Global repression of exotoxin synthesis by staphylococcal superantigens

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Virulent *Staphylococcus aureus* strains typically produce and secrete large quantities of many extracellular proteins involved in pathogenesis. Such strains cause the classical staphylococcal lesion—local tissue destruction and aggressive inflammation accompanied by the massive influx of polymorphonuclear leukocytes, leading to the formation of pus. Most strains causing toxic shock syndrome, however, produce and secrete very small quantities of most exoproteins although they elaborate high levels of toxic shock syndrome toxin-1 (TSST-1). These strains cause local infections that are remarkably apurulent although potentially fatal owing to the superantigen. We have analyzed this disparity and have found that TSST-1 itself is a negative global regulator of exoprotein gene transcription. TSST-1 not only represses most exoprotein genes but determines its own high expression level by autorepression. We report also that a second superantigen, enterotoxin B, has similar regulatory properties.

The pathogenicity of *Staphylococcus aureus* is multifactorial, generally involving a large number of extracellular proteins. Some of these proteins, including cytotoxins and exoenzymes, are secreted; others, including protein A and various adhesins, remain attached to the cell wall. Together, these proteins enable the organism to evade host defenses, adhere to host cells and intercellular matrix molecules, invade or destroy host cells, and spread within the tissues. Their production is governed by a complex network of regulatory functions whose expression *in vitro* is temporally programmed and largely depends, directly or indirectly, on environmental signals. It is assumed that the regulatory functions tune the expression of pathogenicity factors to achieve patterns that are optimal for local spatial and temporal adaptations.

At the same time, there is a subset of staphylococcal diseases whose primary cause is a single toxin. The most important of these toxinoses is toxic shock syndrome (TSS), caused most often by TSS toxin-1 (TSST-1) and less frequently by other superantigens (SAgs) such as enterotoxin A, enterotoxin B (SEB), enterotoxin C, or enterotoxin D. These and other staphylococcal SAgs are encoded by accessory genetic elements, including plasmids, prophages, and mobile pathogenicity islands (SaPIs) (1) and often are produced at relatively high levels (2). Remarkably, however, TSS-producing strains generally produce very little in the way of other extracellular toxins and other proteins (2).

Although TSS occurs most commonly in association with menstrual tampons, it was first described in connection with skin infections in children (3) and continues to be seen in extra-vaginal infections, often postsurgical (4). A remarkable feature of infections caused by TSST-1-producing staphylococcal strains is that they are largely apurulent and lack inflammation and tissue destruction characteristic of staphylococcal infections. Consequently, these cases often go unrecognized until several days after the symptoms of TSS have developed (5).

These features of TSS and the producing organisms prompted us to investigate the regulation of extracellular protein production in TSS strains. We report here that TSST-1 strongly represses the production of essentially all other exoproteins, acting at the level of transcription, and that in addition, it controls its own synthesis, acting as an autorepressor. We find also that SEB behaves very similarly. The possible relation of these findings to the clinical features of infections caused by *S. aureus* strains producing TSST-1 and possibly those producing other SAgs is discussed.

Materials and Methods

Bacterial Strains and Plasmids. The bacterial strains and plasmids used in this study are listed in Table 1.

Media and Growth Conditions. *S. aureus* cultures were grown in CY broth (6) without glucose, supplemented with glycerol phosphate (0.1 M), or on GL agar (6) with suitable antibiotic. Liquid cultures were shaken at 37°C and monitored turbidometrically with a Klett–Summerson (New York) photoelectric colorimeter at 540 nm.

β-Lactamase Measurements. Starting at low bacterial density, *S. aureus* cultures were grown in CY broth (6) without glucose. Samples were collected hourly. One portion of the sample was used to determine bacterial density and the other was quickly frozen and kept for β-lactamase measurements. β-Lactamase measurements were done on equalized samples by using nitrocefin as a substrate (7). Growth rates of the strains under study were indistinguishable within experimental error.

Generation of Murine Subcutaneous Abscess. Bacterial strains RN4282 and RN6938 were grown to midexponential phase, $\approx 10^9$ cells/ml, washed, and resuspended in PBS to 10^9 cells/ml. Bacterial suspensions were mixed with cytodex beads as described by Barg *et al.* (8), and 140 ml of beads + cells, containing 10^8 cells, was injected s.c. in the flank area of hairless mice, strain SKH 1. Mice were monitored daily by visual inspection of the injection site.

DNA Procedures. DNA fragments were amplified by PCR (see Table 2). Primers contained either *Pst*I or *Kpn*I restriction sites at 5′ ends. Templates were chromosomal DNA isolated from strain RN4282 in the case of *tst* gene, S6 in the case of *seb* gene, and RN6734 in the case of *lukS* and *spr* genes. Plasmid and chromosomal DNA were isolated by using a QIAprep Spin Miniprep Kit with minor modifications. PCR-amplified DNA fragments were purified by using a QIAquick PCR Purification Kit. Both kits were obtained from Qiagen (Valencia, CA). PCR products were cut with *Kpn*I and *Pst*I and cloned into the multiple cloning sites of pRN5543 or pRN7034. Clones in pRN7034 shuttle vector were electroporated into *Escherichia coli* and afterward transformed into RN4220 (9). Clones in pRN5543 were transformed to *S. aureus* RN4220. All plasmids were transferred from RN4220 to other strains by transduction with staphylococcal phage 80α (9). Frame-shift mutagenesis was performed by using a QuikChange XL Site-Directed Mutagenesis Kit according to the manufacturer's instructions (Stratagene). Primers were obtained from Integrated DNA Technologies (Coralville, IA). Primer sequences are listed in Table 2.

