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Detection and Measurement of Staphylococcal Enterotoxin-Like K (SEl-K) Secretion by *Staphylococcus aureus* Clinical Isolates

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Staphylococcal enterotoxin-like K (SEl-K) is a potent mitogen that elicits T-cell proliferation and cytokine production at very low concentrations. However, unlike the classical enterotoxins SEB and toxic shock syndrome toxin 1 (TSST-1), the gene for SEl-K is commonly present in more than half of all *Staphylococcus aureus* clinical isolates and is present in almost all USA300 community-acquired methicillin-resistant *S. aureus* (CA-MRSA) isolates. Sequencing of the *sel-k* gene in over 20 clinical isolates and comparative analysis with all 14 published *sel-k* sequences indicate that there are at least 6 variants of the *sel-k* gene, including one that is conserved among all examined USA300 strains. Additionally, we have developed a highly sensitive enzyme-linked immunosorbent assay (ELISA) that specifically detects and measures SEl-K protein in culture supernatants and biological fluids. Quantification of *in vitro* SEl-K secretion by various *S. aureus* isolates using this novel capture ELISA revealed detectable amounts of SEl-K secretion by all isolates, with the highest secretion levels being exhibited by MRSA strains that coexpress SEB. *In vivo* secretion was measured in a murine thigh abscess model, where similar levels of SEl-K accumulation were noted regardless of whether the infecting strain exhibited high or low secretion of SEl-K *in vitro*. We conclude that SEl-K is commonly expressed in the setting of staphylococcal infection, in significant amounts. SEl-K should be further explored as a target for passive immunotherapy against complicated *S. aureus* infection.

Staphylococcus aureus can express a large and diverse repertoire of virulence factors, including many different surface proteins, enzymes, and secreted toxins (1). Among the most potent of these virulence factors are the members of a family of secreted, heat-stable proteins known as enterotoxins. More than 20 staphylococcal enterotoxins (SEs) have been discovered that demonstrate superantigenic (SAg) properties against T cells (2, 3). Superantigens can activate 20% to 50% of the T cell population by bypassing the traditional pathway of major histocompatibility complex (MHC)-dependent presentation of antigens to T cell receptors (TCRs). Instead, superantigens bind simultaneously to MHC class II (MHC-II) and TCRs, outside their antigen-binding groove, resulting in an excessive inflammatory response that can lead to toxic shock, multiorgan failure, and death. Superantigens are also associated with other immune-mediated diseases, including Kawasaki disease, atopic dermatitis, and chronic rhinosinusitis (4).

The classical superantigens toxic shock syndrome toxin 1 (TSST-1), SEA, and SEB have been extensively studied due to their causative roles in toxic shock syndrome and food poisoning (5). However, advancements in sequencing methodologies have enabled the discovery of many more SEs whose roles in pathogenesis remain unknown (6). One of these toxins, SEl-K, has been shown to exhibit superantigenic properties, including Vβ-specific T cell activation, pyrogenicity, emesis, and lethality in primates (7–9). Epidemiological studies have demonstrated the SEl-K-encoding genes to be among the most common SE genes in *S. aureus* clinical isolates (10–14). Additionally, SEl-K is the only SE gene to our knowledge that is significantly associated with community-acquired methicillin-resistant *S. aureus* (CA-MRSA) strains of several clonal lineages (10, 15, 16). Interestingly, the neighboring SEl-Q enterotoxin is not significantly associated with MRSA (10). However, studies examining the role of SEl-K in staphylococcal pathogenesis have been hampered by a lack of sensitive methods to measure expression of this toxin *in vivo*. Here we report the development of a sensitive and specific immunoassay for the measurement of SEl-K in biological fluids and demonstrate, for the first time, expression and accumulation of SEl-K in the setting of staphylococcal infection. To our knowledge, this is the first systematic investigation of SEl-K secretion and gene variation in clinical *S. aureus* strains. The implications of our results are discussed.

MATERIALS AND METHODS

Toxins. Purified toxins SEA, SEB, and TSST-1 were purchased from Toxin Technology (Sarasota, FL) in accordance with CDC biosafety regulations.

Purification of SEl-K. The full-length *sel-k* gene from USA300 clinical isolate W-83b, encoding residues 1 to 219, was subcloned into H-MBT-T vector (17) using primers sel-k_BamH1_F (3′-GGGGGATCCCAAGGT GATATAGGAATTGATAAT-5′) and sel-k_Sal1_R (3′-GGGGTCGACT TATATCGTTTCTTTATAAGAAATATCGAC-5′). H-MBP-T SEl-K plasmid was then transformed into XL10 *Escherichia coli* (Stratagene) and purified by standard methods for sequencing. After sequence verification, the H-MBP-T SEl-K plasmid was transformed into BL21 *E. coli* (Stratagene) for protein expression. Cells were grown for 5 h at 37°C in LB-ampicillin (LB-amp) media after induction with 0.5 mM IPTG (isopropyl-β-D-thiogalactopyranoside) at an optical density at 600 nm (OD_{600}) of 0.6. Cells were harvested, resuspended in 20 mM Tris (pH 7.5) with protease inhibitor tablets (Roche), and then lysed by sonication. The sonicated suspension was centrifuged at 16,000 rpm for 30 min, and the supernatant was syringe filtered with a 0.2-μm-pore-size filter to eliminate cellular debris. Filtered supernatant was flowed through a 5-ml column of Talon affinity resin (Clontech), and the MBP:SEl-K fusion protein

