may play a role in previously unexplained cases of these illnesses.

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REFERENCES