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Abbreviations: TSS, toxic shock syndrome; TSST-1, TSS toxin-1; SAg, superantigen; SEB, enterotoxin B; SaPI, mobile pathogenicity island.

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Table 1. Strains and plasmids used in this study

	Relevant characteristics	Reference/ source
Strains		
S6	*seb*$^+$ strain	31
RN4220	Restriction-deficient mutant of strain 8325-4	32
RN4282	Clinical isolate, contains SaPI1, *tst*$^+$	32
RN6734	*agr*$^+$, 8325-4 derivative	
RN6938	RN4282 derivative, SaPI1::*tetM*, *tst*$^-$	14
RN9131	RN6734 derivative containing SaPI1 and *tst* gene	
Plasmids		
pRN5543	pC194 derivative, pUC19 polylinker, Cm^r	12
pRN7034	Shuttle vector containing promoterless *blaZ* gene, Em^r	Unpublished
pRN7040	pRN7034::*lukS-blaZ* transcriptional fusion	This work
pRN7041	pRN7034::*spr-blaZ* transcriptional fusion	This work
pRN7044	pRN7034::*tst*(a.1)*blaZ* transcriptional fusion	This work
pRN7045	pRN7034::*tst*(a.8)*blaZ* transcriptional fusion	This work
pRN7056	pRN7034::*tst*(a.5)*blaZ* transcriptional fusion	This work
pRN7112	pRN7034::*seb*(b.1)*blaZ* transcriptional fusion	This work
pRN7114	pRN5543::*seb*, intact *seb* gene	This work
pRN7116	pRN5543::*seb*(b.2), truncated *seb* gene	This work
pRN7118	pRN5543::*tst*(a.5), truncated *tst* gene	This work
pRN7119	pRN5543::*tst*(a.8), intact *tst* gene	This work
pRN7123	pRN5543::*tst*(FSh), *tst* gene with frame-shift mutation	This work
pRN7126	pRN7034::*tst*(FSh)*blaZ* transcriptional fusion	This work

Protein Procedures. PAGE with SDS was performed according to Laemmli (10). β-Lactamase was assayed by the nitrocefin method (11) adapted for the microtiter format (7).

Results

Effect of *tst* on Exoprotein Patterns. To test for the effect of *tst* on the overall pattern of exoprotein production by staphylococci, we compared postexponential phase culture supernatants of TSST-1-producing and nonproducing strains as shown in Fig. 1. As can be seen, the supernatant of an RN4282 (TSST-1-producing) culture (Fig. 1, lanes 3) contains very little in the way of exoproteins, with the exception of the strong TSST-1 band, whereas the RN6734 (non-TSST-1-producing) supernatant (Fig. 1, lanes 4) contains many exoproteins in considerable quantities, as has been observed (12), but lacks TSST-1. Exoprotein patterns similar to that of RN4282 have been observed for several other naturally occurring TSS strains (not shown). SaPI1, encoding TSST-1, is responsible for this effect. Thus transfer of SaPI1 to RN6734, resulting in strain RN9131 (13), caused a reduction in overall exoprotein production comparable to that seen with RN4282 (Fig. 1, lanes 5). In contrast, inactivation of *tst* in RN4282 (RN6938) by the insertion of *tetM* (14) had the opposite effect, sharply enhancing exoprotein production in that strain (Fig. 1, lanes 1). When SaPI1*tst*::*tetM* was moved to RN6734, it had no effect on the exoprotein pattern (not shown). The SaPI1-specific inhibition of exoprotein synthesis could represent repression by TSST-1 itself or, because SaPI1 contains some 25 other ORFs, a polar effect of the *tetM* insertion on a downstream gene. The latter possibility was ruled out by complementation tests with a plasmid containing the cloned *tst* but no other SaPI1 material. For this test, a plasmid containing the cloned *tst* gene, pRN7119, was introduced into two strains, the *tst*-negative RN6734 (Fig. 1, lanes 6) and a strain containing SaPI1 with the above-mentioned *tetM* insertion in *tst*, RN6938 (Fig. 1, lanes 2). In each case, the cloned *tst* gene caused the same reduction in exoprotein production as that caused by the intact pathogenicity island, showing that the *tst* gene and not some downstream SaPI1 gene was responsible. We note that SaPI1 encodes two other SAgs, SEK and SEL (15), and conclude that neither of these has a demonstrable effect on exoprotein production. These results demonstrate additionally that the inhibitory effect of TSST-1 does not significantly depend on gene dosage.