Received 9 February 2014 Returned for modification 21 March 2014
Accepted 30 April 2014
Published ahead of print 7 May 2014
Editor: B. A. Forbes
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doi:10.1128/JCM.00387-14

TABLE 1 Strain list[a]

Strain[b]	*mecA*	SCC	ACME	MLST	Toxins[c]	SEl-K (ng/ml)[d]
MSSA						
B-88	−	MSSA	−	CC 8	*sed,sel-j, sel-k, sel-p, sel-r*	2
B-3	−	MSSA	−	CC 8	*sec, sed, sel-j, sel-k, sel-l, sel-p, sel-r, sel-u, tsst-1*	2
B-63	−	MSSA	−	CC 8	*sed, sel-j, sel-k, sel-r*	2
W-133	−	MSSA	−	CC 8	*pvl, sed, sel-j, sel-k, sel-r*	70
W-84	−	MSSA	−	CC 8	*pvl, sed, sel-j, sel-k, sel-p, sel-r*	96
B-41b	−	MSSA	−	CC 8	*sej, sel-k, sel-p, sel-r*	2
W-136	−	MSSA	−	CC 8	*seb, sel-j, sel-k, sel-q, sel-r*	582
W-67	−	MSSA	−	CC 8	*sea, seb, sel-k, sel-p, sel-q, sel-r, sel-u*	993
W-183	−	MSSA	−	CC 8	*seb, sel-i, sel-k, sel-p, sel-r*	2
W-162	−	MSSA	−	CC 59	*seb, sed, sel-k, sel-p, sel-r*	327
W-100a	−	MSSA	−	CC 59	*seb, sel-k*	7
W-116a	−	MSSA	−	CC 20	*seb, sel-g, sel-k, sel-m, sel-n, sel-p, sel-r*	2
MRSA						
W-112b	+	IV	+	CC 8	*sel-k, sel-r, sel-u*	90
B-46a	+	IV	−	CC 8	*sed, sel-j, sel-k, sel-m, sel-p, sel-r, sel-u*	2
W-110a	+	IV	−	CC 8	*sel-k, sel-r, sel-u*	7
B-74b	+	IV	−	CC 8	*sel-k, sel-p, sel-r, sel-u*	41
W-75a	+	IV	−	CC 8	*sed, sel-j, sel-k, sel-p, sel-r, sel-u*	69
W-165	+	II	−	CC 5	*sed, sel-g, sel-i, sel-j, sel-k, sel-m*	1
B-45	+	IV	−	CC 8	*seb, sed, sel-j, sel-k, sel-m, sel-p, sel-r, sel-u*	350
B-2	+	IV	−	CC 8	*seb, sec, sed, sej, sel-k, sel-l, sel-r, sel-u, tsst-1*	641
B-4	+	IV	−	CC 8	*seb, sec, sed, sel-k, sel-p, sel-r, sel-u*	745
B-47a	+	IV	−	CC 8	*seb, sel-k, sel-p, sel-r, sel-u*	647
B-86	+	IV	−	CC 8	*seb, sel-k, sel-p, sel-r*	385
B-62b	+	IV	−	CC 8	*sea, seb, sel-k, sel-r, sel-u*	704
W-132	+	IV	−	ND	*seb, sel-k, sel-r*	688
B-1	+	IV	−	CC5	*sec, sed, sel-i, sel-j, sel-l, sel-m, sel-n, sel-r, sel-u, tsst-1*	0
USA300						
W-144	+	IV	+	CC 8	*pvl, sel-j, sel-k, sel-r*	82
W-129	+	IV	+	CC 8	*pvl, sel-k, sel-q, sel-r*	67
W-98a	+	IV	+	CC 8	*pvl, sel-k, sel-q*	69
W-130	+	IV	+	CC 8	*pvl, sel-k, sel-r*	52
W-118	+	IV	+	CC 8	*pvl, sed, sel-k, sel-p, sel-r*	90
W-123	+	IV	+	CC 8	*pvl, sel-k, sel-p, sel-r*	81
W-85b	+	IV	+	CC 8	*pvl, sel-k, sel-p, sel-r*	44