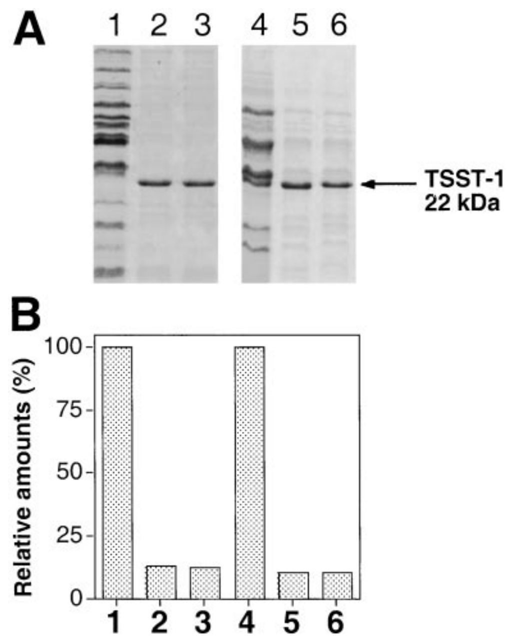

Fig. 1. Effect of *tst* on exoprotein patterns. (*A*) Bacteria were grown to early stationary phase in CYGP in the absence of glucose, and supernatants were trichloroacetic acid-precipitated and analyzed by SDS/PAGE. Gels were stained with Coomassie brilliant blue and scanned. The following samples are shown: RN4282 with SaPI1 *tst*::*tetM* insertional inactivation (RN6938) (lane 1), RN4282 *tst*::*tetM* containing pRN5543::*tst*(a.8) (pRN7119), expressing full-length *tst* (lane 2), RN4282 with SaPI1 carrying intact *tst* gene (lane 3), RN6734 (lane 4), RN6734 (SaPI1) strain (RN9131), expressing *tst* (lane 5), and RN6734 containing pRN5543::*tst*(a.8) (pRN7119) and expressing *tst* (lane 6). Location of TSST-1 protein is indicated by the arrow. (*B*) Scanning data were used to compare the amounts of exoproteins produced by the various strains. Values for RN6938 (lane 1) and RN6734 (lane 4) were set to 100%. Amounts of exoproteins produced by derivative strains were normalized to this value, after subtracting the amount of TSST-1.

Table 2. Primers used in this study

Primer	Sequence (5′–3′)
tst a	GTTGCTGCAGACTCACACTTTGTTTTTTGC
tst 1	GTTGGGTACCAAAATCTGTAGCGATTGTC
tst 2	GTTGGGTACCGTTTGTAGATGCTTTTGC
tst 3	GAAGGGTACCAAGGAATTATCTAAAACTTCAC
tst 4	GTTGGGTACCAGGGCTATAATAAGG
tst 5	GTTGGGTACCTTCAGTATTTGTAACGCCAC
tst 6	GTTGGGTACCTTTTTATCGAACTTTGGC
tst 7	GTTGGGTACCGTCATTCATTGTTATTTTCC
tst(FSh)1	GGGGAAAAAGTGACTTAAACACAAAAAGAACTAAAAAAAGC
tst(FSh)2	GCTTTTTTTAGTTCTTTTTGTGTTTAAGTCACTTTTTCCCC
lukS 1	GTTGCTGCAGGCCTCATAACATTAAATTATTTTATCG
lukS 2	AAAAAAGAATTCATAACGGATTGGCAAGAGGG
seb b	GTTGCTGCAGTGTTGTTAAAGATGTTTTCG
seb 1	GTTGGGTACCATTTTTTATCTCCTTTATCC
seb 2	GTTGGGTACCGTTTGTCAGTTTGATGCG
spr 1	GTTGCTGCAGATTGTTCTTCGAAACTTAAGCACTC
spr 2	GTTGGAATTCTTGCTGTTTGCTTGACTGCG

Mapping the Locus of Inhibition. The region of *tst* responsible for this inhibition was localized by deletion analysis. The cloned *tst* gene and a set of PCR-generated 3′ deletion derivatives were subcloned to a pC194-based vector, pRN5543 (9), transduced to the standard TSST-1-negative strain, RN6734, and analyzed for their effects on

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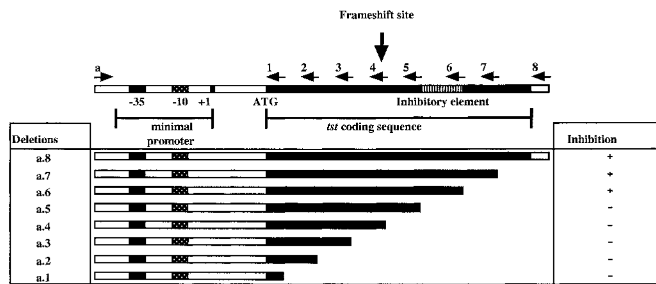

Fig. 2. Mapping the locus of inhibition. At the top is a diagram of the SaPI1 *tst* locus, showing the promoter, the coding sequence (heavy black line), the inhibitory region (gray hatching), and the site of a frame-shift mutation. Deletions, introduced by the PCR, using primers listed in Table 2, are shown below, with the heavy black line indicating the extent of the remaining *tst* DNA. The effect of the progressive 3′ deletions on exoprotein expression is indicated by + for exoprotein inhibition or − for loss of the inhibitory activity. Numbers at the left, designating the individual deletions, are used throughout the text.

overall exoprotein synthesis. The results of this analysis are shown in Fig. 2. The column at the right indicates whether a particular deletion derivative inhibited overall exoprotein synthesis (+) or did not (−). With this set of deletions, there was an all-or-none effect with respect to the inhibition phenomenon—the exoprotein patterns observed resembled that of the standard TSST-1-producing strain, RN4282, or that of the TSST-1-negative RN6734. On the basis of these results, it is concluded that a 30-aa region of the *tst* gene, from 91–120 of the mature protein, shown in gray on the map in Fig. 2, is essential for the inhibition of exoprotein synthesis.

Nature of the Effector Molecule. We next addressed the question of whether the inhibitory element was the gene itself, the mRNA, or the protein product. Here, we introduced a frame shift at nucleotide position 305 of the *tst* coding sequence, 5′ to the inhibitory region, using PCR (see Fig. 2). This frame shift created a nonsense codon 6 nt downstream. The mutated gene was cloned to vector plasmid pRN5543, generating pRN7123, which was transduced to RN6734 for testing. The frame-shift mutation eliminated the inhibitory effect of the gene on overall exoprotein production (not shown), suggesting that the protein itself is responsible for the inhibition. This notion was confirmed by studies with *tst*::*blaZ* fusions (see below).

Effect of *tst* on Exoprotein Gene Transcription. To test the possibility that the *tst*-specific inhibition of exoprotein synthesis was at the level of transcription, we made (in *E. coli*) transcriptional fusions of a staphylococcal β-lactamase gene (*blaZ*) to the promoters of two of the individual exoprotein genes, *lukS* (encoding the S subunit of leukocidin; ref. 16) and *spr* (encoding V8 serine protease; ref. 17), using a shuttle vector pRN7034, which contains *blaZ* lacking its promoter. The resulting plasmids, pRN7040 and pRN7041, respectively, were transferred to *S. aureus* strain RN4220 by protoplast transformation, and thence to RN6734 derivatives containing an inhibitory *tst* fragment, a noninhibitory fragment, and the frame-shifted *tst*, all cloned to pRN5543. As shown in Fig. 3 *a* and *b*, the larger *tst* fragment inhibited both of the exoprotein gene promoters, whereas neither the shorter *tst* fragment nor the frame-shifted *tst* had any effect in comparison to the vector alone. Therefore, *tst* acts by inhibiting transcription of the exoprotein genes.

***tst* Autoregulation.** We had noted earlier that strains containing SaPI1 produced approximately the same level of TSST-1 as strains containing *tst* cloned to a high copy plasmid (see Fig. 1, lanes 5 and 6), suggesting that TSST-1 is an autorepressor as well as a repressor of other exoprotein genes. To test for autorepression, we prepared plasmids in which intact *tst* (a.8, see Fig. 2), a 3′ deletion derivative (a.5) and the frame-shift mutant (FSh) were transcriptionally fused to the β-lactamase reporter by using the vector plasmid, pRN7034, resulting in pRN7045, pRN7056, and pRN7126, respectively. Measurements of the β-lactamase activities of RN6734 derivatives containing these plasmids, during growth in CY medium (9) (omitting glucose, which has been reported to inhibit toxin production; ref. 18) are shown in Fig. 3*d*. As can be seen, the β-lactamase activity of the plasmid containing the truncated *tst* gene (a.5) was sharply elevated in comparison to that of the plasmid with *tst* intact (a.8). Similarly, the frame-shifted derivative (FSh) also showed a sharp elevation of β-lactamase activity. Because β-lactamase production with the frame-shift mutant was stimulated to the same extent as with the short *tst* fragment (a.5), the loss of inhibitory activity by the mutant could have resulted from rapid degradation of the frame-shifted *tst* mRNA only in the very unlikely event that there was a specific cleavage of the fused transcript just upstream of the *blaZ* translational start, followed by rapid degradation of the upstream (*tst*) fragment only. We conclude, therefore, that the inhibitory factor is the TSST-1 protein itself, and not the mRNA or DNA. The addition of pure TSST-1 protein to a growing culture had no effect on β-lactamase production by a short *tst* fragment (a.1) fused to *blaZ*, pRN7044, (not shown), indicating that the inhibitory behavior of the *tst* product is intracellular and presumably represents the TSST-1 precursor, because the mature form is not normally free in the cytoplasm.