[a] ACME, arginine catabolic mobile element; CC, clonal complex; ND, not determined; *pvl*, Panton-Valentine leukocidin (PVL). USA300 data were defined by pulsed-field gel electrophoresis and determination of CC 8, SCCmec IV, and positivity for both PVL and the ACME.
[b] W, wound isolate; B, blood isolate.
[c] Toxin profiles were previously determined by PCR amplification with primer sets for 19 different enterotoxins.
[d] SEl-K secretion was measured by capture ELISA, using MAbs 4G3 and 10C12, after 16 h of growth in BHI at 37°C with shaking at 220 RPM.

was eluted by 200 mM imidazole. The eluted fusion protein was digested with thrombin overnight at 4°C to cleave the H-maltose binding protein (MBP) tag, and the excess imidazole was removed by dialysis into 20 mM Tris (pH 7.5). The MBP fusion tag and other impurities were removed by a series of subsequent washes through Talon affinity resin and then amylase resin columns. The fractions which contained SEl-K were pooled and passed through a size exclusion column preequilibrated with buffer (20 mM Tris [pH 7.5] with 10 mM dithiothreitol [DTT]) to remove high-molecular-weight soluble aggregates. The protein was dialyzed into phosphate-buffered saline (PBS) to remove excess Tris and DTT and was found to be >99% pure by SDS-PAGE. SEl-K stocks were prepared at 1 mg/ml in 10% glycerol, and aliquots were stored at −20°C.

MAbs. Monoclonal antibodies (MAbs) to SEl-K were generated from SEl-K-immunized BALB/c mice in the Hybridoma Facility of Albert Einstein College of Medicine as described here. All mice were immunized with full-length SEl-K (purchased from Toxin Technologies, Sarasota, FL) in complete Freund's adjuvant (CFA). The mouse with the highest Ab titer for SEl-K was selected for spleen harvest and hybridoma generation. Hybridoma supernatants were screened for reactivity to SEl-K by an enzyme-linked immunosorbent assay (ELISA), with positive reactivity being defined as absorbance 3-fold higher than the background value. Six MAbs (4G3, 4H3, 5G2, 6G1, 9H2, and 10C12) were selected and used in this study. Specificity of MAbs for SEl-K was determined by Western blotting and ELISA according to standard methods. MAbs were harvested from concentrated hybridoma supernatants, and their concentrations were determined by ELISA using commercial antibody standards (18).

***Staphylococcus aureus* strains.** *S. aureus* isolates from bacteremic patients were obtained from three hospitals in the Bronx, NY, and were previously genotyped by PCR for 19 SE genes and classified by pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), *spa* typing, and staphylococcal cassette chromosome *mec* (SCC*mec*) typing (10). See Table 1 for a more detailed description of each strain. Isolates were stored in −80°C, grown on blood agar plates, and confirmed as *S. aureus* by a standard laboratory protocol.

Sequence analysis of variant *sel-k* alleles. Genomic DNA was isolated from 23 previously described clinical isolates of *S. aureus* (6 USA300