The *tst* Promoter Is the Target of TSST-1 Repression. We have mapped the *tst* transcription start point by the standard primer extension method. Assuming that the transcript is not posttranscriptionally processed or cleaved *in vitro* during the extraction procedure, this method has localized the *tst* promoter to the position shown in Fig. 2. Accordingly, we cloned a synthetic 45-mer (−45 to +1) to pRN7034, creating a P_{tst}::*blaZ* fusion. Transcriptional activity of this 45-mer was regulated similarly to the other *tst*::*blaZ* fusions, but had a maximum activity level of about 50% that of larger fragments cloned to the same vector (not shown). This finding suggests that one or more regulatory elements are missing from the 45-nt segment. Nevertheless, as shown in Fig. 3*c*, the intact *tst* gene (a.8) caused a substantial reduction in the β-lactamase activity of the cloned promoter in trans. These results establish that TSST-1 is an autorepressor as well as a transinhibitor of the production of other exoproteins, the autorepression effect is at the level of transcription, and the autorepression target lies within a 45-nt segment containing the *tst* promoter.

Effect of *seb* on Expression of Exoprotein Genes. A further question was whether other SAgs have inhibitory activity similar to that of TSST-1. An obvious candidate was SEB, which is encoded by SaPI3, closely related to SaPI1 (15), occupies the same chromosomal site (see the COL genome, www.tigr.org), causes TSS, and has been observed, when cloned to a high copy plasmid, to inhibit production of other exoproteins (19). The *seb* gene and a derivative with a large 3′ deletion (b.2) were each cloned to pRN5543, generating pRN7114 and pRN7116, respectively, and tested for their effects on overall exoprotein synthesis in RN6734. As can be seen (Fig. 4*a*), the intact *seb* gene (lane 2) caused a dramatic decrease in overall exoprotein production, similar to that seen with *tst*, whereas the deletion derivative (lane 3) had no effect in comparison to the parental *seb*-negative strain, RN6734 (lane 1).

Autoregulatory Activity of *seb* Gene. Using a third derivative in which a promoter-containing segment with no *seb* coding sequence (b.1) was cloned to the β-lactamase fusion vector (pRN7034), generating pRN7112. We note that the full-length *seb* gene inhibited β-lactamase synthesis in trans in comparison to the (b.2) deletion (see Fig. 4*b*), confirming that *seb* is also an autorepressor and showing that the 3′ half of the gene is essential for autorepression. Again, as with *tst*, the cross-repression of other exoprotein