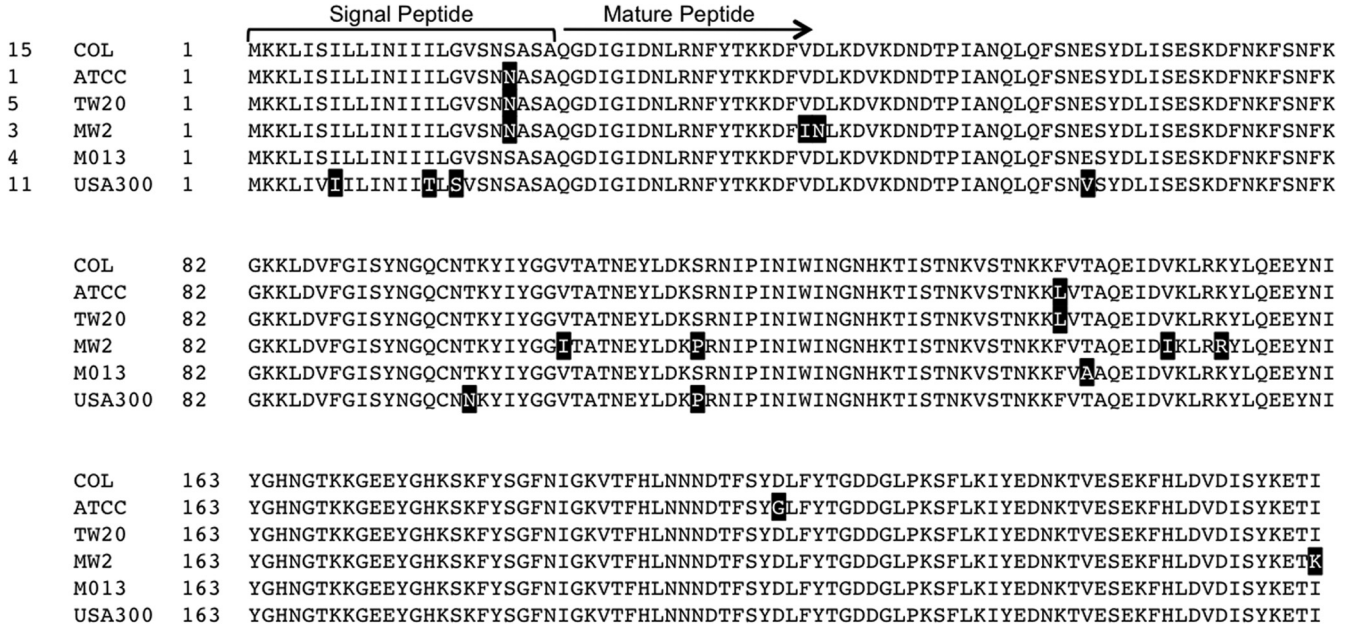

FIG 1 Alignment of 6 variant SEl-K alleles. The COL strain sequence of SEl-K was used as the standard to which all other sequences are compared. The number to the left of each strain name represents the number of strains that have been found to have that corresponding sequence. The number to the right of each strain name indicates amino acid positions. Highlighted residues represent residues that differ from the COL strain sequence. The arrow indicates the cleavage site between the signal peptide and the mature, secreted SEl-K peptide.

strains, 12 non-USA300 MRSA strains, and 5 methicillin-sensitive *S. aureus* [MSSA] strains) and used as the template for amplification of the *sel-k* gene by PCR with the primers sel-k_seq_F (5′-CGACATCCAAATGGAA TTTCTCAGACTCTACAG-3′) and sel-k_seq_R (5′-GCAGAGAATTTT CATTTGGATGTAGAGATTTCATATGAG-3′). An additional 18 *sel-k* nucleotide sequences were obtained from NCBI GenBank. Predicted translated amino acid sequences were determined using the ExPASy Translation Tool, and amino acid sequence alignments were made using the ClustalW2 program.

SDS-PAGE and Western blotting. Purified toxins SEA, SEB, SEl-K, and TSST-1 at 100 ng each were dissolved in 30 μl of sample buffer with β-mercaptoethanol (βME) and boiled for 6 min. After centrifugation for 10 s, the toxins were resolved on a 10% SDS-polyacrylamide gel, and the fractionated proteins were transferred from the gel onto a polyvinylidene difluoride (PVDF) membrane (Millipore) in a semidry transblot apparatus. The membrane was blocked with 5% milk–PBS–Tween for 2 h. The blots were washed and incubated with a 1:1,000 dilution of MAbs at a concentration of 2 mg/ml for 1 h. Later, the blots were washed with PBS-Tween and further incubated with horseradish-peroxidase (HRP)-conjugated goat anti-mouse IgG (1:10,000). After washing, development was performed by a chemiluminescence method according to the instructions of the manufacturer (Fisher Scientific).

Mouse experiments. The thigh infection model was described elsewhere (19). Briefly, female BALB/c mice 6 to 8 weeks of age were anesthetized, and a small incision was made into the lateral aspect of the quadriceps muscle. The thigh wound was then inoculated with 10^5 CFU (in 5 μl of PBS) of either MRSA strain W-132 or USA300 strain W-85b, and a suture was embedded to provide a foreign-body substrate for bacterial attachment. On day 5, mice were euthanized, and abscesses were excised. The abscesses were homogenized in 1 ml of PBS, analyzed by ELISA for SEl-K quantification, and plated for CFU determination.

Ethics statement. Animal experiments were performed with the approval of the Albert Einstein College of Medicine Animal Institute Committee in accordance with their rules and regulations.

Statistical analysis. Standard curves, concentrations, and trend lines were generated and calculated using Excel software (Microsoft). Statistical analysis was performed with Student's *t* tests (two-tailed, unpaired) and analysis of variance (ANOVA) with a posttest using Prism 6 software.

RESULTS

Clinical isolates of *S. aureus* encode variant SEl-K toxins. The gene encoding SEl-K was sequenced in 25 strains of *S. aureus* (4 MSSA strains and 21 MRSA strains, 9 of which were USA300 clones) from our published collection of clinical isolates (10). Deduced translated amino acid sequences were aligned with all 14 *sel-k* gene sequences currently published on NIH databases. Comparative analysis of all 39 sequences revealed the existence of 6 SEl-K toxin alleles (Fig. 1). Moreover, the predicted amino acid sequences of SEl-K in all examined USA300 strains, including the two published USA300 sequences, were found to be 100% identical and contained 5 amino acid mutations, including 3 in the signal peptide and 2 in the coding region, that were unique to the USA300 clone.