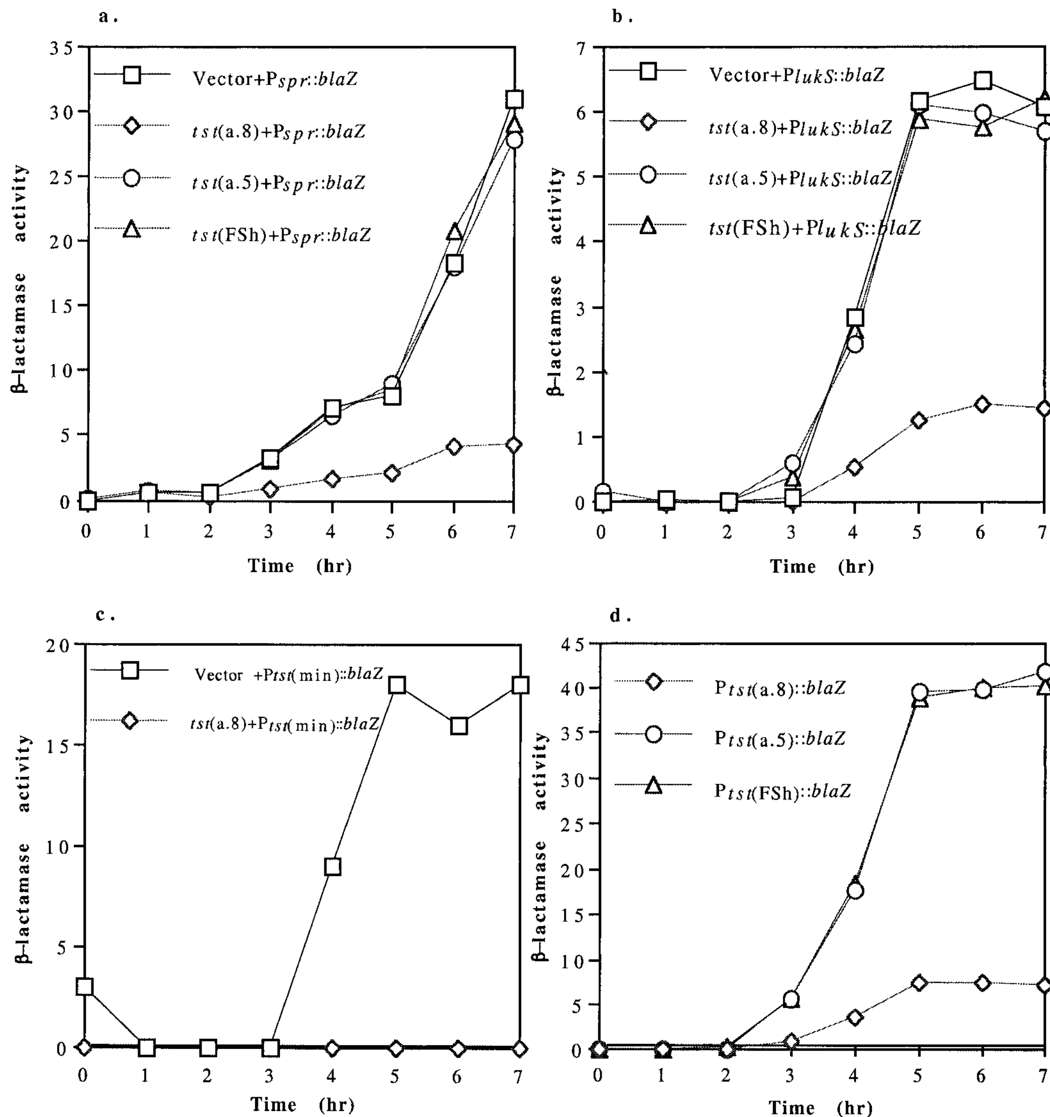

Fig. 3. Effect of *tst* on exoprotein gene transcription. pRN5543 derivatives containing the intact *tst* gene (a.8) (pRN7119), a *tst* deletion, *tst*(a.5) (pRN7118), or *tst* with the frame shift mutation *tst*(FSh) (pRN7123) were transduced to separate RN6734 clones each with one of the following *blaZ* transcriptional fusions: (*a*) P_{spr}::*blaZ* (pRN7041) and (*b*) P_{lukS}::*blaZ* (pRN7040), with promoter-containing fragments of *spr* and *lukS*, respectively, or (*c*) $P_{tst(min)}$::*blaZ* (with minimal promoter sequence of *tst*, defined as a +1 to −45 fragment, in respect to start of transcription). Expression of the reporter gene was monitored during growth at 37°C. (*d*) β-Lactamase expression kinetics for *blaZ* fusions with the intact *tst*, P_{tst}(a.8)::*blaZ* (pRN7045), truncated *tst*, P_{tst}(a.5)::*blaZ* (pRN7056), and frame-shifted *tst*, P_{tst}(FSh)::*blaZ* (pRN7126).

genes seems to be colocalized with the autorepression activity, although in this case, the resolution is low, because only one 3′ deletion has so far been made.

Finally, *tst* and *seb* are mutual cross-repressors (Fig. 4*c*), although cross-repression was not seen when both proteins are intact, presumably because autorepression sets a level of expression that is not affected by a second, cross-reacting autorepressor.

Inhibition of Inflammation by TSST-1 *in Vivo*. As noted, superficial infections with TSST-1-producing strains are often apurulent; in view of the profound inhibition of exoprotein production by our standard TSST-1-producing strain *in vitro*, we sought to correlate these observations by using a simple experimental murine infection, namely the skin abscess model of Barg *et al.* (8). In this experiment, hairless mice were injected s.c., in the flank region, with 10^8 midexponential phase cells of either RN4282 (tst^+) or RN6938 (*tst*::*tetM*) and the site of injection inspected daily. As can be seen in Fig. 5, the *tst*-knockout strain, RN6938, produced the usual s.c. abscess, that was readily apparent after 1 day, and by the fourth day had sloughed and drained. In contrast, the tst^+ strain, RN4282, produced no visible lesion after 1 day, and by the fourth day, had produced a small swelling in one of three mice only, consistent with the clinical observations. These results are not consistent with the results of Molne and Tarkowski (20) who have observed a strong inflammatory response after intracutaneous injection of 10^8 RN4282 organisms in mice.

Discussion

In this report, we describe a paradigm for the regulation of toxin gene expression in bacteria, namely down-regulation by the toxins themselves. The consequences of this regulation are that with strains producing certain SAgs there is a virtual absence of extracellular proteins in culture supernatants, whereas the SAg itself is present at a high and constant level. We have found that two of the major staphylococcal SAgs, TSST-1 and SEB, display this behavior, and that they block or strongly repress exoprotein production at the level of transcription, while regulating their own production as autorepressors. We have established that the