Clinical isolates of *S. aureus* secrete SEl-K *in vitro*. A panel of six stable hybridoma cell lines, each secreting a distinct anti-SEl-K MAb (four IgG1-specific and two IgG2b-specific cell lines), was generated by fusion of myeloma cells with splenic B cells from SEl-K-immunized mice. The hybridoma clones producing MAbs with the highest affinity to SEl-K were identified by an ELISA screen and subcloned to ensure that each clone produced MAbs of a single isotype. A Western blot analysis in which the individual MAbs were probed against a panel of purified enterotoxins (under denaturing conditions) revealed binding by all six MAbs to epitopes on SEl-K (Fig. 2A). None of the MAbs recognized epitopes on SEB or TSST-1. However, MAbs 4G3 and 4H3 did exhibit cross-reactivity with an epitope of SEA. Three clinical isolates of *S. aureus*, W-85b, W-132, and B-1 (Table 1), were grown overnight in brain heart infusion (BHI), and the resulting supernatants were probed with the 6 anti-SEl-K MAbs individually.

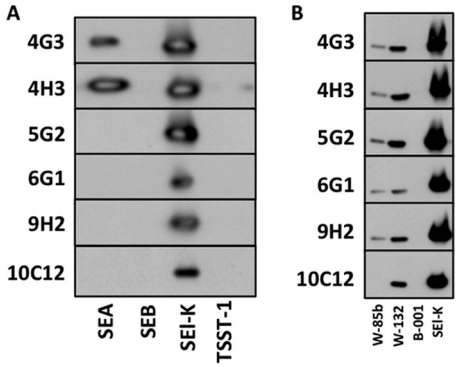

FIG 2 Detection of SEl-K secretion *in vitro*. (A) All six MAbs were individually probed by Western blotting against purified enterotoxins SEA, SEB, SEl-K, and TSST-1 at 100 ng each. (B) A panel of supernatants from overnight cultures of 3 *S. aureus* clinical isolates (W-85b [a USA300 strain that is PCR positive for *sel-k*, *sel-p*, and *sel-r*], W-132 [an MRSA strain that is PCR positive for *sel-k*, *seb*, and *sel-r*], and B-1 [an MRSA strain that is PCR positive for 10 different SEs but not *sel-k*]) were probed by Western blotting using each of the 6 anti-SEl-K MAbs. Identical overnight growth curves were observed for all strains. Purified SEl-K (100 ng) served as a positive control in the right-most lane.

USA300 strain W-85b and MRSA strain W-132, both verified by PCR to carry the *sel-k* gene, produced supernatants that bound the anti-SEl-K MAbs (Fig. 2B). USA300 strain W-85b also exhibited less overnight secretion of SEl-K than MRSA strain W-132, as measured by all 6 MAbs. No cross-reaction was observed with the overnight supernatant from MRSA strain B-1, which contains 10 different enterotoxin genes (*sec*, *sed*, *sel-i*, *sel-j*, *sel-l*, *sel-m*, *sel-n*, *sel-r*, *sel-u*, and *tsst-1*) but not *sel-k*.

Development of ELISA to measure SEl-K secretion. We evaluated our anti-SEl-K MAbs in combinations of two, and in different sandwich ELISA configurations, to identify the combination(s) that produced the most sensitive ELISA for the measurement of SEl-K. The combinations of MAbs 10C12 with 4G3, and 10C12 with 4H3, regardless of which MAb in the combination was used for capture or detection of SEl-K, produced ELISAs with sensitivities of down to 500 pg/ml of SEl-K (Table 2). The other combinations did not yield standard curves good enough to allow fitting of trend lines for quantification of SEl-K either because they bound to toxin only at high concentrations of SEl-K or because they exhibited competitive binding for the epitope(s) on SEl-K (data not shown). The ELISA configuration used in all subsequent SEl-K quantification experiments, with 4G3 as the capture Ab and 10C12 as the detection Ab, is outlined in Fig. 3A. An ELISA-based assay in which 10C12 and 4G3 were added simultaneously to SEl-K revealed no competition, from which we conclude that they bind different epitopes on SEl-K (Fig. 3B). To evaluate the specificity of our capture ELISA for SEl-K, we assayed equivalent amounts of other staphylococcal enterotoxins, including SEA, SEB, and TSST-1. Our data demonstrate that the ELISA is specific for SEl-K (Fig. 3C). As expected, the ELISA was not sensitive to SEA, even at high concentrations, and became marginally sensitive to SEB or TSST-1 only at concentrations greater than 1 μg/ml.

Measurement of SEl-K secretion in culture supernatants. Next, we tested the utility of our capture ELISA in measuring *in vitro* secretion of SEl-K by *S. aureus*. Thirty clinical isolates that carry the *sel-k* gene were selected for this experiment and grouped according to their SE profile and clone type (6 strains per group). *S. aureus* strains were grown overnight (16 h) in BHI. SEl-K concentrations in supernatants were measured by capture ELISA. MRSA strains that carry both *sel-k and seb* secreted significantly more SEl-K (350 to 750 ng/ml) than strains that do not cocarry the *seb* genes (1 to 100 ng/ml) (Fig. 4). These differences in SEl-K secretion are not a product of growth characteristics that differed between strains, as the growth curves obtained for all strains were equal (data not shown).

Measurement of *in vivo* SEl-K secretion in murine abscesses. Next, we applied our capture ELISA to the detection of SEl-K in abscesses resulting from infection with *S. aureus*. Two groups of mice (5 mice per group) were infected with 10^5 CFU of either the *in vitro* high producer of SEl-K, MRSA strain W-132, or the *in vitro* low producer, USA300 strain W-85b, resulting in a localized abscess after 5 days (Fig. 5A). An accumulation of SEl-K of between 3 and 6 ng/ml was measured in all 10 abscesses regardless of the infecting *S. aureus* strain (Fig. 5B). The levels of CFU in the abscesses between these two groups were comparable (Fig. 5C). Likewise, in a single sample of surgically removed human abscess, our ELISA was able to detect 1 ng/ml of SEl-K. The MRSA strain cultured from this human abscess produced 100 ng/ml SEl-K when grown overnight in BHI (data not shown). In summary, these data confirm that our assay can quantify secretion of SEl-K in infected tissue and indicate that *in vitro* secretion may not correlate with *in vivo* secretion.

DISCUSSION

Several epidemiological studies have shown that *sel-k* is one of the most prevalent enterotoxin genes in clinical isolates of *S. aureus* (10, 12–16). Moreover, in several of these studies, SEl-K was significantly associated with CA-MRSA strains (10, 15, 16), which included USA300, USA400, and other clones. The USA300 clone in particular is now a major epidemic strain in several countries

TABLE 2 Sensitivities of different capture ELISAs tested

Capture MAb (isotype)	Detection MAb	Sensitivity (ng/ml)[a]
5G2 (IgG1)	4G3	**333**
	4H3	ND
6G1 (IgG1)	4G3	ND
	4H3	ND
9H2 (IgG1)	4G3	ND
	4H3	ND
10C12 (IgG1)	4G3	**0.5**
	4H3	**0.5**
4G3 (IgG2b)	10C12	**0.5**
	5G2	**37**
	6G1	ND
	9H2	ND
4H3 (IgG2b)	10C12	**0.5**
	5G2	ND
	6G1	ND
	9H2	ND

[a] Sensitivity is defined by the lowest concentration of SEl-K from which the starting concentration (1 μg/ml) could be calculated by using the linear-fit standard curve generated in each ELISA. Antibody combinations without a sensitivity value listed did not successfully bind SEl-K in this assay; hence, calculations were not done (ND).

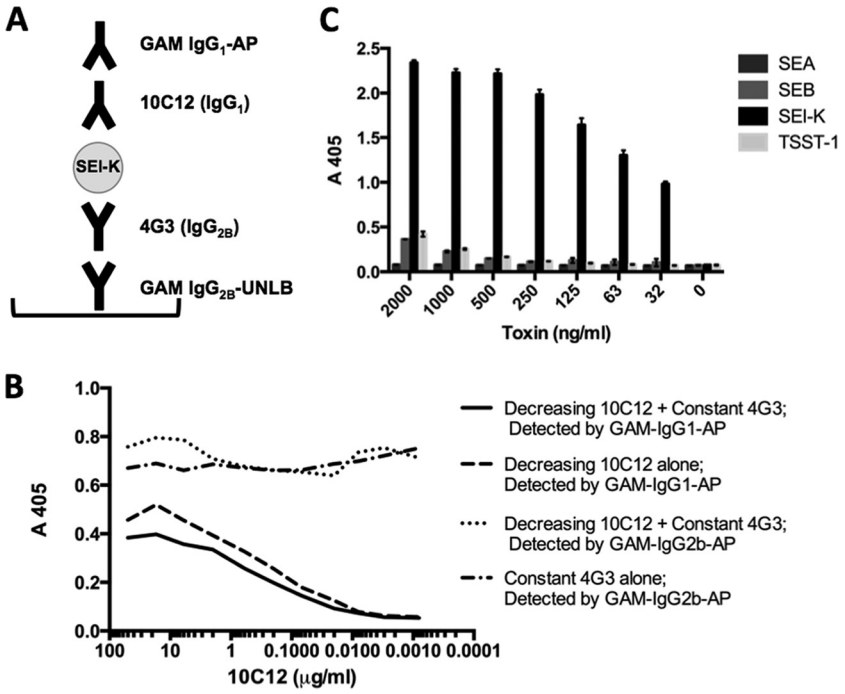

FIG 3 Development of SEl-K capture ELISA. (A) Configuration of sandwich ELISA for quantification of SEl-K. GAM, goat anti-mouse; AP, alkaline phosphatase; UNLB, unlabeled. GAM antibodies were purchased from SouthernBiotech (Birmingham, AL). (B) Competition assay. A 96-well ELISA plate was coated with 25 ng of SEl-K per well, blocked with 1% bovine serum albumin (BSA), and then treated with MAbs 10C12 (IgG1) and 4G3 (IgG2b) simultaneously. The experiments were performed in duplicate. (C) Specificity assay. A panel of purified enterotoxins, at concentrations equal to that of SEl-K, was assayed by capture ELISA with MAbs 4G3 and 10C12. The experiment was conducted in triplicate, and the error bars reflect standard deviations.