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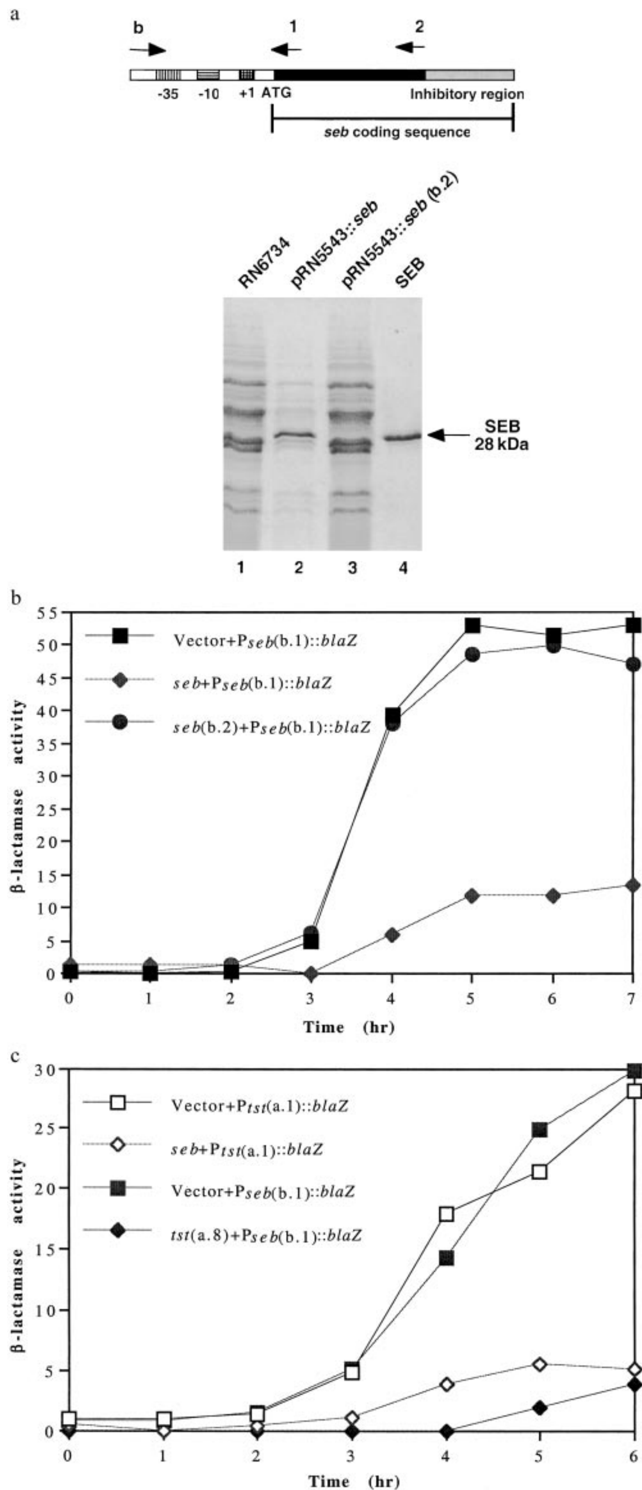

Fig. 4. Effect of *seb* on expression of exoprotein genes. (*a*) Cultures of RN6734 containing the intact cloned *seb*, pRN5543::*seb* (pRN7114) or a derivative with a large 3′ deletion, pRN5543::*seb*(b.2) (pRN7116), were grown to early stationary phase, and the culture supernatants were analyzed for exoprotein patterns as in Fig. 1. Location of SEB protein is indicated by the arrow. (*b*) The plasmids used in *a* were introduced into a RN6734 derivative containing the P_{seb}(b.1)::*blaZ* transcriptional fusion (pRN7112), and the kinetics of β-lactamase synthesis were monitored throughout growth. (*c*) pRN5543 derivatives containing either *tst*(a.8) (pRN7119) or intact *seb* (pRN7114) were introduced into RN6734 derivatives containing either P_{seb}(b.1)::*blaZ* (pRN7112) or P_{tst}(a.1)::*blaZ* (pRN7044), and β-lactamase activity was measured as in *b*.

cytoplasmic (precursor) form of each protein, rather than the DNA or mRNA is the regulatory effector for transrepression of the other exoproteins as well as for its own repression and that a short internal segment of the protein is required. For the *tst* gene, we have shown that a 45-nt segment containing the gene's promoter contains the target of repression. The target for *seb* has been localized to a somewhat larger segment, which contains the promoter plus the region from −35 to −90, a region that apparently binds a positively acting transcription factor and is required for expression (19). The inhibitory effect of *seb* on exoprotein production has previously been seen, in strain S6, with the gene cloned to a multicopy vector but not with a single chromosomal copy (21). This effect was attributed by the authors to titration of a positive transcription factor by the 5′ region of the cloned gene; our results rule this out because 3′ deletions eliminate the effect without altering the gene dosage of the 5′ region. Because S6 is a hyperproducer of RNAIII (21, 22), the *agr* effector, RNAIII overproduction may override the repressive effect of *seb* in single copy. Although TSST-1 and SEB are likely to act by the same mechanism, and TSST-1 shows 20–30% identity to SEB, sequence comparison has not suggested any obvious commonality. However, SAgs show remarkably similar folds and we believe that a fine-structure map of the inhibitory regions will reveal whether or not a common structure is involved.

Not all SAgs have this inhibitory activity. Thus the expression of enterotoxins K and L has no visible effect on the exoprotein patterns of the producing organisms. Because neither of these has been shown to have any clinically relevant activity, the significance of their lack of regulatory activity is uncertain. However, preliminary observations suggest that enterotoxin A, a well-characterized SAg that is an important cause of food poisoning as well as of extra-vaginal TSS, also lacks autorepressive and cross-repressive activity. It may be relevant that neither enterotoxin A (23) nor SEK (24) is regulated by *agr*. Perhaps the regulatory properties of *tst* and *seb*, which are *agr*-regulated, are related to the larger regulatory network to which they belong. Consistent with this possibility is our inability to demonstrate direct binding of purified TSST-1 to the *tst* promoter, suggesting that one or more intermediary factors may be involved. Any such intermediary factor(s) could well belong to the overall regulatory network that governs exoprotein production in *S. aureus*. Identification of these factors is currently a high priority.