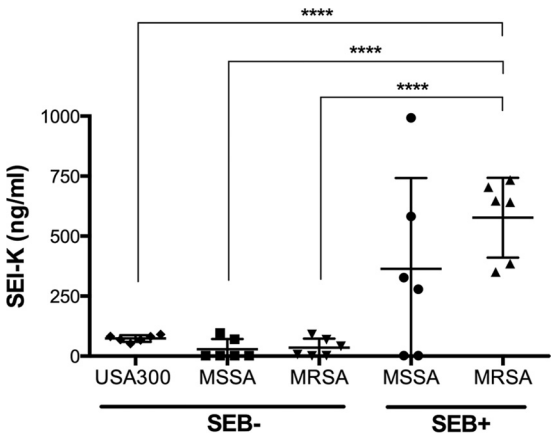

FIG 4 Measurement of *in vitro* SEl-K secretion. A total of 30 clinical isolates of *S. aureus* (6 different strains per group) were grown overnight in BHI, and capture ELISA, performed with MAbs 4G3 and 10C12, was used to measure SEl-K. USA300 strains: W-98a, W-118, W-123, W-129, W-130, and W-144. MSSA/B$^-$ strains: B-3, B-41b, B-63, W-84, B-88, and W-133. MRSA/B$^-$ strains: B-46a, B-74b, W-75a, W-110a, W-112b, and W-165. MSSA/B$^+$ strains: W-67, W-100a, W-116a, W-136, W-162, and W-183. MRSA/B$^+$ strains: B-2, B-4, B-45, B-47a, B-62b, and B-86. Each point represents the analysis of a single strain (performed in duplicate). The lower detection limit of the ELISA was 500 pg/ml. The horizontal bars represent the means ± standard deviations. Four asterisks (****) indicate a significant difference ($P > 0.00001$) between results determined for the SEl-K/SEB-copositive MRSA strains and strains that do not coexpress SEB. Statistically significant differences between the groups were determined by ANOVA with a posttest ($P < 0.0001$). See Table 1 for the exact measurements of overnight SEl-K secretions for each strain.

over 5 continents, and its clinical and epidemiological features, internationally, mirror those observed in the United States (20). The cause of hypervirulence and rapid transmission of USA300 strains relative to other staphylococcal strains is still been actively investigated (21). Importantly, we have generated anti-SEl-K MAbs that now provide a tool that facilitates the study of SEl-K expression *in vivo* and its strain-dependent variability. Such MAbs could potentially be further developed for adjunctive therapy in the treatment of *S. aureus* disease as described for SEB-specific MAbs (22).

Our analysis of predicted amino acid sequences from 39 *S. aureus* strains revealed the existence of at least 6 variants of SEl-K, including one that is conserved and unique to all examined USA300 strains. Naturally occurring variations in the coding region of TSST-1, SEB, and SEC have been shown to result in altered biological properties, including mitogenicity, immunogenicity, and lethality (23–25). Potential differences in biological properties between these 6 SEl-K variants must be explored, especially in regard to USA300 strains, which carry the gene encoding SEl-K in almost all cases and carry conserved unique mutations in their signal and coding regions.

Three of these mutations are located in a presumed signal peptide as determined on the basis of results from sequence prediction program Signal IP v1.1 (7). It is conceivable that these signal peptide mutations contribute to the consistently low secretion of SEl-K exhibited by all USA300 strains *in vitro*. Future studies are needed to determine if these USA300-specific sequence alterations in the *sel-k* gene are relevant for expression levels. Also plausible, however, is that SEl-K expression is regulated by factors encoded in the same pathogenicity island in which SEB is cocarried. Several

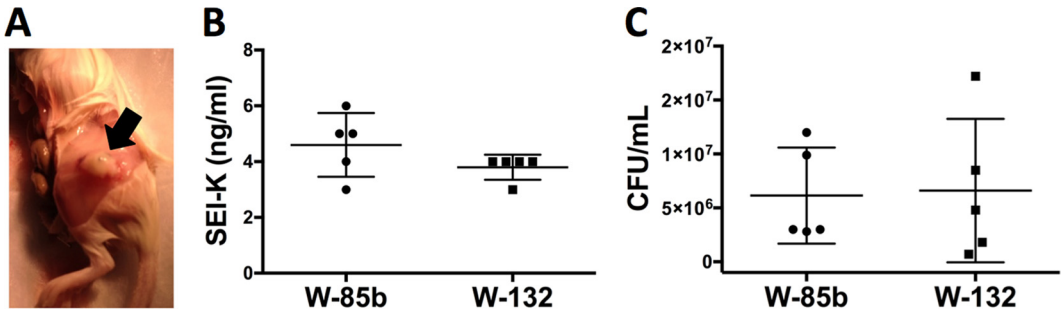

FIG 5 Detection of SEl-K in the murine thigh abscess model. (A) Representative abscess (arrow) of a mouse that was infected with 10^5 CFU of *S. aureus* clinical isolate W-85b or W132 in 5 μl of PBS. (B) Measurement of SEl-K concentrations in mouse thigh abscesses. Each point represents the analysis of one mouse abscess (performed in duplicate) by ELISA with MAbs 4G3 and 10C12. Horizontal lines represent means ± standard deviations. The lower detection limit of the ELISA was 500 pg/ml. (C) Levels of CFU in the abscesses were not significantly different between the two groups.

epidemiological studies have shown that SEl-K and SEB are often encoded on the same mobile genetic elements, namely, *S. aureus* pathogenicity island 1 (SaPI1), SaPI2, SaPI3, and SaPI5 (16, 26–28). Moreover, our *in vitro* data show that strains carrying both *sel-k* and *seb* expressed SEl-K at significantly higher levels than those that do not. Regulatory factors SaeRS and σ(B) have been shown to enhance and inhibit SEB transcription, respectively, but to have no effect on SEl-K. Thus, additional studies are needed to identify the regulatory factors of SEl-K (27).

Interestingly, despite a 10-fold difference between MRSA strain W-132 and USA300 strain W-85b in their levels of *in vitro* SEl-K secretion, infection of mice with either of these 2 strains resulted in equal accumulations of SEl-K in our murine thigh abscess model. This finding is consistent with studies on the regulation of SEs, which have also revealed that the *in vivo* expression of SEs may not correlate with *in vitro* observations (26, 29). One reason could be that the pathogen burden and/or the *in vivo* growth conditions potentially affect toxin production (30–32). Further experimentation is needed to identify the factors that influence SE expression *in vivo*.

The contribution of SEl-K to *S. aureus* pathogenesis is not well understood. However, the available data indicate that SEl-K is a potent stimulator of T cells, and now we demonstrate that the toxin is abundantly expressed in a large of portion of *S. aureus* strains. SEl-K accumulates in abscesses to similar degrees regardless of whether the infecting strain exhibits low or high secretion of SEl-K *in vitro*. This finding supports the notion that SEl-K, similarly to SEB, is not degraded or cleared from an abscess *in vivo*. Thus, our findings suggest that SEl-K may play a previously underappreciated role in *S. aureus*-mediated skin and soft-tissue infection. This finding is consistent with previous findings that documented SEl-K secretion in 14 of 36 *S. aureus* clinical isolates by Western blotting (7). However, the SEl-K secretion was not quantified and none of the strains were USA300 clones.

S. aureus is the second-most-common pathogen recovered in nosocomial bloodstream infections in the United States, and emergent USA300 strains in particular are reported to cause the majority of complicated skin and soft-tissue infections (33–36). Despite sensitivity to antibiotics, complicated *S. aureus* infections still have high mortality and treatment failure rates (37, 38). Several studies demonstrate that neutralization of SAgs with drugs or Abs improves outcomes in both intoxication and infection models (39–43). Specifically, a recent study from our laboratory performed with MAbs specific to SEB provides encouraging data with respect to the development of such Abs as adjunctive therapy for complicated *S. aureus* infections (44). Given that several anti-infective MAbs are now FDA licensed, more efforts should be focused on developing additional MAbs as therapeutics to neutralize SEs (45, 46). Because SEs are heat stable and resistant to enzymatic degradation, they are expected to persist over time in an abscess even if pathogen growth is suppressed by antibiotic treatment. Therefore, in the setting of a complicated skin and soft-tissue infection with deep-seated abscess formation, possibly also complicated by osteomyelitis, passive immunotherapy that targets SEl-K in combination with other commonly expressed toxins may enhance the therapeutic efficacy of current antibiotic therapies and improve outcomes. Our SEl-K-specific capture ELISA may prove helpful in examining such future clinical investigations.

ACKNOWLEDGMENTS

This work was supported by the NIH National Institute of Allergy and Infectious Diseases, grant U54-AI057158, and NIH MSTP training grant T3GM07288.

We thank Manxia Fan, Susan Buhl, and the staff at the Hybridoma Facility at the Albert Einstein College of Medicine for their technical help.

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