As the target sequences responsive to the newly observed self-inhibition of TSST-1 synthesis and to the well-established *agr* activation (unpublished results) are both located within the promoter regions of the toxin gene, we have not yet been able to separate them genetically, either from each other or from function of the promoter itself. Indeed, the *agr*-responsive element of *sed*, which encodes enterotoxin D, has recently been mapped also to within the minimal *sed* promoter (25). Whether the *agr* and autorepression targets coincide for *seb* remains to be determined.

In sum, we have observed that at least two of the important staphylococcal SAgs, TSST-1 and SEB, strongly inhibit their own synthesis as well as that of many other exoproteins. Although the autorepression and trans inhibition functions of the two SAgs seem to colocalize within the respective coding sequences, there is reason to believe that they are biologically distinct and may involve different functional pathways. Thus, trans inhibition could be relevant to the pathogenic strategy of the organism: it will be recalled that infections with TSST-1-producing staphylococci are rapidly fatal in several animal models as well as in susceptible humans (26, 27), and that local infections with TSST-1-producing strains typically lack the ebullient inflammatory response that is highly characteristic of staphylococcal disease (28, 29). We have confirmed this effect in a simple murine s.c. abscess model, where the TSST-1-producing strain produced essentially no significant lesion whereas the knockout strain produced the typical *S. aureus* abscess. Because among the affected exoproteins are proteases, the

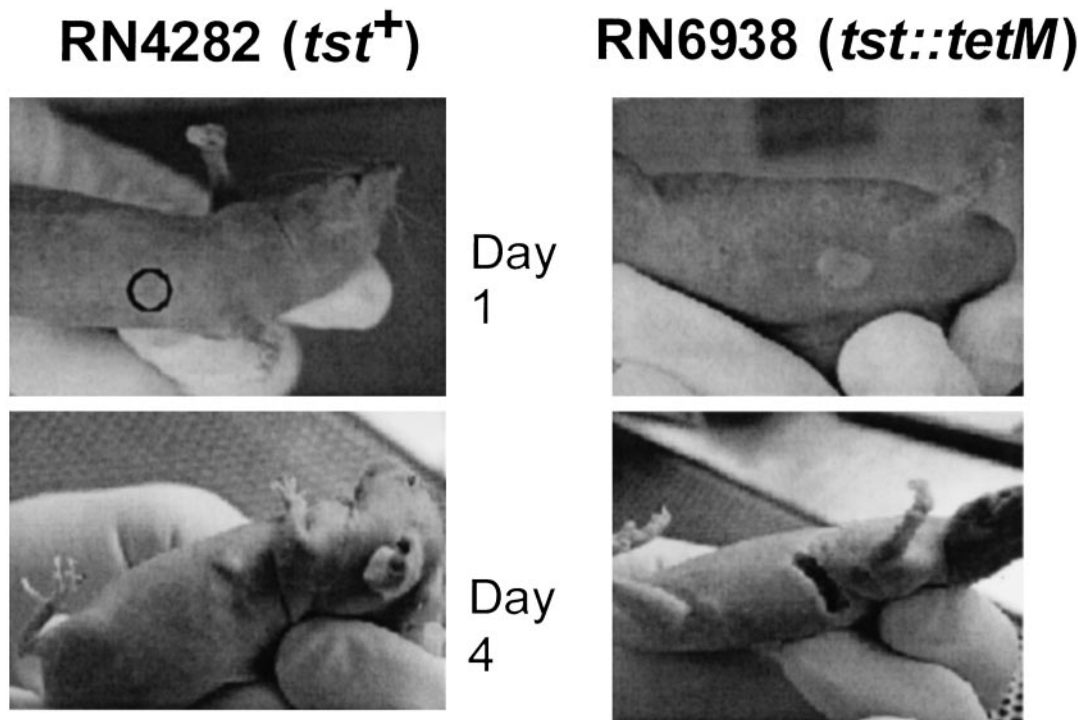

Fig. 5. Inhibition of inflammation by TSST-1-producing strain *in vivo*. Three mice were injected with either TSST-1-producing strain (RN4282) or isogenic *tst*$^{-}$ strain (RN6938). Mice were inspected daily. Results shown are representative of each group.

inhibitory effect of the SAg might lead to a higher effective tissue concentration of TSST-1 owing to reduced proteolysis. The possible relevance of this to pathogenesis remains to be determined.

The suppression of inflammation by TSST-1 has been attributed to the induction of tumor necrosis factor α by the SAg (29). Alternatively (or additionally) it could be a consequence of the sharp reduction in exoproteins, which have an important role in excitation of the inflammatory response, thus retarding elimination of the organism and permitting the elaboration of a lethal dose of the SAg. We note that one of these proteins, lipase, is cytotactic for polymorphonuclear leukocytes (30). Whether other staphylococcal exoproteins are also cytotactic is presently unknown.

Regarding autorepression, perhaps the precursors of these two SAgs are toxic to the organism at elevated levels and autorepression prevents their accumulation. Study of the effects of TSST-1 and SEB on the comparative histopathology of experimental infections, in comparison with SAgs that do not repress other exoproteins, is expected to clarify the role of these toxins and their regulatory properties with respect to the inflammatory response and to the biology and/or pathogenicity of the organism.

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